A Study of the substrate-binding site in hog kidney diamine oxidase.

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SUMMARY Purified diamine oxidase from hog kidney has been shown to oxidize a series of compounds related to diamines, in which the amino group is replaced by a group bearing a formal positive charge, e.g. dimethylsulfonium, isothiuronium, quaternary ammonium, imidazolium or pyridinium. Usually the rate of oxidation was about half that of the parent diamine. This is interpreted as being consistent with a mechanism of substrate binding in which the positively charged substrate is attracted to a negatively charged binding site, but not with an alternative scheme involving the substrate acting as nucleophile towards an electrophilic binding site on the enzyme.

INTRODUCTION Hog kidney diamine oxidase [E C 1.4.3.6] is known to oxidize diamines more readily than monoamines. This has led to the suggestion that the amino group not being oxidized is involved in binding the substrate to the enzyme at a substrate-binding site some distance from the oxidizing site. This substrate-binding site has been regarded as an electrophilic residue[2] or as a negative charge.[3,4]

To settle this problem we have synthesized a number of novel compounds of the type shown in figure 1, where one amino group in a diamine is replaced by a group bearing a formal positive charge, and tested them as possible substrates.

MATERIALS and METHODS The enzyme used was prepared as previously reported[4], except that the final DEAE-cellulose column

Fig. 1 $X[CH_2]_nNH_2$

Typical substrates of diamine oxidase have $X=NH_2$ and n=3-6. In this work n was varied from 1 - 6 and X was $-\overset{+}{N}[CH_3]_3$, $-\overset{+}{S}[CH_3]_2$, $-\overset{+}{S}=C[NH_2]_2$, p $C_6H_5-CH_2-\overset{+}{N}[CH_3]_3$, p $C_6H_5-CH_2-\overset{+}{S}[CH_3]_2$ p $C_6H_5-CH_2-\overset{+}{S}=C[NH_2]_2$, N-Methyl pyridinium and N, N′ -Dimethylimidazolium.

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was replaced by a DEAE-Sephadex [A50] column followed by a Sephadex [G200] column. The final specific activity was 0.98 units mg^{-1}. [One unit giving an oxygen uptake of 11.2μl min^{-1} with cadaverine under standard conditions]. Oxidation of the substrates was followed by Warburg manometry at 37^o using air as the gaseous phase, and 0.1M potassium phosphate buffer pH 7.0. Substrates were used either as hydrobromides or hydrochlorides, since we discovered that the rate of oxidation is independent of the anion used at the concentrations employed.

RESULTS Table 1 lists the compounds used together with the rate of oxidation expressed as a percentage of that for the corresponding diamine. It is apparent from this table that when one amino group of a diamine is replaced by a dimethylsulphonium, isothiuronium, or N,N′-dimethylimidazolium group the rate of oxidation is usually decreased by 20-70%. Quaternization of the pyridine ring does not alter the rate appreciably, while substitution of an amino group by a trimethylammonium group effectively abolishes oxidation as previous workers have reported.[5]

DISCUSSION If, during the interaction between enzyme and substrate, a protonated amino group of the substrate is attached to a negative charge on the enzyme, then if one amino group of a diamine is replaced by a group bearing a formal positive charge,

Table 1

Oxidation of amines by diamine oxidase [0.1 units] A final volume of 1 ml 0.1M phosphate buffer pH 7.0 was used containing catalase [0.2 units] and 10 mM substrate at 37°.

Compound	Initial rate of O_2 uptake in μl min-1	Oxidation as a percentage of that of the parent diamine
1,3-Diaminopropane	0.274	100
3-Aminopropyldimethylsulfonium bromide	0.119	43.4
S,3-Aminopropylisothiuronium bromide	0.203	74.1
3-Aminopropyltrimethylammonium bromide	0	0
1,4-Diaminobutane [putrescine]	1.025	100
4-Aminobutyldimethylsulfonium bromide	0.938	91.5
S,4-Aminobutylisothiuronium bromide	0.384	37.4
4-Aminobutyltrimethylammonium bromide	0	0
1,5-Diaminopentane [cadaverine]	1.200	100
5-Aminopentyldimethylsulfonium bromide	0.484	40.3
S,5-Aminopentylisothiuronium bromide	0.406	33.8
5-Aminopentyltrimethylammonium bromide	0	0
1,6-Diaminohexane	0.712	100
6-Aminohexyldimethylsulfonium bromide	0.212	29.8
S,6-Aminohexylisothiuronium bromide	0.166	23.3
6-Aminohexyltrimethylammonium bromide	0	0
Histamine	0.336	100
1,3-Dimethyl-4[5]-2'-aminoethylimidazolium bromide	0.102	30.3
p-Bis[aminomethyl]benzene [p-xylylene bromide]	0.856	100
p-Aminomethylbenzyldimethylsulfonium bromide	0.545	63.6
p-Aminomethylbenzylisothiuronium bromide	0.362	42.2
p-Aminomethylbenzyltrimethylammonium bromide	0	0
2-Aminomethylpyridine	0.124	100
1-Methyl-2-aminomethylpyridinium bromide	0.146	117.5
3-Aminomethylpyridine	0.195	100
1-Methyl-3-aminomethylpyridinium bromide	0.174	89.2
4-Aminomethylpyridine	0.206	100
1-Methyl-4-aminomethylpyridinium bromide	0.206	100
2-[2'-Aminoethyl]-pyridine	0.141	100
1-Methyl-2-[2'aminoethyl]-pyridinium bromide	0.137	97

the resulting substrate should be oxidised at a diminished rate. Only one amino group can now be oxidized, although the positively charged group can still contribute to binding. If the substrate-binding site had an identical affinity for protonated amino groups and the various positively charged species, then a diminution of oxidation rate by 50% would be expected, corresponding to a 50% decrease in favourably orientated interactions between enzyme and substrate. Any altered affinity would lead to some deviation from this figure of 50%. We submit that our results are in agreement with those theoretical consideration for all compounds tested except for the pyridinium and trimethyl-ammonium compounds.

The high rates of oxidation observed for pyridinium compounds could be due to the fact that the pyridine nitrogen would not normally be completely protonated at pH 7.0 and quaternisation would effectively increase the concentration of a species corresponding to the pyridinium cation.

In the trimethylammonium compounds it must be supposed that attraction between the negatively charged substrate-binding site and positively charged substrate is decreased, due to the positively charged nitrogen being surrounded by hydrophobic methyl groups.

Since none of these compounds can act as nucleophiles at an electrophilic residue away from the oxidizing site, this alternative mechanism must be ruled out.

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