Interference of carbidopa and other catechols with reactions catalyzed by peroxidases

Interference of carbidopa and other catechols with reactions catalyzed by peroxidases

Beata Gąsowska-Bajger[a], Yuki Nishigaya[b], Krystyna Hirsz-Wiktorzak[a], Anna Rybczyńska[a], Toshimasa Yamazaki[b], and Hubert Wojtasek[a*]

[a]Faculty of Chemistry
Opole University
Ul. Oleska 48
45-052 Opole
Poland

[b]Advanced Analysis Center
National Agriculture and Food Research Organization (NARO)
2-1-2 Kannondai
Tsukuba, Ibaraki, 305-8602
Japan

[*]To whom correspondence should be addressed:
Faculty of Chemistry
Opole University
Ul. Oleska 48
45-052 Opole
Poland
phone: +48 77 452 7122
fax: +48 77 452 7101
e-mail: Hubert.Wojtasek@uni.opole.pl

Keywords: carbidopa; catechol; hydrazine; peroxidase; enzymatic assay interference;

Parkinson's disease

Abstract

Background: A number of compounds, including ascorbic acid, catecholamines, flavonoids, *p*-diphenols and hydrazine derivatives have been reported to interfere with peroxidase-based medical diagnostic tests (Trinder reaction) but the mechanisms of these effects have not been fully elucidated.

Methods: Reactions of bovine myeloperoxidase with *o*-dianisidine, bovine lactoperoxidase with ABTS and horse radish peroxidase with 4-aminoantipyrine/phenol in the presence of carbidopa, an anti-Parkinsonian drug, and other catechols, including L-dopa, were monitored spectrophotometrically and by measuring hydrogen peroxide consumption.

Results: Chromophore formation in all three enzyme/substrate systems was blocked in the presence of carbidopa and other catechols. However, the rates of hydrogen peroxide consumption were not much affected. Irreversible enzyme inhibition was also insignificant.

Conclusions: Tested compounds reduced the oxidation products or intermediates of model substrates thus preventing chromophore formation. This interference may affect interpretation of results of diagnostic tests in samples from patients with Parkinson's disease treated with carbidopa and L-dopa.

General significance: This mechanism allows prediction of interference in peroxidase-based diagnostic tests for other compounds, including drugs and natural products.

1. Introduction

Peroxidases are heme-containing oxidoreductases catalyzing the oxidation of a variety of substrates with hydrogen peroxide. Mammalian peroxidases include enzymes involved primarily in defense reactions (myeloperoxidase, MPO; eosinophil peroxidase, EPO; lactoperoxidase, LPO) and thyroid hormone biosynthesis (thyroid peroxidase, TPO) [1]. The antimicrobial activity of MPO, EPO, and LPO results from their ability to convert halides, thiocyanate, and nitrite to compounds (e.g. hypohalous acids) that are highly toxic to invading microorganisms [2, 3]. These enzymes also participate in the metabolism of xenobiotics, including drugs [4], and some of them, particularly MPO, have been implicated in the pathogenesis of many diseases, such as atherosclerosis, Alzheimer's disease, Parkinson's disease, and cancer [5, 6]. Therefore substantial efforts have been made to develop inhibitors of MPO with perspectives for clinical applications [7, 8].

Hydrazine derivatives have been widely used in medicine and include the primary anti-tuberculosis drug isoniazid, the anti-cancer agent procarbazine, the anti-Parkinsonians carbidopa and benserazide, and the older generation anti-depressants iproniazid and phenelzine [9]. Peroxidases oxidize hydrazine derivatives generating radical products that generally inactivate these enzymes by covalent binding to the heme prosthetic group or the polypeptide chain [10]. The effect of phenylhydrazine has been particularly well studied on horseradish peroxidase (HRP) [11], LPO [12] and lacrimal-gland peroxidase [13]. Substantial efforts have also been made to elucidate the interactions of peroxidases with isoniazid [14-17] in attempts to understand the action of this drug on the *Mycobacterium tuberculosis* catalase-peroxidase (MtCP, KatG) [18]. Inhibition of peroxidases by isoniazid also led to investigations of these enzymes with a number of benzoic acid hydrazides [19-22].

We have recently synthesized a potential tyrosinase-activated anti-melanoma prodrug with a hydrazine linker [23]. Before designing such prodrugs we tested the reaction of

carbidopa ((2*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid, **1**, Fig. 1) to determine the reaction pathway following the oxidation of a compound containing both a hydrazine group and a catechol moiety by tyrosinase [24]. However, since prodrugs with such a structure, before reaching the target tissues, would be exposed to the oxidative systems in the blood, we also decided to test their oxidation by mammalian peroxidases. It has been shown that carbidopa is oxidized by horseradish peroxidase [25] but interactions of this drug

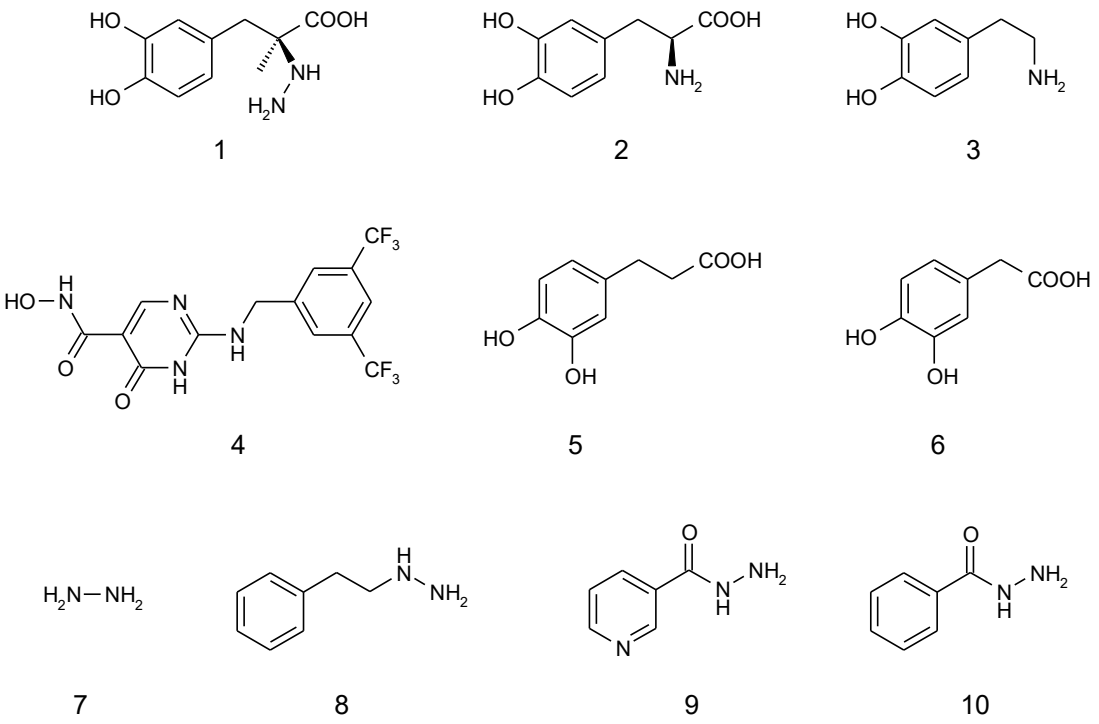

Figure 1. Structures of compounds used in this study: 1 – (*S*)-carbidopa ((2*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid; 2 – L-dopa ((*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid), 3 – dopamine (2-(3,4-dihydroxyphenyl)ethylamine); 4 – HX1 (2-(3,5-bistrifluoromethylbenzylamino)-6-oxo-1*H*-pyrimidine-5-carbohydroxamic acid; 5 – 3-(3,4-dihydroxyphenyl)propanoic acid; 6 – 3,4-dihydroxyphenylacetic acid; 7 – hydrazine; 8 – phenelzine (2-phenylethylhydrazine); 9 – isoniazid (isonicotinic acid hydrazide); 10 – benzhydrazide.

with mammalian peroxidases have not been studied so far. Here we show that HRP, bovine MPO and LPO oxidize carbidopa to the same products as tyrosinase. We also show that this drug and other catechol-containing compounds (**2**, **3**, **5**, and **6**, Fig. 1) interfere with the oxidation of standard peroxidase substrates, such as *o*-dianisidine, ABTS, and 4-amino-antipyrine/phenol. This effect results primarily from reduction of the products or intermediates of the oxidation of these substrates not from reversible or irreversible inhibition of these enzymes.

2. Materials and Methods

2.1. Reagents

Carbidopa was obtained from Sochinaz SA (Vionnaz, Switzerland). HRP, bovine LPO, *o*-dianisidine, 4-aminoantipyrine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), L-3,4-dihydroxyphenylalanine (L-dopa), dopamine, 3-(3,4-dihydroxyphenyl)propanoic acid, 3,4-dihydroxyphenylacetic acid, phenelzine, isoniazid, and HPLC grade acetonitrile were purchased from Sigma-Aldrich. Hydrogen peroxide, phenol, hydrazine hydrate, xylenol orange, buffer components and solvents were from Avantor Performance Materials (Gliwice, Poland), SDS was from Fluka, and benzhydrazide from Alfa Aesar. MPO was purified from bovine spleen according to published procedures [26, 27]. Tyrosinase was isolated from mushrooms as described previously [28].

