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HISTAMINE AND RELATED COMPOUNDS AS SUBSTRATES OF DIAMINE OXIDASE (HISTAMINASE)

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Abstract—The variation of the rate of oxidation of histamine derivatives, diamines, a monoamine and aminoalkylonium compounds by pig kidney diamine oxidase with alteration of pH has been studied. Two types of behaviour of reaction velocity as a function of pH are shown; some substrates having a straightforward optimum pH value around pH 7·4 and others showing an uneven curve instead of a smooth maximum around this pH value. This could be related to inhibition by high substrate concentration as shown by the variation of substrate inhibition with pH. Implications of these findings in the interpretation of the mechanism of binding and oxidation of histamine derivatives by diamine oxidase are discussed. In particular, the side chain $\alpha\beta$ dehydrogenation mechanism for the oxidation of histamine is criticised since histamine derivatives lacking β hydrogens were found to be substrates of DAO.

The effect of pH on the oxidation of diamines by pig kidney diamine oxidase (histaminase, pyridoxal-dependent amine oxidase E.C. 1.4.3.6) has only been investigated in a perfunctory manner. Blaschko *et al.* [1] described a pH curve with cadaverine showing the rate of oxidation still increasing up to pH 8·0 whereas histamine showed a maximum rate of oxidation at pH 6·0. This was interpreted as due to the necessity for substrate to be in dicationic form before reaction with the enzyme. Mondovì *et al.* [2] give a straightforward cadaverine oxidation plot with maximal oxidation at pH 7·0 and using a spectrophotometric method, we have found the pH optimum for oxidation pf *p*-dimethylaminomethylbenzylamine to be 7·2.

Recently we have studied the oxidation of diamines and congeners [3, 4] and have synthesized diamine analogues in which one amine group is replaced by a group bearing a formal positive charge. It seemed that these compounds would be useful in deciding the degree to which the substrate is normally protonated when it interacts with the enzyme since, unlike the parent amine, the onium group cannot lose its positive charge as the pH is raised. This paper describes the oxidation of 1,3-diaminopropane, 1,4-diaminobutane (putrescine), 1,5-diaminopentane (cadaverine), 1,6-diaminohexane, histamine, 1,3-dimethyl-4(5)-(2'-aminoethyl)imidazolium bromide (quaternaryhistamine), 5-aminopent-1-yldimethylsulphonium bromide (dimethylsulphonium-cadaverine), 5-aminopentan-1-ol and *p*-bis(aminomethyl)-benzene (*p*-xylylene diamine) by pig kidney diamine oxidase as a function of pH. Also a number of histamine derivatives have been studied at pH 7·0.

MATERIALS AND METHODS

Preliminary investigations showed that the pH profiles were not markedly altered by variation in ionic strength or nature of the buffer salts.

Enzyme preparation. This was as previously described [4], the average specific activity being 1·2 i.u./mg protein.

Spectrophotometric estimations. A Cary 118C spectrophotometer was used as previously described [5].

Oxidation of substrates. Warburg manometry was used at 37° with air as the gaseous phase. 0·1 M buffers were used thus: pH 5·0–5·5 (citrate); 6·0–8·0 (phosphate) and 8·5–10·5 (borate). A final volume of 1·0 ml was used, containing enzyme (0·1 unit), catalase (0·2 units) and final substrate concentrations of 1·0, 3·0, 10·0, 30·0 mM in all cases with other concentrations as appropriate.

Substrates. These were as previously described [4] except that 4(5)-aminomethylimidazole and 3-methylhistamine were synthesized by standard methods [6, 7]. In addition, the following histamine derivatives were provided by Smith, Kline & French: 2-, and 4-methylhistamine; 4(5)(3'-aminoprop-1-yl)imidazole, 4(5)(4'-aminobut-1-yl)imidazole and β,β-dimethylhistamine.

