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TEMPORARY SUPPRESSION OF DIAMINE OXIDASE (HISTAMINASE) ACTIVITY BY CYSTEAMINE

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Abstract—Diamine oxidase (histaminase) oxidizes cystamine at a rate comparable with other conventional substrates. On the other hand diamine oxidase is strongly inhibited by cysteamine, i.e. the reduced product of cystamine. When diamine oxidase is incubated with cysteamine an unknown thermostable catalytic factor converts cysteamine into cystamine which is then destroyed by the oxidase. The oxidation of cystamine by the oxidase does not start unless the first oxidation of cysteamine to cystamine is completed. This phenomenon is indicated by a typical diphasic oxidation curve when cysteamine is incubated with diamine oxidase, either alone or in the presence of other substrates. The oxidation of cysteamine to cystamine produced in the first step of the reaction converts the inhibitor into the substrate of the same enzyme. As a consequence the inhibitor disappears at the end of the process and the activity of diamine oxidase is fully regenerated. It is suggested that this peculiar process may have some importance in the mechanism of the biological and radiobiological effects of cysteamine.

IT IS known that cystamine is a substrate for diamine oxidase as good as other diamines like cadaverine, putrescine, and histamine.[1–3] We have observed that when cysteamine is used as substrate for diamine oxidase in the place of cystamine, a strong inhibition ensues which lasts as long as cysteamine remains in the reduced state. Being a thiol compound, cysteamine is readily oxidized to the disulphide form (cystamine) in the presence of traces of metal ions and other catalysts present in tissues and tissue extracts.[4, 5] As a consequence of this oxidation the inhibitor is converted into the substrate of the same enzyme that it had formerly inhibited and is destroyed. The inhibition of diamine oxidase by cysteamine is thus only transient and some of the biological effects of cysteamine could be ascribed to the temporary suppression of diamine oxidase (histaminase) activity.

Cysteamine is one of the best protecting agents against radiation injury.[6] Although a number of details have been elucidated, the precise mode of action of cysteamine in reducing radiation damage is still unknown and it would be of interest to study the contribution of the temporary suppression of histaminase activity on the radioprotection activity of cysteamine.

MATERIALS AND METHODS

The compounds used in the present work were of commercial origin. Diamine oxidase was prepared from hog kidney by the following procedure: 500 g of frozen hog kidney were homogenized for 5 min in a Waring blendor with 600 ml water. After centrifugation at 5000 rev/min for 1 hr the supernatant was fractionated with ammonium sulphate and the precipitate between 33 and 60 per cent saturation was

collected, dialysed against distilled water adjusted to pH 7·2 with Na_2CO_3, and freeze-dried.

The oxidation of substrates was followed in a conventional Warburg apparatus. Each vessel contained: 50 mg lyophilized diamine oxidase dissolved in 2 ml 0·1 M pH 7·4 phosphate buffer; substrates dissolved in 0·3 ml water placed in the side arm; water with eventual additions to a total volume of 3 ml. The centre well contained 0·2 ml 10% NaOH; the gas phase was air; temp. = 38 °C. The enzyme alone showed a slight O_2 uptake which was subtracted from the reported values.

Disappearance of thiol groups was followed by the method of Folin-Marenzi with the adaptation indicated by Shinohara.[7]

RESULTS

The incubation of cysteamine with diamine oxidase results in an O_2 uptake curve which is unusual (curve 2 of Fig. 1) and different from that of cystamine (curve 1 of the same figure). While the curve for cystamine is exponential as expected for the straight oxidation of a substrate, that for cysteamine is clearly made up of two distinct stages representing two oxidative processes. The first stage leads to the uptake of slightly more than 5 μmoles of O_2 (theoretical value 112 μl). As soon as the first oxidation is finished a second oxidation starts. The two oxidative processes can be readily distinguished since the second oxidation does not start until the first is complete. When the same amount of cysteamine is incubated with the enzyme together with 10 μmoles of cystamine (curve 3) the diphasic character of the curve is retained, although the rate of the two oxidation steps and the total O_2 uptake of the second oxidation are changed.

The approximate value of 5 μmoles O_2 for 20 μmoles of cysteamine consumed in the first oxidation of curves 2 and 3 suggested that the first oxidative process could be ascribed to the oxidation of cysteamine to cystamine, by an unknown catalytic factor, which requires exactly 0·5 atoms of O_2 per mole of cysteamine oxidized:

$$2\ \begin{matrix}CH_2\text{—}SH\\ |\\ CH_2\text{—}NH_2\end{matrix} \xrightarrow{+O} \begin{matrix}CH_2\text{—}S\text{—}S\text{—}CH_2\\ |\qquad\quad |\\ CH_2\text{—}NH_2\quad CH_2\text{—}NH_2\end{matrix} + H_2O$$

This hypothesis is supported by the disappearance of —SH groups from the incubation mixture in the course of the first oxidative step as indicated by curve 6. The O_2 uptake of the second oxidative step of curve 2 is higher than that of the first, and, although in the time of the observation the oxidation is not ceased, it approximates that obtained by incubating the enzyme with 10 μmoles of cystamine alone (curve 1).