2.2. Spectrophotometric analysis of the oxidation of carbidopa by peroxidases and tyrosinase

Solutions of carbidopa were prepared fresh daily in 1 mM phosphoric acid to prevent autooxidation. Reactions with peroxidases were carried out at room temperature in 3 ml of 50 mM Sörensen buffer, pH 6.0, with 10-500 μM carbidopa in the presence of equimolar concentration of H_2O_2 and 20 to 500 nM enzymes. The concentration of the enzymes was calculated from the absorbance of their Soret bands using their extinction coefficients: 91000 $M^{-1}\times cm^{-1}$ at 430 nm for bovine MPO [21], 112000 $M^{-1}\times cm^{-1}$ at 412 nm for bovine LPO [29], and 102000 $M^{-1}\times cm^{-1}$ at 403 nm for HRP [30]. Reactions were initiated by the addition of either hydrogen peroxide or the enzymes. Reactions with tyrosinase were carried out in 50 mM sodium phosphate buffer, pH 6.8. The concentration of tyrosinase was determined using the Bradford assay with bovine serum albumin as a standard. UV/Vis

spectra from 250 to 700 nm were recorded in a Jasco V-650 UV-Vis spectrophotometer immediately after mixing the reagents and then in 1 or 2 min intervals.

2.3. HPLC Analysis

The reactions with peroxidases were carried out in 0.5 ml of 50 mM Sörensen buffer, pH 6.0, with 500 μM carbidopa in the presence of 500 μM H_2O_2 and 50 to 500 nM enzymes. Samples were incubated at room temperature for 0-120 min and then the enzymes were removed from the reaction mixtures by ultrafiltration on Microcons Ultracel YM-10 (Merck Millipore). The filtrates were then separated at 30 °C on an Accucore C_{18} analytical column (100 mm length, 2.1 mm diameter, 2.6 μm particle size, Thermo Scientific) with a guard column connected to a Dionex UltiMate 3000 HPLC instrument equipped with a UV-Vis photodiode array detector at a flow rate of 0.2 ml/min. Samples were injected into a 5 μl loop and then eluted with the following program: 5% acetonitrile in water for 3 min, 5-50% gradient of acetonitrile in water for 14 min, followed by 90% acetonitrile in water for 1 min. The identity of all products was confirmed by LC-MS analysis on a Dionex UltiMate 3000 HPLC connected to a Bruker Daltonik micrOTOF-Q II mass spectrometer with ESI ionization in the positive or negative ion mode. The mass spectrometer was calibrated with sodium formate clusters. 6,7-Dihydroxy-3-methylcinnoline and 3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid were prepared by oxidation of carbidopa with tyrosinase in a large scale reaction (50 mg), purified as described previously [24] and used as standards.

2.4. Oxidation of model substrates in the presence of carbidopa and other catechols

The peroxidatic activity of bovine MPO was measured with *o*-dianisidine. The reaction mixture consisted of 50 mM Sörensen buffer, pH 6.0, 100 μM *o*-dianisidine, 50 μM H_2O_2, 2-20 nM enzyme, and various concentrations of carbidopa, L-dopa, 3-(3,4-dihydroxyphenyl)propanoic acid or 3,4-dihydroxyphenylacetic acid (1-40 μM). Changes in absorbance at 460 nm resulting from the oxidation of *o*-dianisidine were measured for 30 min.

The peroxidatic activity of bovine LPO was measured with either *o*-dianisidine under conditions identical to reactions with bovine MPO or 2,2-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) diammonium salt. The reactions with 25 μM ABTS were carried out in 50 mM Sörensen buffer, pH 6.0, with 25 μM H_2O_2, and 1-10 nM LPO. Absorbance of the oxidized ABTS product ($ABTS^{+\bullet}$) at 415 nm was monitored for 30 min. The same catechols as for MPO were applied at the same concentrations.

The activity of horseradish peroxidase was tested in 50 mM phosphate buffer, pH 7.0 with 50 μM 4-aminoantipyrine, 1 mM phenol, 50 μM H_2O_2, 0.5 or 1.0 μg of HRP, and various concentrations of carbidopa, L-dopa, dopamine, 3-(3,4-dihydroxyphenyl)propanoic acid or 3,4-dihydroxyphenylacetic acid. The tested compounds were applied at 10-160 μM. Spectra from 250 to 700 nm were recorded in a Jasco V-650 UV-Vis spectrophotometer immediately after mixing the reagents and then in 1 min intervals for 30 min. Additionally the same reactions were performed and the absorbance at 505 nm was measured for 10-30 min.

Hydrogen peroxide consumption was measured under the same conditions as the reactions monitored spectrophotometrically with the modified xylenol orange method including sorbitol [31]. Aliquots of the reaction mixtures were removed at 1 min intervals, mixed with the xylenol orange reagent, incubated in the dark for 30 min and the absorbance was measured at 560 nm. Under the reaction conditions (25 mM sulfuric acid) the catechols used in this study did not interfere with this assay (Fig. S1).

2.5. Analysis of meriquinone reduction

Reduction of meriquinone, the intermediate in the oxidation of *o*-dianisidine by peroxidases, was performed according to published procedures [32, 33] with slight modifications. Reactions were carried out at room temperature in 50 mM sodium acetate buffer, pH 4.0, with 100 μM *o*-dianisidine, 100 μM H_2O_2, 20 nM MPO in the presence of 200 μM SDS (1 mM SDS used previously in this assay with HRP [32, 33] strongly inhibited

bovine MPO) for 10 min. After that time, when the absorbance of meriquinone at 395 nM stabilized, the tested compounds at concentration 10 μM, 25 μM or 50 μM were added. The following compounds were analyzed: carbidopa, L-dopa, 3-(3,4-dihydroxyphenyl)propanoic acid, 3,4-dihydroxyphenylacetic acid, hydrazine, phenelzine, benzhydrazide, and isoniazid. Changes in absorbance at 395 nm were then measured for 10-30 min.

2.6. Analysis of irreversible enzyme inhibition

Bovine MPO or LPO at 1.5 μM concentration were incubated with various concentrations of carbidopa (15-1500 μM) in the presence of equimolar concentration of H_2O_2 in 50 mM Sörensen buffer, pH 6.0 in a final volume of 0.5 mL for 1 h at room temperature and then the reaction components were separated by dialysis overnight (20-22 h) at 4°C. Originally the enzymes were separated from the low molecular mass components by gel filtration on Sephadex G-25 columns. However, large part of the enzyme was retained on the column, which indicated substantial protein cross-linking, as would be expected during oxidation of a compound containing both a catechol and a hydrazine group. This method was therefore abandoned. After dialysis the protein concentration was determined spectrophotometrically with the Bradford method and the UV-Vis spectra of the enzymes in the 250-700 nm range were also recorded. The peroxidatic activity was then measured with *o*-dianisidine as described above.

2.7. Molecular modeling

Molecular docking experiments of carbidopa to human MPO (PDB ID: 1D2V) [34] and bovine LPO (PDB ID: 3Q9K) were carried out using AutoDock Vina 1.1.2 [35] and AutoDockTools 1.5.6 [36] with standard protocols. The crystal structures with the highest resolutions among those deposited in the Protein Data Bank were chosen as the template structures. A 28 × 30 × 28 Å grid box with the level of exhaustiveness set at 20 was used to cover all the space of ligand-binding pocket around the heme. The ionized form of the ligand

generated by the wash command of Molecular Operating Environment (MOE) software (MOE version 2014.09; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2014) was used for the docking experiments. The rotatable bonds of the small molecules were applied as default settings. The graphical representations were prepared by PyMOL (version 1.7.0.1, Schrödinger, LLC.).

3. Results

3.1. Oxidation of carbidopa by peroxidases and tyrosinase

Spectrophotometric analysis of mixtures of bovine MPO or LPO with carbidopa showed slow changes of absorbance in the visible and near-UV region with a maximum of absorption at ca. 365 nm (Fig. S2A and B), seen previously during reactions of this compound with HRP [25] (Fig. S2C). These spectra were very similar to the spectrum of the product obtained after oxidation of carbidopa by tyrosinase (Fig. S2D), which we have previously identified as 6,7-dihydroxy-3-methylcinnoline [24]. The reaction rates were, however, much smaller than in the case of tyrosinase (35-fold for bovine MPO and 16-fold for bovine LPO) and also smaller than for HRP (11-fold for bovine MPO and 5.2-fold for bovine LPO, based solely on the absorption at 365 nm). We also analyzed the reaction mixtures by HPLC to determine their composition. The reaction mixture with tyrosinase contained products identified previously [24], i.e. 6,7-dihydroxy-3-methylcinnoline, 3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid, and 3,4-dihydroxyphenylacetone (Fig. S3D). These compounds were also present in reaction mixtures with peroxidases, although in much smaller quantities (Fig. S3A-C), as was expected from the spectrophotometric analysis.