Substrate inhibition. In the present case, plots of v against log S were symmetrical bell-shaped curves while plots of $1/v$ against S became linear. Therefore,

Table 1. Variation with pH of apparent K_m (mM) and V_{max} observed (μl min^{-1}) [from $v/(v/s)$ plots] and α (μM^{-1}) [from $(1/v)/s$ plots] for pig kidney diamine oxidase

Compound	Parameter	pH 5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5
1	K_m	—	3·42	6·61	2·14	0·51	2·18	4·78	0·35	12·9	4·85	5·50	12·2
	V_{max}	—	0·24	0·37	0·42	0·40	0·31	0·82	0·59	0·93	1·21	1·10	1·47
2	K_m	—	2·70	1·26	1·08	0·49	1·28	0·11	—	—	0·25	1·25	0·70
	V_{max}	—	0·29	0·80	0·85	1·08	1·24	1·23	0·78	0·56	0·65	0·59	0·63
3	K_m	—	1·54	4·61	1·25	1·25	0·65	0·81	0	0·29	0·30	0·54	1·22
	V_{max}	0	0·24	0·68	0·98	1·35	1·44	1·20	0·38	0·40	0·36	0·57	0·41
4	K_m	—	—	0·31	0·30	1·32	0·71	—	—	0·13	—	—	—
	V_{max}	—	—	0·20	0·24	0·80	0·53	0·76	0·30	0·23	0·23	0·20	0·30
	α	—	—	3·2	1·0	5·7	9·8	14	24	1·2	18	89	50
5	K_m	—	—	—	73·9	36·5	23·2	9·9	18·4	5·2	1·7	15	6·9
	V_{max}	—	—	—	0·34	0·45	0·66	0·84	0·55	0·43	0·57	0·58	0·30
6	K_m	—	0·61	1·13	0·74	0·7	0·43	0·13	0·05	0·25	0·14	0·41	2·64
	V_{max}	0	0·46	0·59	0·54	0·42	0·43	0·47	0·21	0·24	0·10	0·20	0·09
	α	—	9·5	22	26	27	26	26	11	1·8	26	12	—
7	K_m	—	0·87	0·66	0·19	0·23	0·56	0·05	0·13	0·23	7·14	5·22	0·89
	V_{max}	0	0·07	0·17	0·29	0·16	0·29	0·16	0·10	0·13	0·09	0·10	0·21
		—	17	20	25	28	36	23	6	24	28	—	7·5
8	K_m	—	—	3·73	0·80	0·67	2·40	1·11	0·44	0·21	0·01	0·15	1·87
	V_{max}	0	0	0·12	0·36	0·56	0·72	0·61	0·24	0·25	0·24	0·17	0·18
9	K_m	0	2·20	1·99	0·89	0·11	0	0·06	0	0	0	1·89	12·60
	V_{max}	0·15	0·31	0·63	0·69	0·71	0·47	0·74	0·31	0·37	0·20	0·13	0·34
	α	2·8	15	29	3·6	7·2	16	19	4·2	2·9	8·6	22	—

Compound: 1—1,3-diaminopropane; 2—1,4-diaminobutane (putrescine); 3—1,5-diaminopentane (cadaverine); 4—1,6-diaminohexane; 5—5-aminopentan-1-ol; 6—4(5)-2′-aminoethylimidazole (histamine); 7—1,3-dimethyl-4(5)-2′-aminoethylimidazolium bromide (quaternary-histamine); 8—5-aminopent-1-yl dimethylsulphonium bromide (dimethylsulphonium-cadaverine); 9—*p*-bis(aminomethyl)-benzene(p-xylylene diamine).

the degree to which the concentration of substrate occurs in the original rate equation is approximately numerator 1 and denominator 2. Therefore, since oxygen was constant in all these experiments, we can write

$$v = \frac{V\,S}{K + S + \alpha S^2}, \quad \text{or} \quad 1/v = \frac{K}{V}\left(\frac{1}{S}\right) + \frac{1}{V}(1 + \alpha s).$$

The coefficient of S^2, i.e. α, can be obtained as the negative reciprocal of the horizontal intercept of the linear asymptote to a plot of $1/v$ against S and is a direct measure of the degree of substrate inhibition. The meaning of this parameter will depend upon the mechanism of substrate inhibition and is the reciprocal of K's which is sometines referred to as the substrate inhibition constant [8, 9].

RESULTS

Table 1 gives the kinetic parameters for substrates as a function of pH and Table 2 gives the kinetic parameters for histamine derivatives at pH 7·0.

At the pH optimum for enzymic reaction (pH approximately 7·5), diamines and amino-alkylonium compounds are present as dications but histamine derivatives would exist with a significant proportion of monocationic species present.

Figure 1 shows the pH profiles at various substrate concentrations for one normal compound and two compounds that do not show smooth maxima.

