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THE ROLE OF PORCINE THYROID PEROXIDASE AND FAD-CONTAINING MONOOXYGENASE IN THE METABOLISM OF 1-METHYL-2-THIOIMIDAZOLE (METHIMAZOLE)

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SUMMARY: The peroxidase and FAD-containing monooxygenase activities of porcine thyroid subcellular preparations were measured and it was observed that FAD-containing monooxygenase activity was considerably lower than that of peroxidase. The end product of 1-methyl-2[^{14}C]thioimidazole oxidation catalysed by thyroid peroxidase was confirmed to be 1-methylimidazole by mass spectrometry. In the presence of thyroid peroxidase 1-methyl-2-thioimidazole would appear initially to be oxidised to bis(1-methylimidazole)-2,2'-disulphide. The extent of oxidation was dependent on the iodide concentration in the reaction mixture.

The thioureylene, 1-methyl-2-thioimidazole(methimazole), which accumulates within the thyroid gland where it inhibits the enzyme thyroid peroxidase (1), is extensively metabolised in man (2) and rat (3). The enzymes thyroid peroxidase, EC 1.11.1.8 (1), FAD-containing monooxygenase, EC 1.14.13.8 (4) and cytochrome P.450-dependent monooxygenase (5) have all been implicated in its metabolism. One of the products of monooxygenase catalysed oxidation of 1-methyl-2-thioimidazole is 1-methylimidazole, which has been proposed to be the end product after sequential oxidation *via* either sulphenic and sulphinic acids (4) or a sulphoxide (5). However with some thiocarbamides such as ethylenethiourea the sulphur atom is replaced by an oxygen atom (6).

Thyroid peroxidase also catalyses the oxidative removal of the sulphur atom of 1-methyl-2-thioimidazole; when 1-methyl-2-[^{35}S]thioimidazole is incubated with purified pig thyroid peroxidase [^{35}S]sulphate is produced (1). Also [^{35}S]sulphate has been shown to be an intrathyroidal metabolite when 1-methyl-2-[^{35}S]thioimidazole is administered to rats (2).

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In this paper evidence is presented to show that the oxidation of 1-methyl-2-thioimidazole to 1-methylimidazole is catalysed by porcine thyroid peroxidase. Also the activities of thyroid peroxidase and FAD-containing monooxygenase in porcine thyroid tissue are reported.

MATERIALS AND METHODS

Thyroid peroxidase studies. A porcine thyroid subcellular preparation was prepared according to the method of Neary et al (7), using fresh thyroid glands. The preparation was further purified (8) by resuspending the pellet in a 1M NaCl, 10mM Tris-HCl, 0.1mM KI buffer, pH 7.4, and stirring the mixture at 4°C for 60 minutes which was subsequently centrifuged at 105,000 x g for 1 hour. The resultant pellet was resuspended in an equal volume of 0.1mM KI, and mixed gently on a wrist-action shaker for 1 hour at 4°C. The suspension was then centrifuged at 105,000 x g for 1 hour and the pellet was retained. The complete washing procedure was repeated. The washed pellet was suspended in 10mM Tris-HCl pH 7.4 buffer containing 0.1mM KI (10mg protein per ml). To every 9 volumes of this suspension was added 1 volume of a 1% w/v Triton X-100 solution in 10mM Tris-HCl pH 7.4 buffer containing 0.1mM KI. The resultant suspension was mixed gently for 15h at 4°C, and then centrifuged at 105,000 x g for 1 hour. The supernatant was concentrated by ultrafiltration using a Diaflo PM 10 membrane in an Amicon ultrafiltration cell at 40 p.s.i. Thyroid peroxidase activity was determined using the guaiacol peroxidation assay (9).

1-Methyl-2-[^{14}C]thioimidazole was incubated for 30 min with the thyroid peroxidase preparation in the presence of a H_2O_2 generating system and KI. The reaction mixtures were extracted 3 times with 3 volumes of chloroform containing 2% v/v ethanol. The extract was reduced in volume, applied on to silica gel TLC plates and developed in either the organic phase of a mixture of chloroform:ammonia solution sg 0.88 (5:1 v/v) or tetrahydrofuran:ammonia solution sg 0.88 (10:0.25 v/v). Radioactive compounds were located using a Berthold Radiochromatogram Scanner.

The oxidation of 1-methyl-2-thioimidazole and the hydrolysis of bis(1-methylimidazole)-2,2'-disulphidewere monitored in the region 230-320nm with a Cary 210 spectrophotometer.