3.2. Spectrophotometric analysis of the influence of carbidopa on the peroxidatic activity of bovine MPO and LPO

Since carbidopa was oxidized very slowly by the bovine enzymes, we decided to investigate its potential to inhibit them using model substrates (*o*-dianisidine and ABTS). In the spectrophotometric analysis the presence of carbidopa resulted in a dramatic decrease of absorbance in reactions with both enzymes (Fig. 2). Essentially no absorption in the range of the oxidation products of *o*-dianisidine in the case of bovine MPO and *o*-dianisidine or ABTS in the case of bovine LPO was detected in the initial phase of the reactions. However, at lower carbidopa concentrations the activity was recovered to a substantial degree after a lag period,

as would be expected for a compound which also served as a substrate for both enzymes. At higher concentrations the lag period was no longer proportional to concentration and was even extended indefinitely (until the end of the measurement time - up to 120 min). These results indicated that carbidopa may act as a suicide substrate, just as other hydrazine derivatives, and the activity may be retained only after a limited turn-over number. This effect may have also been caused by disruption of the peroxidase cycle. While carbidopa may have been readily oxidized by compound I, it may have been a poor substrate for compound II leading to accumulation of the latter, as it has been demonstrated for a number of compounds, such as indole and tryptamine derivatives [1].

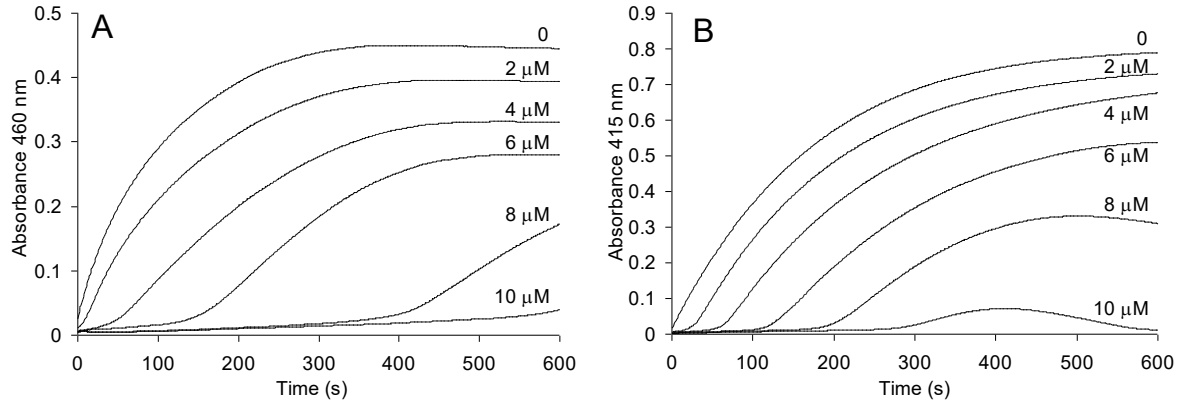

Figure 2. Influence of carbidopa on the peroxidatic activity of bovine MPO (A) and LPO (B) measured spectrophotometrically. The oxidation of 100 μM *o*-dianisidine with 50 μM H_2O_2 and 5 nM MPO was monitored at 460 nm (A) and the oxidation of 25 μM ABTS with 25 μM H_2O_2 and 2 nM LPO was monitored at 415 nm (B) in the absence (0) or presence of carbidopa at concentrations shown next to each curve. The concentrations of the enzymes and carbidopa were chosen to best show the changes in the lag period.

3.3. Irreversible inhibition of bovine peroxidases by carbidopa

Prolonged incubation of bovine MPO and PLO with carbidopa and H_2O_2 led to partial loss of activity, but the concentration of carbidopa necessary to achieve this effect was quite high. Even at the substrate to enzyme ratio equal 1000:1 (1.5 mM:1.5 μM) both enzymes still retained substantial activity (Fig. 3). The inhibitory effect was slightly stronger for bovine MPO than for bovine LPO, confirming the results of the oxidation of carbidopa by both enzymes (spectrophotometric and chromatographic analysis). Partition ratios [37] calculated from these results were 1240 and 1460 for bovine MPO and bovine LPO, respectively. These numbers are quite high when compared to other irreversible peroxidase inhibitors, including hydrazine derivatives. For phenelzine and HRP the partition ratio was 11 [38], and for the very effective irreversible thioxanthine inhibitor TX2 and human MPO – only 0.2 [39]. The UV-Vis spectra of the enzymes recorded after incubation with carbidopa and dialysis have shown little changes in the heme Soret bands (data not shown), which indicated that the prosthetic group was not destroyed to a significant degree.

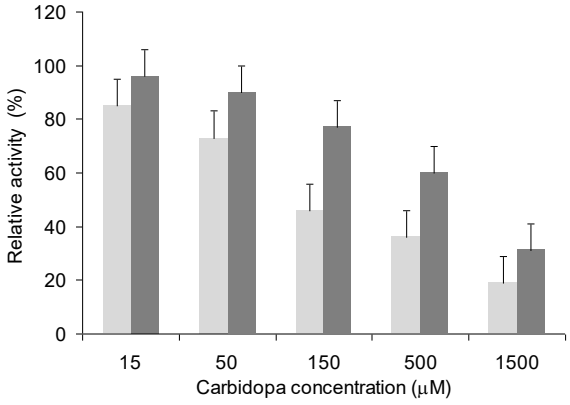

Figure 3. Irreversible inactivation of 1.5 μM bovine MPO (▒) and bovine LPO (▒) determined after incubation with carbidopa for 1 h followed by dialysis. Enzyme activity was monitored with *o*-dianisidine as a substrate. The activity was calculated with respect to a control reaction with the enzyme which was treated in the same way in the absence of carbidopa and H_2O_2.

3.4. Molecular docking of carbidopa to human MPO and bovine LPO

The relatively inefficient irreversible inhibition of MPO and LPO by carbidopa can be attributed to the presence of the *o*-diphenol moiety, which may preferentially interact with the active site preventing the oxidation of the hydrazine group followed by covalent modification of the enzymes' active sites. The hydrophobic character of the access channel may lead to preferential orientation of carbidopa with the catechol group directed towards the heme center. Formation of 6,7-dihydroxy-3-methylcinnoline by both enzymes, which proceeds via the *o*-quinone intermediate, also seemed to support this hypothesis. This mode of oxidation of carbidopa could explain, at least in part, the lack of its reported carcinogenic effects. We performed molecular docking experiments of carbidopa to human MPO (PDB ID: 1D2V) [34] and bovine LPO (PDB ID: 3Q9K) using AutoDock Vina 1.1.2 [35]. Since no crystal structures are available for bovine MPO we have used human MPO which has 87% identity with bovine MPO. In the best-scored structures the orientation of carbidopa was indeed as we expected – with the catechol group directed towards the heme forming a network of hydrogen bonds (Fig. S4 and S5). While a single structure with a binding energy of -7.1 kcal×mol^{-1} was obtained for human MPO, the experiments with bovine LPO provided two top-scored structures with equal binding energies of -6.8 kcal×mol^{-1}. The observation of the two equally favored ligand orientations in bovine LPO suggests that the reversible interaction of carbidopa with the heme distal side of this enzyme is weaker than with the human MPO. The detailed results of these experiments are presented in the Supplementary Material.

3.5. Changes in H_2O_2 concentration during oxidation of model substrates by bovine MPO and LPO in the presence of carbidopa

Since we excluded irreversible inhibition of MPO and LPO by carbidopa, we looked for other mechanisms that could explain the lack of chromophore formation during reactions

with *o*-dianisidine and ABTS (Fig. 2). It may have resulted from preferential oxidation of carbidopa by peroxidases until its depletion or reduction of the oxidation products of standard substrates. This effect was particularly likely for $ABTS^{+\bullet}$, which is frequently used to determine antioxidant activity [40] and is readily reduced by a number of compounds, including polyphenols [41, 42].