Table 2. Kinetic parameters for histamine derivatives in air at pH 7·0

Compound	V_{max} (μl O_2 min^{-1})	K_m (mM)
Histamine	0·42	0·7
4(5)-Aminomethylimidazole	0·23	0·1
4(5)(3′-Aminoprop-1-yl)imidazole	0·32	0·24
4(5)(4′-Aminobut-1-yl)imidazole	0·26	0·21
2-Methylhistamine	0·64	0·32
3-Methylhistamine	0·53	0·54
4-Methylhistamine	0·29	0·56
1,3-Dimethyl-4(5)(2′-aminoethyl)imidazole	0·16	0·23
β,β-Dimethylhistamine	0·12	0·15
N-Methylhistamine	—	—

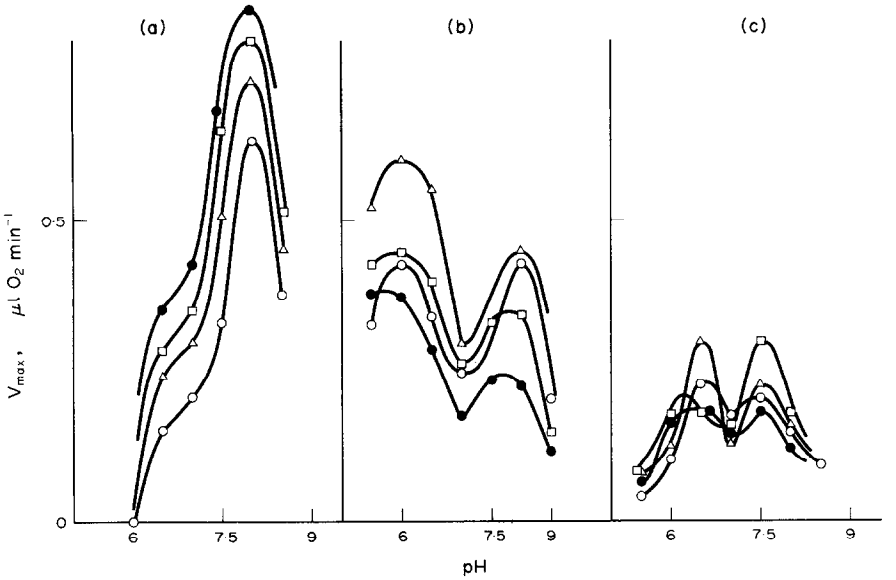


Fig. 1. The effect of substrate concentration on maximum velocity. (a) 5-Aminopentanol at ○ (20 mM), △ (30 mM), □ (50 mM) and ● (70 mM). (b) Histamine at ○ (1 mM), △ (3·0 mM), □ (10 mM) and ● (30 mM). (c) Quaternary histamine ○ (1 mM), △ (3·0 mM), □ (10 mM) and ● (30 mM).

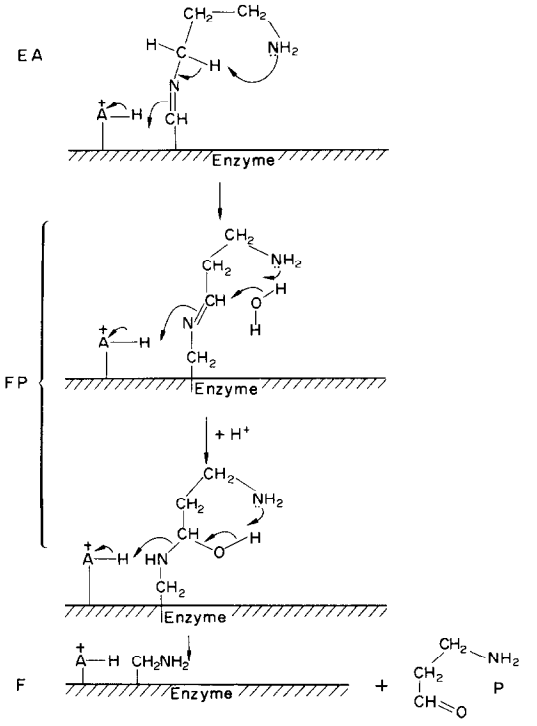


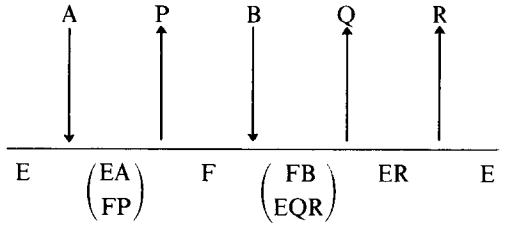
Fig. 2. Proposed mechanism for the sequence EA → FP → F + P for 1,3-diaminopropane to account for rate enhancement at high pH values. In EA the aldehyde group of pyridoxal is shown as having formed a Schiff base with the amino group to be oxidized. In FP a prototropic shift has converted this into a Schiff base between pyridoxamine and the aminoaldehyde product 3-aminopropionaldehyde.