A reasonable explanation of the diphasic character of curve 2 is that the first portion of the curve may represent the oxidation of cysteamine to cystamine, while the second portion may represent the oxidation of cystamine, produced in the first step, by diamine oxidase. In order to understand the sharp separation of the two oxidative processes it is necessary to invoke a strong inhibition of diamine oxidase by cysteamine. Actually if one postulates that cysteamine inhibits diamine oxidase, the second oxidative process, representing the oxidation of cystamine by the enzyme, does not start until the last trace of cysteamine is present. The experiments to be reported below offer evidence on the correctness of this hypothesis.

The catalytic systems present in tissues and tissue extracts capable of oxidizing thiols like cysteamine to the respective disulphides are metal ions and haemoproteins of various natures.[4, 5] Isoniazid, which is a strong inhibitor of diamine oxidase,[8] is not known to impair the oxidation of thiols by their usual catalysts. If the above interpretation is correct, the addition of isoniazid to the incubation mixture of diamine

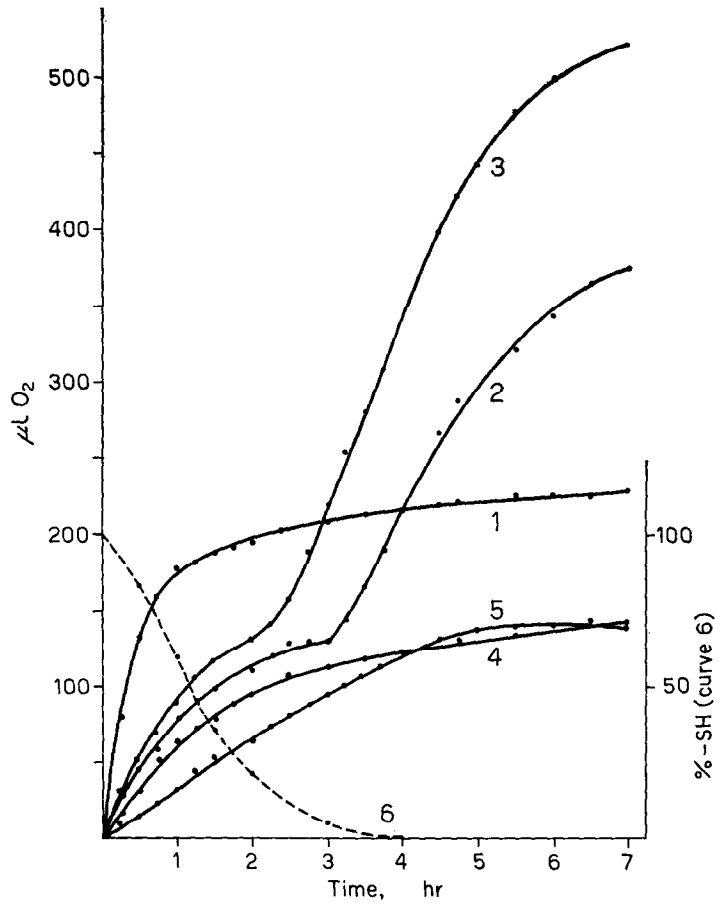

FIG. 1. Oxidation of cysteamine and cystamine by hog kidney diamine oxidase at pH 7·4 temp. 38 °C.
(1) 10 μmoles of cystamine.
(2) 20 μmoles of cysteamine.
(3) 10 μmoles of cystamine + 20 μmoles of cysteamine.
(4) 20 μmoles of cysteamine + 5 μmoles of isoniazid.
(5) 20 μmoles of cysteamine (enzyme boiled).
(6) disappearance of —SH groups in an incubate like that used for curve 2 (ordinates at right).
For experimental details see text.

oxidase with cysteamine should abolish the second step of the oxidative process and leave unmodified the first step. The observation of curve 4 reported in Fig. 1 does not leave any doubt on the correctness of the interpretation formulated above. As a further check, cysteamine was incubated with the enzyme kept in boiling water for 10 min (curve 5). Also in this case the second step alone has been abolished, indicating

the destruction of diamine oxidase activity by the heating procedure. This is a further indication that the first oxidation is catalysed by a system different from diamine oxidase. The survival of the first oxidation to the heating procedure suggests that it may be catalysed by a metal ion.

The oxidation of cadaverine (pentamethylenediamine) and histamine by diamine oxidase is also impaired by the presence of cysteamine (Fig. 2). The curves obtained