To determine which of these two mechanisms is involved we monitored hydrogen peroxide consumption during the oxidation of *o*-dianisidine by MPO and ABTS by LPO in the presence of carbidopa. Changes in H_2O_2 concentration did not correspond to changes in absorbance. In the reactions with MPO and *o*-dianisidine moderate decrease in the reaction rates was observed in the presence of carbidopa and the effect was proportional to its concentration but no lag periods were observed (Fig. 4A). These results demonstrated that in this case both competitive oxidation of carbidopa (at a rate lower than for *o*-dianisidine) and reduction of the oxidation product of *o*-dianisidine (causing lack of absorbance at 460 nm

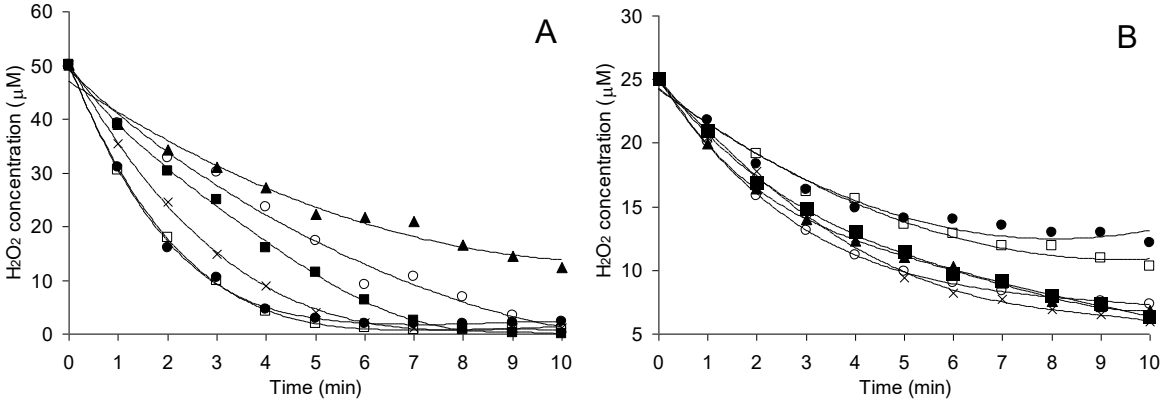

Figure 4. Changes in H_2O_2 concentration during oxidation of *o*-dianisidine by bovine MPO (A) and ABTS by bovine LPO (B) measured by the modified xylenol orange assay. Reactions were carried out under conditions identical as in Figure 1 in the absence (●) or presence of 2 μM (□), 4 μM (×), 6 μM (■), 8 μM (○), and 10 μM (▲) carbidopa.

during the lag period) took place. In reactions with LPO and ABTS a slight increase in the reaction rate was observed in the presence of carbidopa, although no correlation with its concentration could be seen (Fig. 4B). This effect can be attributed to reduction of $ABTS^{+\bullet}$ back to ABTS, whose concentration is maintained close to the initial value until carbidopa is consumed. Also, the final values of H_2O_2 concentration were lower in the presence of carbidopa than in its absence.

3.6. Reduction of meriquinone by carbidopa and other catechols

When we oxidized ABTS with bovine LPO and added carbidopa to the reaction mixture it was decolorized immediately. This effect could not be reproduced with *o*-dianisidine, which demonstrated that it was not the final oxidation product, which was reduced by carbidopa but one of the reaction intermediates. Such an intermediate, a charge transfer complex between the substrate and the quinonediimine product named meriquinone [43], may be stabilized by SDS at low pH [32]. We have therefore generated meriquinone in reactions of *o*-dianisidine with MPO and tested the effect of carbidopa on its concentration. Results of this experiment clearly demonstrated that carbidopa could reduce this intermediate (Fig. 5). To determine the role of each of the reducing groups in carbidopa (catechol and hydrazine) we performed this analysis with several catechols (L-dopa, 3-(3,4-dihydroxyphenyl)propanoic acid, 3,4-dihydroxyphenylacetic acid) and with hydrazine and several of its derivatives (phenelzine, benzhydrazide, isoniazid). The effect of all catechol-containing compounds was similar to that of carbidopa, while the influence of hydrazine and its derivatives was small or negligible (Fig. S6). We also analyzed the oxidation of *o*-dianisidine by peroxidases in the presence of these catechols under standard assay conditions. In all cases lack of absorbance at 460 nm in the initial phase of the reactions followed by its appearance after a lag period was observed (Fig. S7A and B).

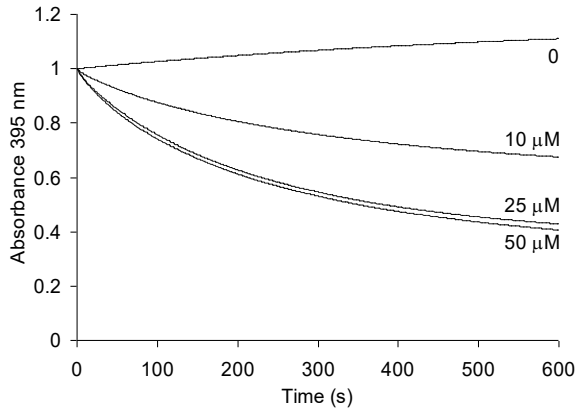

Figure 5. Reduction of meriquinone by carbidopa. After oxidation of 100 μM *o*-dianisidine with 100 μM H_2O_2 and 20 nM MPO in the presence of 200 μM SDS in 50 mM acetate buffer, pH 4.0 for 10 min (time 0) the reaction was monitored at 395 nm in the absence of carbidopa (0) or in its presence at concentrations shown next to each curve.

3.7. Influence of carbidopa and other catechols on the oxidation of 4-aminoantipyrine by HRP

It therefore became obvious that carbidopa and the other tested catechols interfered with bovine MPO and LPO-catalyzed reactions with both *o*-dianisidine and ABTS as substrates by reducing their oxidation products or intermediates leading to changes in absorbance which did not reflect changes in reaction rates. We have therefore decided to study their effects on reactions catalyzed by horseradish peroxidase with 4-aminoantipyrine as a substrate. This reaction is applied in many medical diagnostic tests and a number of compounds have been reported to interfere with it. Such an effect was first discovered for tolazamide with three substrate systems: 4-aminoantipyrine, guaiacum, and ABTS [44], and later for another hydrazine derivative – gliclazide [45]. It was also reported for N-acetylcysteine [46], acetaminophen with MBTH used as a substrate [47], and is particularly well documented for ascorbic acid [48-50]. Most importantly, however, such interference was also described for catechol-containing compounds – first for dopamine, which interfered with

creatinine measurements [51], and later for dopamine and dobutamine, which affected detection of several analytes (cholesterol, triglycerides, and uric acid) [52, 53]. More recently such an effect was also reported for norepinephrine and epinephrine, although for these two compounds physiological and therapeutic concentrations are too low to cause significant interference in diagnostic tests [54].

When we carried out oxidation of 4-aminoantipyrine/phenol by HRP in the presence of carbidopa and monitored the reactions spectrophotometrically, we obtained results very similar to those observed for *o*-dianisidine/MPO and ABTS/LPO – a lag period in the increase of absorbance of the quinoneimine chromophore and a decrease in the final absorbance proportional to carbidopa concentration (Fig. 6). However, measurements of changes in hydrogen peroxide concentration again have shown that the reaction rates were essentially unaffected by the presence of carbidopa (Fig. 7). These results demonstrate that in this system (HRP/4-aminoantipyrine/phenol) carbidopa is not an effective competing substrate as it was for MPO with *o*-dianisidine as a substrate (Fig. 4A) and the observed decrease in the rate of

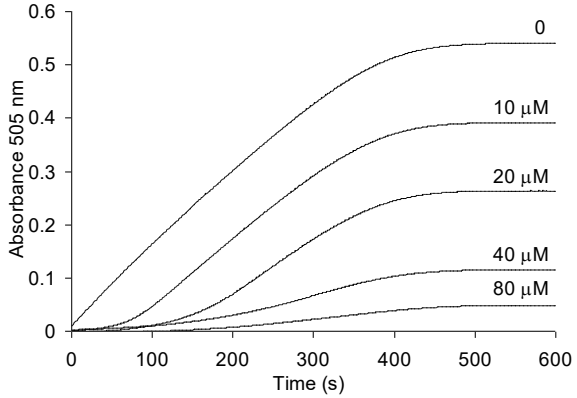

Figure 6. Changes of absorbance at 505 nm during oxidation of 50 μM 4-aminoantipyrine and 1 mM phenol with 100 μM H_2O_2 by 0.5 μg of HRP in the absence (0) or presence of carbidopa at concentrations shown next to each curve. The concentrations of the enzyme and carbidopa were chosen to best show the changes in the lag period.

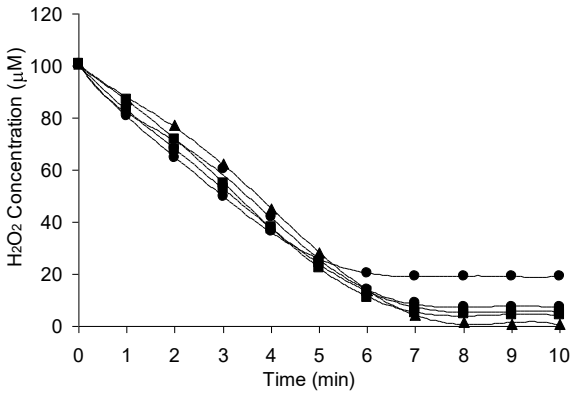

Figure 7. Changes in H_2O_2 concentration during oxidation of 50 μM 4-aminoantipyrine and 1 mM phenol with 100 μM H_2O_2 by 0.5 μg of HRP in the absence (●) or presence of 10 μM (□), 20 μM (○), 40 μM (■), and 80 μM (▲) carbidopa.

formation and the final concentration of antipyrilquinoneimine are due primarily to the reduction of the reaction intermediates (most likely the antipyril radical) by this compound.