Figure 2 gives the explanation for the pronounced increase in reaction velocity found with 1,3-diaminopropane at higher pH values and discussed in the text.

Figure 3 shows the spectral changes occurring during the oxidation of 4(5)-aminomethylimidazole by DAO. A linear change in absorbance at 257 nm occurs for several hours and the spectrum resulting is identical to that of 4-formylimidazole.

DISCUSSION

Kinetic studies of diamine oxidase from pig kidney [10, 11] and human placenta [12] have indicated a Ping Pong mechanism for these enzymes. The first substrate to add is the amine (A) followed by aminoaldehyde (P) release and addition of the second substrate (B). Sequential release of H_2O_2 (Q) and NH_3 (R) then follow as in the following scheme:



A negative charge on the enzyme is responsible for substrate [3, 4] binding and also mono and bis onium compounds inhibit by binding end on at this site [13, 14]. The rate limiting step in the oxidation of *p*-dimethylaminomethylbenzylamine has been elucidated by kinetic isotope and medium effects [11, 12] and found to involve a prototropic shift. Since the

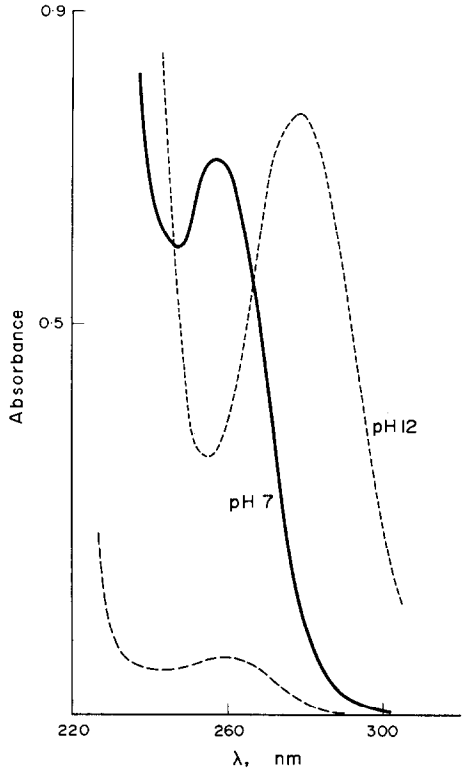


Fig. 3. Oxidation of 4(5)-aminomethylimidazole by diamine oxidase. Dashed line indicates the u.v. absorption spectrum of a solution containing enzyme 0·1 unit and 4(5)-aminomethylimidazole (3 mM) in phosphate buffer (0·1 M, pH 7·0). The solid line is the absorption spectrum after standing for 10 h in air at 20° and the dotted line is the spectrum of the same solution after the solution had been adjusted to pH 12·0. 4(5)-Formylimidazole has spectral behaviour identical to the solid line and undergoes the same bathochromic and hyperchromic shift at higher pH values.

second half of the catalytic sequence is the same for all substrates, then the effects of substrate structure on reaction velocity must be effects on the nature of the transition state for the prototropic shift. Since the kinetic isotope effect is present throughout the pH range [11], we feel that there is no need to postulate a change in rate limiting step with pH.

Cadaverine and dimethylsulphonium-cadaverine. Whilst it is admitted that a knowledge of the ionic form of any ionizable substrate at the pH optimum does not easily allow conclusions to be made concerning the nature of the enzyme-substrate complexes due to the rapidity with which protonation and deprotonation occur, we suggest that the present findings favour the hypothesis that only the state of ionization of the amino group being oxidized need to be considered. The other amino group remains protonated throughout the reaction as shown by the similar pH profiles given by cadaverine and the dimethylsulphonium analogue (Table 1) which has a formal positive charge throughout the pH range studied. We can presume that the amino group to be oxidized is normally protonated at the pH optimum and will interact in this form with the enzyme, although deprotonation will precede Schiff base formation.