FAD-containing monooxygenase studies. Porcine thyroid glands which were obtained within 3 min of death were immersed immediately in ice-cold 0.05M Tris-HCl 0.25M sucrose buffer, pH 7.4. After removal of fat and connective tissue, the glands were dried and homogenised in 2 volumes of 1.5% w/v KCl-0.01M HEPES buffer, pH 7.4 for 2.5 min, comprising 15 sec homogenisation periods and 15 sec cooling intervals. The homogenate was filtered through gauze and the filtrate centrifuged at 9,000 x g for 20 min. The resultant supernatant was centrifuged at 105,000 x g for 90 min to obtain the microsomal pellet which was resuspended in 0.05M Tris-HCl, 0.1M KCl buffer, pH 7.4 containing bovine serum albumin (1% w/v). This suspension was centrifuged at 37,000 x g for 45 min, and the microsomal pellet obtained was resuspended in the Tris-HCl/KCl buffer and centrifuged at 37,000 x g for 45 min to obtain the final microsomal pellet. The method of Ziegler & Pettit (10) using N,N-dimethylaniline to determine FAD-monooxygenase activity was modified. The N-oxide of N,N-dimethylaniline was also extracted from the reaction mixtures and analysed by TLC using a published method (11). 1-Methyl-2-thioimidazole was also used as a substrate.

1-Methyl-2[^{14}C]thioimidazole was prepared according to a literature method (12). Bis(1-methylimidazole)-2,2'-disulphide was synthesised by oxidising 1-methyl-2-thioimidazole in an aqueous solution of KI containing sodium hydrogen carbonate (13). N,N-Dimethylaniline-N-oxide was synthesised according to the method of Craig and Purushothaman (14).

High resolution mass spectra were obtained on an AE1-MS9 mass spectrometer, using a cold probe.

1-Methyl-2-thioimidazole and N-N-dimethylaniline (redistilled under vacuum) were obtained from BDH, Poole, Dorset; n-octylamine, guaiacol, 1-methylimidazole, NADPH, glucose, glucose oxidase (type V from Aspergillus niger) from Sigma Chemical Co., Poole, Dorset.

RESULTS

The FAD-containing monooxygenase activity of porcine thyroid microsomes was markedly lower than the peroxidase activity (Table 1). No FAD-containing monooxygenase activity could be detected with 1-methyl-2-thioimidazole as substrate. Moreover, FAD-containing monooxygenase activity could be detected only in the presence of n-octylamine and NADPH. TLC confirmed the presence of the N-oxide metabolite when N,N-dimethylaniline was incubated with the thyroid microsomes.

Only one radioactive compound was detected in the chloroform extracts of the test reaction mixture when 1-methyl-2[^{14}C]thioimidazole was incubated with the thyroid peroxidase preparation containing 0.1mM KI (Fig. 1). The Rf values of this peak in two developing solvent systems were similar to that observed for 1-methylimidazole.

Several radioactive compounds were detected in a test reaction mixture containing 2.5μM KI. The Rf values of two of these peaks were the same as those observed for 1-methyl-2-thioimidazole and 1-methylimidazole. Another radioactive peak had an Rf value the same as a value observed for one of the spots due to bis(1-methylimidazole)-2-2'-disulphide. The radioactive material present in the test extracts was subsequently confirmed by high resolution mass spectrometry to be 1-methylimidazole, with a M^+ m/z 82.053.

TABLE 1. FAD-monooxygenase and peroxidase activities of porcine thyroid subcellular preparations.

FAD-monooxygenase	0.18 ± 0.03 nmole N,N-dimethylaniline-N-oxide formed min^{-1} mg^{-1} protein.
Peroxidase	10.33 ± 2.39 nmole 1-methyl-2-thioimidazole oxidised min^{-1} mg^{-1} protein 120 ± 20 nmole guaiacol oxidised min^{-1} mg^{-1} protein.

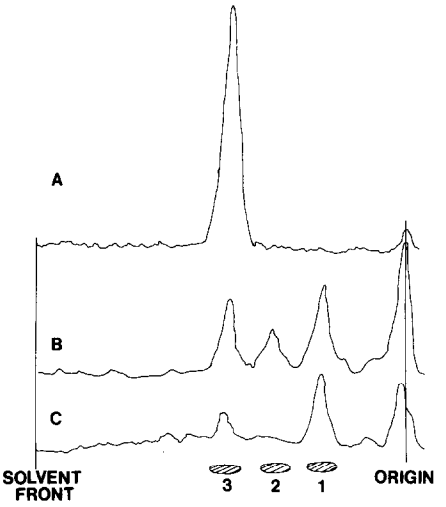

Figure 1 Radiochromatograms of chloroform extracts of incubation mixtures containing thyroid peroxidase preparation.

Incubation conditions.

A 1-Methyl-2-[^{14}C]thioimidazole (12μM:21.5 μCi mg^{-1}) was incubated with a porcine thyroid peroxidase preparation (activity 0.2 guaiacol units ml^{-1}) in 0.05M Tris-HCl buffer pH 7 containing a H_2O_2 generating system (glucose, 5mM; glucose oxidase; 5mU ml^{-1}) and KI (0.1mM).

B Conditions as for A but containing 2.5 μM KI.

C Conditions as for A but with heat denatured thyroid peroxidase preparation.