Similar results were also obtained for other catechols tested before with MPO and *o*-dianisidine and LPO with *o*-dianisidine and ABTS as substrates (L-dopa, 3-(3,4-dihydroxyphenyl)propanoic acid, 3,4-dihydroxyphenylacetic acid). For comparison we also performed this analysis for dopamine, whose effects on HRP-catalyzed reactions with 4-aminoantipyrine/phenol were previously reported [51-53, 55, 54]. Although the lag phases were not as clear as in the case of carbidopa, the rates of increase of absorbance at 505 nm and their final values were lower in the presence of all catechols than in their absence (Fig. S8). The differences between the shapes of these curves can be explained by the absorption spectra of the oxidation products of carbidopa an the other catechols. While the oxidation products of carbidopa formed during the measurement period absorb very weakly at 505 nm, spectra of the oxidation products of the other catechols overlap in large part with the spectrum of the antipyrilquinoneimine (Fig. S9). These data show how complex the influence of catechols on the peroxidase catalyzed reactions may be. The value of absorbance will depend

on the measurement time, the rates of the reactions between catechols and the intermediates/products of the oxidation of standard substrates, the rates of transformation of the *o*-quinones formed (cyclization or decomposition), absorption spectra of the oxidation products of catechols, and the ratio of absorption coefficients of the oxidation products of standard substrates and catechols at the detection wavelength.

To illustrate the influence of some of these factors we recorded the UV-Vis spectra during oxidation of 4-aminoantipyrine and phenol with HRP in the presence of carbidopa, L-dopa and dopamine (Fig. 8). In reactions with carbidopa much slower increase of absorbance at 505 nm was accompanied by an increase of absorbance at 365 nm, the maximum of absorption for 6,7-dihydroxy-3-methylcinnoline, one of the oxidation products of carbidopa [24] (Fig. 8B), at a rate much greater than for carbidopa alone (Fig. S9A). In the case of L-dopa the spectra corresponded to dopachrome (maximum at 475 nm), showing that essentially only this chromophore was formed (Fig. 8C), again at a rate much greater than for L-dopa alone (Fig. S9B). This conclusion is supported by the final value of absorbance in this reaction (0.1734), which corresponds almost exactly to 50 μM dopachrome (extinction coefficient at 475 nm equal 3600 $M^{-1}\times cm^{-1}$) showing that oxidation (indirect) of L-dopa completely depletes hydrogen peroxide from the reaction medium (2 equivalents of H_2O_2 are required for oxidation of L-dopa to dopachrome). The spectra for reactions with dopamine indicated formation of a mixture of chromophores (dopaminochrome and antipyrilquinoneimine) although formation of a different chromophore (e.g. an oxidized adduct of 4-aminoantipyrine to dopamine) cannot be excluded on the basis of UV-Vis spectra alone, since the maximum of absorption shifted to ca. 495 nm (Fig. 8D). This difference in behavior between L-dopa and dopamine may be explained by the difference in the pK_a values of their amino groups (8.72 for L-dopa and 10.2 for dopamine [56]), which will result in much

slower cyclization of dopaminequinone than dopaquinone (ca. 30 ×) and may allow competing reactions (e.g. addition of 4-aminoantipyrine to dopaminequinone) to occur.

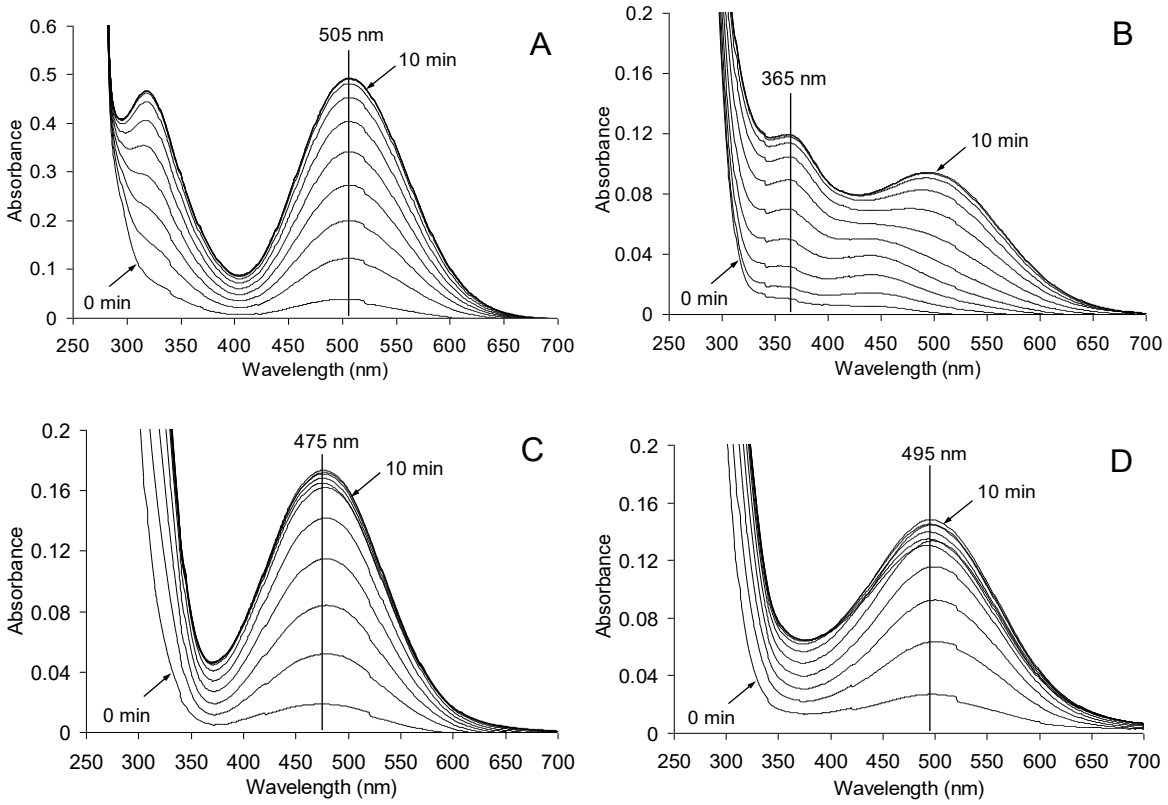

Figure 8. UV-Vis spectra recorded during oxidation of 50 μM 4-aminoantipyrine and 1 mM phenol with 100 μM H_2O_2 by 0.5 μg of HRP in the absence of other compounds (A) and in the presence of 80 μM carbidopa (B), 80 μM L-dopa (C), and 80 μM dopamine (D). Spectra were recorded immediately after addition of the enzyme (0 min) and then in 1 min intervals for 10 min.

Addition of any of the catechols to the formed chromophore (antipyrilquinoneimine) caused little changes in absorbance (less than 5% decrease within 15 min), as previously demonstrated for dopamine [55].

4. Discussion

There are numerous reports describing the effects of polyphenols, such as flavonoids or gallic acid and its derivatives on the activity of peroxidases [57-62]. These studies usually rely on measuring the concentration of oxidized products of standard substrates, such as tetramethylbenzidine, *o*-dianisidine or ABTS for peroxidatic activity. In the case of chlorinating activity of myleoperoxidase chlorination of monochlorodimedone or taurine, oxidation or chlorination of LDL is usually measured. Rarely, however, these experiments are accompanied by monitoring the concentration of hydrogen peroxide and the slower changes in the concentration of the oxidized products are therefore interpreted as enzyme inhibition. However, when the tested compounds may act as antioxidants, inhibition of the enzyme cannot be distinguished by such methods from its antioxidant effects. Inhibition of dityrosine formation by myeloperoxidase was reported for ferulic acid, caffeic acid, curcumin and quercetin. In this case quenching of the generated tyrosyl radical was simply excluded based on the fact that the concentration of tyrosine (0.2 mM) was higher than the concentration of the antioxidants (0.1 mM). The rate of tyrosine oxidation, however, was not determined [58]. Another report, however, clearly demonstrated scavenging of tyrosyl radicals by quercetin, its derivatives and other flavonoids [63]. When the influence of flavonoids, such as rutin, taxifolin, epicatechin, quercetin, and kaempferol, on the oxidation LDL by MPO in the presence of nitrite was studied, a lag period in the appearance of peroxidized lipids was observed. This effect was very similar to the results obtained by us during oxidation of *o*-dianisidine by MPO and ABTS by LPO in the presence of carbidopa and other catechols and was attributed to the scavenging of the MPO-derived NO_2 radical by flavonoids [64]. Quercetin and some of its metabolites were shown to inhibit neutrophil-mediated LDL oxidation and this effect was also attributed to inhibition of myeloperoxidase [60]. Interestingly, modification of quercetin at either 3 or 3’ position did not affect the inhibitory

potency, but conjugations at both positions significantly reduced this activity. We have recently shown that the presence of a catechol in flavonoid ring B or conjugated phenol groups at positions 3 and 4' enables their redox reaction with *o*-quinones thus leading to apparent inhibition of tyrosinase [65]. Results obtained with myeloperoxidase [60] indicate a similar phenomenon in this case.