Histamine and quaternary-histamine. It has been suggested that the pH optimum at pH 6·0 for histamine is due to the need for dication formation to precede reaction with the enzyme [1]. This work disproves this explanation since ring quaternization does not alter the shape of the V_{max} vs pH plot(s) (Fig. 1). The following explanation is offered: histamine and quaternary-histamine would show a similar pH optimum to cadaverine, around pH 7·5, since ionizations in this region promote breakdown of the enzyme–substrate complexes favouring the forward reaction. However, ionizations in this region also promote substrate inhibition (Table 1) and this is sufficient to cause an overall slight reduction in reaction velocity in this pH range for substrates showing strong substrate inhibition, producing uneven pH profiles.

The special case of 1,3-*diaminopropane.* Here the V_{max} vs pH plot is of the type without a smooth peak at pH 7·4, but the rapid increase in V_{max} as higher pH values are approached remains to be explained. This may be due to an ionization of the substrate amino group other than the one being oxidized since the pH values for the deprotonation of amino groups is being approached and the ionization is likely to be one on an enzyme–substrate complex. A possible explanation of this is now given and illustrated in Fig. 2.

The sequence E → F involves the formation of a Schiff base, prototropic shift and release of an aldehyde by hydrolysis. We can assume that the last step will be the general acid–base catalysed elimination of aldehyde from a tetrahedral carbinolamine intermediate formed by nucleophilic addition of water to a protonated imine (or its equivalent). In the special case of 1,3-diaminopropane, it is possible for the amino group not being oxidized to assist in this sequence at several possible points by means of a cyclic transition state as shown in Fig. 3. This rate-enhancement can only occur when the amino group becomes nucleophilic, i.e. loses a proton, which explains why this occurs only at higher pH values. No other compound that we have used could react in this way and 1,2-diaminoethane is a poor substrate for this enzyme.

The mechanism of oxidation of histamine and derivatives. It has been claimed recently that during the oxidation of histamine by diamine oxidase, the side chain protons adjacent to the imidazole ring are lost [15]. Thus, β-side chain tritiated histamine has been widely used as a substrate for determining diamine oxidase in biological fluids [16, 17] by measuring the tritiated water produced. This raises the possibility that histamine oxidation goes by a different mechanism than that of other amines and suggests a re-examination of an αβ dehydrogenation mechanism [18]. From the fact that a large number of benzylamine derivatives [3–5] and amines with blocked β positions [19] are oxidized by DAO, it had been concluded that this mechanism

was not possible. Zeller has recently criticised the side chain β proton loss mechanism on similar grounds [20] although, using purified DAO, we have not been able to substantiate his claim for *p*-nitrobenzylamine as a good substrate for DAO.

The family of histamine derivatives all show similar kinetic behaviour although there are differences in K_m and V_{max}. The Eadie plots all have shallow lines and a considerable region of curvature where substrate inhibition occurs but there are no indications to suggest that histamine is oxidized by a special mechanism. Two compounds in particular illuminate the question of the $\alpha\beta$ dehydrogenation mechanism, namely, β,β-dimethylhistamine and 4(5)-aminomethylimidazole where such a process is not possible.

β,β-Dimethylhistamine is a poor substrate (Table 2). This is in accord with current views on the conformation of histamine derivatives in solution [21–23] and the structure function relationships among histamine derivatives. 4(5)-Aminomethylimidazole is more closely similar to histamine as a substrate and, since it produces an aldehyde with a distinct absorption spectrum [24], has now been studied spectrophotometrically as well as manometrically. When this compound is incubated with DAO, there is a linear rate of change of absorbance at 257 nm and a well defined maximum begins to appear in the absorption spectrum (Fig. 3). This spectrum undergoes a bathochromic and hyperchromic shift at higher pH values which is identical to that given by authentic 4(5)-formylimidazole [24].

We conclude that there is no need to invoke a special mechanism for the oxidation of histamine by DAO and that the release of tritium from β tritiated histamine is not an essential part of the reaction sequence but rather represents an artefact due to exchange of the product or enzyme product complex with solvent. It is also of interest to note that stereospecific release of α tritium from histamine occurs during oxidation with DAO [25].

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