The reaction mixtures were incubated at 37°C for 30 min. Developing solvent = chloroform:ammonia solution sg 0.88 (5:1 v/v)

1 = 1-methyl-2-thioimidazole; 1 & 2 = bis(1-methylimidazole)-2, 2'-disulphide; 3 = 1-methylimidazole.

The ultraviolet spectrum obtained when methimazole was incubated with the thyroid peroxidase preparation showed an initial bathochromic shift (Fig. 2B). It can be seen also that an isobestic point may be present at 270 nm, similar to that observed when bis(1-methylimidazole)-2,2'-disulphide is in equilibrium with 1-methyl-2-thioimidazole (Fig. 2A).

DISCUSSION

It is known that 1-methylimidazole is a product of 1-methyl-2-thioimidazole metabolism *in vivo* in rat (3) and *in vitro* with guinea pig and rat liver microsomal preparations possessing either cytochrome P.450-dependent monooxygenase activity (5) or FAD-containing monooxygenase activity (4).

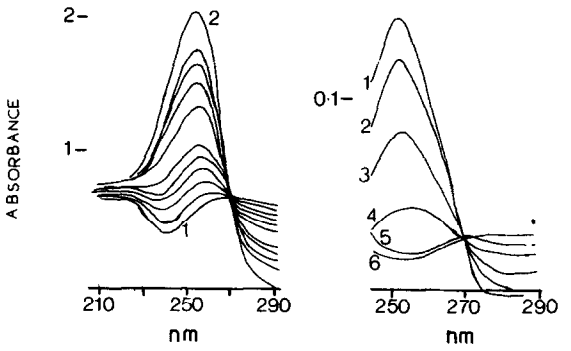

Figure 2 A. Absorption spectral changes with time of bis(1-methylimidazole)-2,2'-disulphide in 0.05M Sorensen's phosphate buffer pH 7.4.

Scan 1 taken at 4 min after sample preparation. Scan 2 taken at 70 min.

B. Absorption spectral changes with time of a reaction mixture containing 1-methyl-2-thioimidazole (10μm) thyroid peroxidase preparation (activity 0.10 guaiacol units ml^{-1}), glucose (5μM), glucose oxidase (21 mU ml^{-1}) and KI (100μM) in 0.09M Sorensen's phosphate buffer pH 7.4.

Scan number with time of recording (min) in brackets 1(0); 2(0.75); 3(1.67); 4(2.53); 5(4.17); 6(5.75).

Various reaction sequences have been proposed for the oxidative removal of the sulphur atom in thioureylenes and other related compounds. One mechanism involves the initial formation of a sulphenic acid which is oxidised further to a sulphinic acid which is hydrolysed, releasing sulphite (4). An alternative mechanism is *via* a sulphoxide which either undergoes carbon-sulphur bond cleavage to release sulphur monoxide (4) or rearranges to form a oxathiiran intermediate, which loses atomic sulphur and incorporates the oxygen into the molecule (4).

Using 1-methyl-2[^{14}C]thioimidazole as substrate for porcine thyroid peroxidase we have shown with the aid of thin-layer chromatography (Fig. 1) and mass spectrometry that 1-methyl-2[^{14}C]imidazole was an oxidation product and not 1-methyl-4-imidazolin-2[^{14}C]one, indicating that the sulphur atom was not replaced by an oxygen atom. Moreover when the iodide concentration in the incubation mixture was reduced to 2.5μM KI, a compound was also observed which was chromatographically similar to bis(1-methylimidazole)-2,2'-disulphide. That there was an incomplete conversion of 1-methyl-2[^{14}C]

thioimidazole to 1-methyl-2[^{14}C]imidazole at low iodide concentration, confirms the observations of others and ourselves that the rate and extent of 1-methyl-2-thioimidazole oxidation catalysed by thyroid peroxidase is dependent on the iodide concentration (1,15).

In the presence of a thyroid preparation possessing FAD-containing monooxygenase activity 1-methyl-2-thioimidazole would be oxidised to 1-methylimidazole via its sulphenic and sulphinic acids. However from preliminary experiments with 1-methyl-2-thioimidazole and a thyroid peroxidase preparation, UV spectra (Fig. 2B) were produced which were similar to those obtained when bis(1-methylimidazole)-2,2'-disulphide was hydrolysed (Fig. 2A); also TLC studies indicate that 1-methyl-2-thioimidazole is oxidised to 1-methylimidazole via a disulphide (Fig. 1), which would support the proposal that the disulphide of a thioureylene is the initial metabolite formed in the presence of thyroid peroxidase and iodide (16). Although Engler et al (15) obtained similar spectra when they incubated a highly purified porcine thyroid peroxidase preparation with 1-methyl-2-thioimidazole they concluded that the initial intermediate was a sulphenyl iodide.

The FAD-containing monooxygenase activity (Table 1) is approximately 10 times higher than has been previously reported (17). It would appear also that thyroid peroxidase is more important than FAD-containing monooxygenase in the thyroidal metabolism of the thioureylenes since FAD-containing monooxygenase activity is considerably lower than that measured for the peroxidase in porcine thyroid microsomes.

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