The effects of polyphenols on the halogenating activity of myeloperoxidase and the pseudohalogenating activity of lactoperoxidase vary. Several studies demonstrated that flavonoids enhance these activities [66-69]. This effect was explained by the ability of these compounds to reduce compound II of MPO and LPO to the native enzyme thus enabling its participation in the (pseudo)halogenating activity. Recently, however, epigallocatechin gallate was shown to inhibit hypochlorous acid formation by myeloperoxidase and neutrophils due to accumulation of compound II [70].

Interference with reactions catalyzed by horseradish peroxidase is of particular importance because of application of this enzyme in numerous commercial diagnostic tests. Several mechanisms have been postulated to explain the effects of various compounds on these reactions. For dopamine and dobutamine the mechanism of this interference was initially not investigated, although it was speculated that dopamine may be oxidized by HRP thus depleting hydrogen peroxide from the reaction medium and preventing formation of antipyrilquinoneimine [52, 53]. Later experiments led to a conclusion that dopamine and 4-aminoantipyrine upon oxidation with peroxidase formed a chromophore with an absorption coefficient smaller than antipyrilquinoneimine thus reducing the final absorbance, while for dobutamine rapid enzymatic oxidation leading to hydrogen peroxide depletion was postulated [55]. However, we consider the postulated mechanism of interference by dopamine insufficient. Although a chromophore resulting from coupling of the oxidized 4-aminoantipyrine with dopamine may form when only these two compounds are present in the

mixture, in the presence of excess of another phenolic substrate (in our experiments the ratio of phenol to dopamine was 12.5 to 100) such a reaction would be marginal. Our results (Fig. 8D) indicate that under such conditions formation of dopaminochrome (resulting from oxidation of dopamine by 4-aminoantipyril radical) predominates and the antipyrilquinoneimine chromophore formed by coupling of oxidized 4-aminoantipyrine with either phenol or dopamine is a minor component – it causes the shit of the maximum of absorption from 475 nm to 495 nm but does not influence much the final value of absorbance, which is close to the value corresponding to dopaminochrome alone. If an adduct between dopamine and the oxidation product of 4-aminoantipyrine were formed, it should have an absorption coefficient comparable to antipyrilquinoneimines formed with other phenolic substrates, many of which are used in commercial diagnostic tests (e.g. phenol, chlorophenol, 3-hydroxybenzoic acid, 2,4,6-tribromo-3-hydroxybenzoic acid) all giving oxidation products with maxima of absorption above 500 nm and much bigger absorption coefficients at this wavelength than dopaminochrome. To us the much greater oxidation rate of dobutamine than dopamine and different mechanisms of interference for these two compounds [55] are also surprising. We have shown that L-dopa and dopamine are oxidized at comparable (very low) rates (Fig. S9B and C) and based on the structure of dobutamine one would expect similar (smaller rather than bigger - because of the larger size of the side chain) oxidation rate of the catechol group in this compound by HRP. We would therefore expect for this compound reactions similar to those observed for carbidopa, L-dopa, dopamine and other catechols, although enzymatic oxidation of the monophenolic part followed by redox exchange with the catechol part cannot be excluded. Appropriate experiments would be required to solve this problem.

Reduction of the oxidation products/intermediates of standard peroxidase substrates may also be responsible for interference in peroxidase-based diagnostic tests observed

previously for other compounds. A study of interference of several flavonoids with the commercial assays used for monitoring free fatty acids and triglycerides demonstrated that the concentrations of these analytes were significantly lowered in the presence of quercetin, quercetin-3-*O*-glucuronide, (+)-catechin, kaempferol, and luteolin [71]. This effect was attributed to inhibition of peroxidase by these compounds. However, lack of inhibitory effects for genistein and naringenin, which do not contain a catechol group or two conjugated phenolic groups in the flavonoid rings B and C, indicates that these two compounds, which cannot form quinonoid-type products upon oxidation [65], may simply be incapable of reducing the oxidation product of 4-aminoantipyrine. Such an effect has been shown for a number of flavonoids and phenolic acids in the oxidation of *o*-dianisidine by horseradish peroxidase [33]. All compounds showing strong interference possessed a catechol group, a 1,2,3-triol group or conjugated phenol groups in flavonoid rings B and C and included, among others, catechin, epigallocatechin, keampferol, morin, fisetin, myricetin, and quercetin. For all these compounds reduction of meriquinone was demonstrated [33]. Such an effect has also been reported for green tea polyphenols [72, 73]. In one of these reports two mechanisms responsible for this interference have been postulated: 1) depletion of hydrogen peroxide caused by direct oxidation of these compounds by peroxidase and 2) conversion of the antipyrilquinoneimine back to substrates (4-aminoantipyrine and 3-hydroxybenzoic acid) [72]. This second reaction, however, is not possible. It was postulated because formation of the chromophore was restored when additional amount of hydrogen peroxide was added to the reaction mixture containing green tea components after prolonged incubation with peroxidase. However, such an effect does not require regeneration of the substrate from the final product – our results show that the substrate (4-aminoantipyrine) is regenerated from the reaction intermediates before coupling with the phenol occurs.

Interference in commercial diagnostic tests utilizing peroxidase catalyzed reactions was also reported for compounds containing a *p*-diphenol moiety. Homogentisic acid, present at large concentration in the serum and urine of patients with alkaptonuria, interfered with tests used for detection of uric acid [74], creatinine [75], and later several more analytes, including cholesterol, triglycerides, and glucose [76]. Although hydrogen peroxide depletion has been postulated as the main cause of interference by this compound, the exact mechanism has not been elucidated. Hemostatic drugs etamsylate and calcium dobesilate (both salts of a *p*-diphenol – 2,5-dihydroxybenzenesulphonic acid) were reported as agents interfering with measurements of creatinine, uric acid, triglycerides, and cholesterol [77-79]. We suggest that all these *p*-diphenols may also reduce the oxidation product of 4-aminoantipyrine, just as catechols do. Structures of major compounds interfering with the peroxidase-based diagnostic tests are shown in Fig. S10.

References

[1] P.G. Furtmuller, M. Zederbauer, W. Jantschko, J. Helm, M. Bogner, C. Jakopitsch, C. Obinger, Active site structure and catalytic mechanisms of human peroxidases, Arch. Biochem. Biophys. 445 (2006) 199-213.

[2] C. Obinger, Chemistry and biology of human peroxidases, Arch. Biochem. Biophys. 445 (2006) 197-8.

[3] J. Arnhold, J. Flemmig, Human myeloperoxidase in innate and acquired immunity, Arch. Biochem. Biophys. 500 (2010) 92-106.

[4] S. Tafazoli, P.J. O'Brien, Peroxidases: a role in the metabolism and side effects of drugs, Drug Discov. Today 10 (2005) 617-25.

[5] M.J. Davies, C.L. Hawkins, D.I. Pattison, M.D. Rees, Mammalian heme peroxidases: from molecular mechanisms to health implications, Antioxid. Redox Signal. 10 (2008) 1199-234.

[6] M.J. Davies, Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention, J. Clin. Biochem. Nutr. 48 (2011) 8-19.

[7] J. Soubhye, I. Aldib, C. Delporte, M. Prevost, F. Dufrasne, P. Van Antwerpen, Myeloperoxidase as a Target for the Treatment of Inflammatory Syndromes: Mechanisms and Structure Activity Relationships of Inhibitors, Curr. Med. Chem. 23 (2016) 3975-4008.

[8] J. Soubhye, M. Gelbcke, P. Van Antwerpen, F. Dufrasne, M.Y. Boufadi, J. Neve, P.G. Furtmuller, C. Obinger, K.Z. Boudjeltia, F. Meyer, From Dynamic Combinatorial Chemistry to in Vivo Evaluation of Reversible and Irreversible Myeloperoxidase Inhibitors, ACS Med. Chem. Lett. 8 (2017) 206-210.

[9] C. Toth, A Review of the Natural Occurence, Synthetic Production an Use of Carcinogenic Hydrazines and Related Chemicals, In vivo 14 (2000) 299-320.

[10] P.R. Ortiz de Montellano, Arylhydrazines as probes of hemoprotein structure and function, Biochimie 77 (1995) 581-93.

[11] M.A. Ator, P.R. Ortiz de Montellano, Protein control of prosthetic heme reactivity. Reaction of substrates with the heme edge of horseradish peroxidase, J. Biol. Chem. 262 (1987) 1542-51.

[12] W.S. Allison, L.C. Swain, S.M. Tracy, L.V. Benitez, The inactivation of lactoperoxidase and the acyl phosphatase activity of oxidized glyceraldehyde-3-phosphate dehydrogenase by phenylhydrazine and phenyldiimide, Arch. Biochem. Biophys. 155 (1973) 400-4.