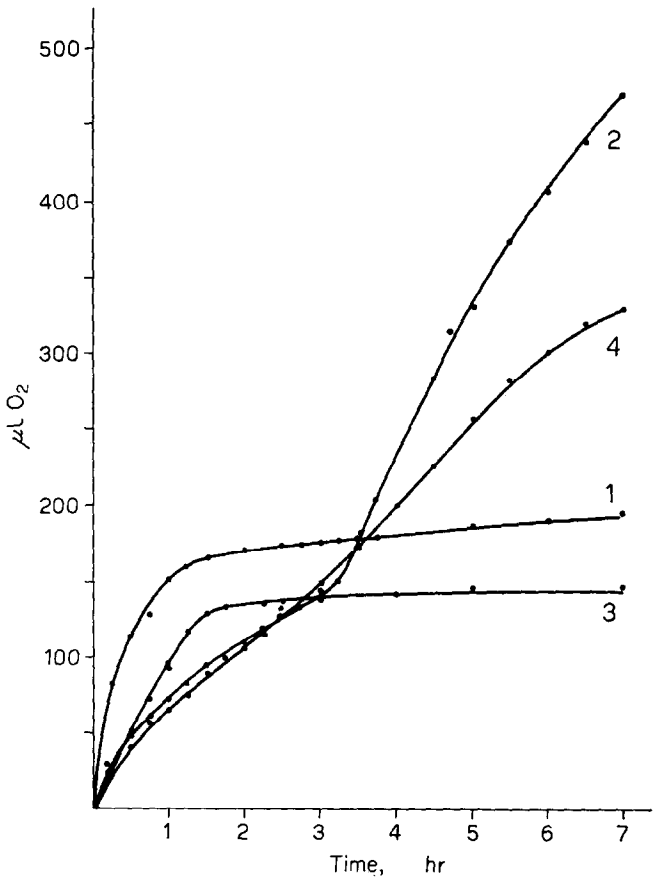

Fig. 2. The effect of cysteamine on the oxidation of cadaverine and histamine by hog kidney diamine oxidase at pH 7·4, temp. 38 °C.

(1) 10 μmoles of cadaverine.
(2) 10 μmoles of cadaverine + 20 μmoles of cysteamine.
(3) 10 μmoles of histamine.
(4) 10 μmoles of histamine + 20 μmoles of cysteamine.
For experimental details see text.

by incubating these two substrates in the presence of cysteamine have the diphasic character expected for the inhibition of the oxidase also when acting on substrates other than cystamine. The two-step type of curve is less evident with histamine than with cadaverine. This result is understood considering the lower rate of oxidation of histamine compared with cystamine and cadaverine, which makes the transition between the two steps less sharp.

Under the same conditions used for cysteamine, cysteine and mercapto-ethanol did not show any inhibitory effect on the oxidation of histamine and cadaverine. The inhibitory effect of cysteine and mercapto-ethanol was observed only when cystamine was the substrate. In this case however the inhibition is explained by a spontaneous thiol disulphide exchange between cystamine and the thiols,[9] yielding the inhibitory cysteamine. Ethanolamine has also been tested and found without effect on the oxidation of cystamine, histamine, and cadaverine by diamine oxidase. These findings clearly indicate that both the —SH and —NH_2 groups must be present in the same molecule in order to display the inhibitory effect, and that a carboxyl adjacent to the —NH_2 group, like in cysteine, abolishes or strongly depresses the inhibition.

DISCUSSION

The inhibition of diamine oxidase (histaminase) by cysteamine is documented by the results of the present investigation. Amino-thiol compounds like penicillamine[10] and cysteine[11] are reported as inhibitors of pyridoxal-enzyme catalysed reactions. The inhibition is explained by the ability of amino-thiols to condense with aldehydic groups yielding thiazolidine rings.[12] Since diamine oxidase is a pyridoxal-enzyme,[8] the described inhibition by cysteamine is understandable in the light of the production of a dissociable inactive thiazolidine ring between cysteamine and the aldehydic group of pyridoxal phosphate.

Some striking differences exist, however, between the inhibition of diamine oxidase by cysteamine and that reported for other amino-thiols on other enzymes. The inhibition of cysteamine is very strong when compared with that by cysteine. Under our experimental conditions, cysteine was found ineffective on the oxidation of diamines by diamine oxidase. The inhibition found by Mardashef[11] on transaminase was apparent with concentration more than thousand times higher than those used for cysteamine in the present work. According to the data reported by du Vigneaud *et al.*[10] the inhibitory effect of penicillamine on transaminase is much higher than that reported for cysteine[11] and is in the range of that found in the present work for cysteamine on diamine oxidase. A remarkable difference between the inhibition of diamine oxidase by cysteamine compared with that of other inhibitors is that cysteamine is readily oxidized to cystamine and when it is oxidized becomes the substrate of the same enzyme which it had formerly inhibited. This fact allows a complete reactivation of the oxidase after a period of inactivation lasting as long as cysteamine is present in the reduced state.

The possible involvement of the above findings with the ability of cysteamine to protect animals against radiation damage may be considered here. Although the evidence for the identity of diamine oxidase with histaminase is not complete[13], nevertheless it is evident from Fig. 2 that the oxidation of histamine is impaired by cysteamine to the same extent as the oxidation of other diamines. Apart from the problem of the possible identity of the two enzymes, this finding suggests that the transient inhibition of histaminase *in vivo* is likely to produce a temporary increase of local histamine concentration which may result in a beneficial effect against radiation injury. Actually histamine has been reported to be increased after cystamine injection in mammals,[14, 15] and histamine is known as a good radioprotector.[16] In those animals

where histamine displays a protective action against radiation, the histamine sparing action of cysteamine may therefore have some significance.

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