[13] A. Mazumdar, S. Adak, R. Chatterjee, R.K. Banerjee, Mechanism-based inactivation of lacrimal-gland peroxidase by phenylhydrazine: a suicidal substrate to probe the active site, Biochem. J. 324 (1997) 713-9.

[14] H.A. Shoeb, B.U. Bowman, Jr., A.C. Ottolenghi, A.J. Merola, Peroxidase-mediated oxidation of isoniazid, Antimicrob. Agents Chemother. 27 (1985) 399-403.

[15] J.M. van Zyl, K. Basson, R.A. Uebel, B.J. van der Walt, Isoniazid-mediated irreversible inhibition of the myeloperoxidase antimicrobial system of the human neutrophil and the effect of thyronines, Biochem. Pharmacol. 38 (1989) 2363-73.

[16] R. Pierattelli, L. Banci, N.A. Eady, J. Bodiguel, J.N. Jones, P.C. Moody, E.L. Raven, B. Jamart-Gregoire, K.A. Brown, Enzyme-catalyzed mechanism of isoniazid activation in class I and class III peroxidases, J. Biol. Chem. 279 (2004) 39000-9.

[17] L.V. Forbes, P.G. Furtmuller, I. Khalilova, R. Turner, C. Obinger, A.J. Kettle, Isoniazid as a substrate and inhibitor of myeloperoxidase: Identification of amine adducts and the influence of superoxide dismutase on their formation, Biochem. Pharmacol. 84 (2012) 949-60.

[18] G.S. Timmins, V. Deretic, Mechanisms of action of isoniazid, Mol. Microbiol. 62 (2006) 1220-7.

[19] A.J. Kettle, C.A. Gedye, M.B. Hampton, C.C. Winterbourn, Inhibition of myeloperoxidase by benzoic acid hydrazides, Biochem. J. 308 (1995) 559-63.

[20] A.J. Kettle, C.A. Gedye, C.C. Winterbourn, Mechanism of inactivation of myeloperoxidase by 4-aminobenzoic acid hydrazide, Biochem. J. 321 (1997) 503-8.

[21] U. Burner, C. Obinger, M. Paumann, P.G. Furtmuller, A.J. Kettle, Transient and steady-state kinetics of the oxidation of substituted benzoic acid hydrazides by myeloperoxidase, J. Biol. Chem. 274 (1999) 9494-502.

[22] S.M. Aitken, M. Ouellet, M.D. Percival, A.M. English, Mechanism of horseradish peroxidase inactivation by benzhydrazide: a critical evaluation of arylhydrazides as peroxidase inhibitors, Biochem. J. 375 (2003) 613-21.

[23] B. Frackowiak-Wojtasek, B. Gasowska-Bajger, M. Mazurek, A. Raniszewska, M. Logghe, T. Cichon, R. Smolarczyk, S. Szala, H. Wojtasek, Synthesis and analysis of activity of a potential anti-melanoma prodrug with a hydrazine linker, Eur. J. Med. Chem. 71 (2014) 98-104.

[24] B. Gasowska-Bajger, B. Frackowiak-Wojtasek, S. Koj, T. Cichon, R. Smolarczyk, S. Szala, H. Wojtasek, Oxidation of carbidopa by tyrosinase and its effect on murine melanoma, Bioorg. Med. Chem. Lett. 19 (2009) 3507-10.

[25] J.L. Munoz-Munoz, F. Garcia-Molina, M. Garcia-Molina, J. Tudela, F. Garcia-Canovas, J.N. Rodriguez-Lopez, Kinetic characterization of the oxidation of carbidopa and benserazide by tyrosinase and peroxidase, Biosci. Biotechnol. Biochem. 73 (2009) 1308-13.

[26] J.C. Davis, B.A. Averill, Isolation from bovine spleen of a green heme protein with properties of myeloperoxidase, J. Biol. Chem. 256 (1981) 5992-6.

[27] M. Ikeda-Saito, Spectroscopic, ligand binding, and enzymatic properties of the spleen green hemeprotein. A comparison with myeloperoxidase, J. Biol. Chem. 260 (1985) 11688-96.

[28] B. Gasowska, P. Kafarski, H. Wojtasek, Interaction of mushroom tyrosinase with aromatic amines, *o*-diamines and *o*-aminophenols, Biochim. Biophys. Acta 1673 (2004) 170-177.

[29] R.P. Ferrari, S. Traversa, L. De Gioia, P. Fantucci, G. Suriano, E.M. Ghibaudi, Catechol(amine)s as probes of lactoperoxidase catalytic site structure: spectroscopic and modeling studies, J. Biol. Inorg. Chem. 4 (1999) 12-20.

[30] G.R. Schonbaum, S. Lo, Interaction of peroxidases with aromatic peracids and alkyl peroxides. Product analysis, J. Biol. Chem. 247 (1972) 3353-60.

[31] C. Gay, J.M. Gebicki, A critical evaluation of the effect of sorbitol on the ferric-xylenol orange hydroperoxide assay, Anal. Biochem. 284 (2000) 217-20.

[32] A.V. Kireyko, I.A. Veselova, T.N. Shekhovtsova, Mechanisms of Peroxidase Oxidation of *o*-Dianisidine, 3,3',5,5'-Tetramethylbenzidine, and *o*-Phenylenediamine in the Presence of Sodium Dodecyl Sulfate, Russ. J. Bioorg. Chem. 32 (2006) 71-77.

[33] A.I. Wong, D. Huang, Assessment of the degree of interference of polyphenolic compounds on glucose oxidation/peroxidase assay, J. Agric. Food Chem. 62 (2014) 4571-6.

[34] T.J. Fiedler, C.A. Davey, R.E. Fenna, X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 A resolution, J. Biol. Chem. 275 (2000) 11964-71.

[35] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2010) 455-461.

[36] M.F. Sanner, Python: a programming language for software integration and development, J. Mol. Graph. Model. 17 (1999) 57-61.

[37] G.C. Knight, S.G. Waley, Inhibition of class C beta-lactamases by (1'*R*,6*R*)-6-(1'-hydroxy)benzylpenicillanic acid SS-dioxide, Biochem. J. 225 (1985) 435-9.

[38] M.A. Ator, S.K. David, P.R. Ortiz de Montellano, Structure and catalytic mechanism of horseradish peroxidase. Regiospecific meso alkylation of the prosthetic heme group by alkylhydrazines, J. Biol. Chem. 262 (1987) 14954-60.

[39] A.K. Tiden, T. Sjogren, M. Svensson, A. Bernlind, R. Senthilmohan, F. Auchere, H. Norman, P.O. Markgren, S. Gustavsson, S. Schmidt, S. Lundquist, L.V. Forbes, N.J. Magon, L.N. Paton, G.N. Jameson, H. Eriksson, A.J. Kettle, 2-Thioxanthines are mechanism-based inactivators of myeloperoxidase that block oxidative stress during inflammation, J. Biol. Chem. 286 (2011) 37578-89.

[40] G. Bartosz, Non-enzymatic antioxidant capacity assays: Limitations of use in biomedicine, Free Radic. Res. 44 (2010) 711-20.

[41] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231-7.

[42] K.J. Reszka, B.E. Britigan, Doxorubicin inhibits oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by a lactoperoxidase/H(2)O(2) system by reacting with ABTS-derived radical, Arch. Biochem. Biophys. 466 (2007) 164-71.

[43] A. Claiborne, I. Fridovich, Chemical and enzymatic intermediates in the peroxidation of *o*-dianisidine by horseradish peroxidase. 1. Spectral properties of the products of dianisidine oxidation, Biochemistry 18 (1979) 2324-9.

[44] P. Sharp, Interference in glucose oxidase-peroxidase blood glucose methods, Clin. Chim. Acta 40 (1972) 115-20.

[45] E. Nakashima, J. Nakamura, Y. Hamada, N. Koh, F. Sakakibara, N. Hotta, Interference by gliclazide in the glucose oxidase/peroxidase method for glucose assay, Diabetes Res. Clin. Pract. 30 (1995) 149-52.

[46] J.R. Genzen, J.J. Hunsaker, L.S. Nelson, B.A. Faine, M.D. Krasowski, N-acetylcysteine interference of Trinder-based assays, Clin. Biochem. 49 (2016) 100-104.

[47] I. Kaufmann-Raab, H.G. Jonen, E. Jahnchen, G.F. Kahl, U. Groth, Interference by acetaminophen in the glucose oxidase-peroxidase method for blood glucose determination, Clin. Chem. 22 (1976) 1729-31.

[48] R.H. White-Stevens, L.R. Stover, Interference by ascorbic acid in test systems involving peroxidase. II. Redox-coupled indicator systems, Clin. Chem. 28 (1982) 589-95.

[49] F. Martinello, E.L. da Silva, Ascorbic acid interference in the measurement of serum biochemical parameters: in vivo and in vitro studies, Clin. Biochem. 39 (2006) 396-403.

[50] F. Martinello, E. Luiz da Silva, Mechanism of ascorbic acid interference in biochemical tests that use peroxide and peroxidase to generate chromophore, Clin. Chim. Acta 373 (2006) 108-16.

[51] J.A. Weber, A.P. van Zanten, Interferences in Current Methods for Measurements of Creatinine, Clin. Chem. 37 (1991) 695-700.

[52] T.M. Daly, K.C. Kempe, M.G. Scott, "Bouncing" creatinine levels, New Engl. J. Med. 334 (1996) 1749-1750.

[53] K.T. Koprowicz, D.S. Ooi, J.G. Donnelly, Influence of dopamine on peroxidase-based assays, Clin. Chem. 42 (1996) 1502.

[54] A.K. Saenger, C. Lockwood, C.L. Snozek, T.C. Milz, B.S. Karon, M.G. Scott, A.S. Jaffe, Catecholamine interference in enzymatic creatinine assays, Clin. Chem. 55 (2009) 1732-6.

[55] B.S. Karon, T.M. Daly, M.G. Scott, Mechanisms of dopamine and dobutamine interference in biochemical tests that use peroxide and peroxidase to generate chromophore, Clin. Chem. 44 (1998) 155-60.

[56] M. Jimenez, F. Garcia-Carmona, F. Garcia-Canovas, J.L. Iborra, J.A. Lozano, F. Martinez, Chemical Intermediates in Dopamine Oxidation by Tyrosinase, and Kinetic-Studies of the Process, Arch. Biochem. Biophys. 235 (1984) 438-448.

[57] J. Pincemail, C. Deby, A. Thirion, M. de Bruyn-Dister, R. Goutier, Human myeloperoxidase activity is inhibited in vitro by quercetin. Comparison with three related compounds, Experientia 44 (1988) 450-3.

[58] Y. Kato, A. Nagao, J. Terao, T. Osawa, Inhibition of myeloperoxidase-catalyzed tyrosylation by phenolic antioxidants in vitro, Biosci. Biotechnol. Biochem. 67 (2003) 1136-9.

[59] R. Wippel, M. Rehn, A.C.F. Gorren, K. Schmidt, B. Mayer, Interference of the polyphenol epicatechin with the biological chemistry of nitric oxide- and peroxynitrite-mediated reactions, Biochem. Pharmacol. 67 (2004) 1285-1295.

[60] W.M. Loke, J.M. Proudfoot, A.J. McKinley, P.W. Needs, P.A. Kroon, J.M. Hodgson, K.D. Croft, Quercetin and its in vivo metabolites inhibit neutrophil-mediated low-density lipoprotein oxidation, J. Agric. Food. Chem. 56 (2008) 3609-15.

[61] L.O. Regasini, J.C. Vellosa, D.H. Silva, M. Furlan, O.M. de Oliveira, N.M. Khalil, I.L. Brunetti, M.C. Young, E.J. Barreiro, V.S. Bolzani, Flavonols from *Pterogyne nitens* and their evaluation as myeloperoxidase inhibitors, Phytochemistry 69 (2008) 1739-44.

[62] B. Nastasijevic, T. Lazarevic-Pasti, S. Dimitrijevic-Brankovic, I. Pasti, A. Vujacic, G. Joksic, V. Vasic, Inhibition of myeloperoxidase and antioxidative activity of *Gentiana lutea* extracts, J. Pharm. Biomed. Anal. 66 (2012) 191-6.

[63] Y. Shiba, T. Kinoshita, H. Chuman, Y. Taketani, E. Takeda, Y. Kato, M. Naito, K. Kawabata, A. Ishisaka, J. Terao, Y. Kawai, Flavonoids as substrates and inhibitors of myeloperoxidase: molecular actions of aglycone and metabolites, Chem. Res. Toxicol. 21 (2008) 1600-9.

[64] V.A. Kostyuk, T. Kraemer, H. Sies, T. Schewe, Myeloperoxidase/nitrite-mediated lipid peroxidation of low-density lipoprotein as modulated by flavonoids, FEBS Lett. 537 (2003) 146-50.

[65] B. Gasowska-Bajger, H. Wojtasek, Reactions of Flavonoids with o-Quinones Interfere with the Spectrophotometric Assay of Tyrosinase Activity, J. Agric. Food Chem. 64 (2016) 5417-5427.

[66] T. Kirchner, J. Flemmig, P.G. Furtmuller, C. Obinger, J. Arnhold, (-)-Epicatechin enhances the chlorinating activity of human myeloperoxidase, Arch. Biochem. Biophys. 495 (2010) 21-7.

[67] J. Flemmig, D. Rusch, M.E. Czerwinska, H.W. Rauwald, J. Arnhold, Components of a standardised olive leaf dry extract (Ph. Eur.) promote hypothiocyanite production by lactoperoxidase, Arch. Biochem. Biophys. 549 (2014) 17-25.

[68] J. Gau, P.G. Furtmuller, C. Obinger, J. Arnhold, J. Flemmig, Enhancing hypothiocyanite production by lactoperoxidase - mechanism and chemical properties of promotors, Biochem. Biophys. Rep. 4 (2015) 257-267.

[69] J. Gau, P.G. Furtmuller, C. Obinger, M. Prevost, P. Van Antwerpen, J. Arnhold, J. Flemmig, Flavonoids as promoters of the (pseudo-)halogenating activity of lactoperoxidase and myeloperoxidase, Free Radic. Biol. Med. 97 (2016) 307-319.

[70] R. Tian, Y. Ding, Y.Y. Peng, N.H. Lu, Inhibition of Myeloperoxidase- and Neutrophil-Mediated Hypochlorous Acid Formation in Vitro and Endothelial Cell Injury by (-)-Epigallocatechin Gallate, J. Agric. Food Chem. 65 (2017) 3198-3203.

[71] E.F. Hoek-van den Hil, K. Beekmann, J. Keijer, P.C. Hollman, I.M. Rietjens, E.M. van Schothorst, Interference of flavonoids with enzymatic assays for the determination of free fatty acid and triglyceride levels, Anal. Bioanal. Chem. 402 (2012) 1389-92.

[72] S. Shaukat, M.A. Waqar, Green tea phenols interference in the glucose oxidase/peroxidase test, J. Food Biochem. 35 (2011) 1170-1185.

[73] H. Xu, X. Leng, M. Wang, G. Zhang, Glucose Measurement in the Presence of Tea Polyphenols, Food Anal. Methods 5 (2012) 1027–1032.

[74] Y. Moriwaki, T. Yamamoto, Y. Nasako, H. Ohata, S. Takahashi, Z. Tsutsumi, J. Yamakita, K. Higashino, 'Pseudohypouricosuria' in alcaptonuria: homogentisic acid interference in the measurement of urinary uric acid with the uricase-peroxidase reaction, Ann. Clin. Biochem. 36 (1999) 501-503.

[75] P.R. Loken, M.J. Magera, W. Introne, S. Tortorelli, D. Gavrilov, D. Oglesbee, P. Rinaldo, D. Matern, K. Raymond, Homogentisic acid interference in routine urine creatinine determination, Mol. Genet. Metab. 100 (2010) 103-4.

[76] S.L. Curtis, N.B. Roberts, L.R. Ranganath, Interferences of homogentisic acid (HGA) on routine clinical chemistry assays in serum and urine and the implications for biochemical monitoring of patients with alkaptonuria, Clin. Biochem. 47 (2014) 640-7.

[77] O. Wiewiorka, M. Dastych, Z. Cermakova, Strong negative interference of ethamsylate (Dicynone (R)) in serum creatinine quantification via enzymatic assay using Trinder reaction, Scand. J. Clin. Lab. Inv. 73 (2013) 449-451.

[78] M. Dastych, O. Wiewiorka, M. Benovska, Ethamsylate (Dicynone (R)) Interference in Determination of Serum Creatinine, Uric Acid, Triglycerides, and Cholesterol in

Assays Involving the Trinder Reaction; In Vivo and In Vitro, Clin. Lab. 60 (2014) 1373-1376.

[79] X.Z. Guo, L.A. Hou, X.Q. Cheng, T.J. Zhang, S.L. Yu, H.L. Fang, L.Y. Xia, Z.H. Qi, X.Z. Qin, L. Zhang, Q. Liu, L. Liu, S.L. Chi, Y.Y. Hao, L. Qiu, Strong Negative Interference by Calcium Dobesilate in Sarcosine Oxidase Assays for Serum Creatinine Involving the Trinder Reaction, Medicine 94 (2015) 1-7.

Acknowledgements

This work was supported by statutory funds from the Polish Ministry of Science and Higher Education. We thank Sochinaz SA, Vionnaz, Switzerland, for the generous gift of a sample of carbidopa and Jerzy Tarnawski for performing the HPLC analysis. The MOE software used in this research was provided by Agriculture, Forestry and Fisheries Research Information Technology Center (AFFRIT), Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan.