INVESTIGATION OF THE SUBSTRATE-BINDING SITE OF ALDEHYDE OXIDASE

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The liver cytosol enzyme, aldehyde oxidase, plays an important role in the oxidation of a number of drugs. Previous work has shown that this hepatic enzyme is very active in vitro towards various unsubstituted azanaphthalenes (Stubley 1979a). The present study describes the effects of substitution on these heterocycles with a view to extending existing knowledge of the substrate-binding site.

The rates of oxidation of the compounds were followed by monitoring spectrophotometrically the reduction of potassium ferricyanide which occurs subsequent to the reduction of the enzyme by substrate. Km values were determined using either crude fractions of enzyme free from cytochrome c or highly purified enzyme fractions prepared by gel chromatography.

Table 1 Compounds Tested as Substrates of Aldehyde Oxidase

Substrate	Km (mol. litre^{-1})	No (-) or little (*) activity	
Quinoline	3×10^{-3}	Pyridine	-
3-Methylquinoline	5×10^{-4}	Pyrazine	-
Isoquinoline	1.96×10^{-4}	Pyrimidine	*
3-Methylisoquinoline	2.47×10^{-4}	Pyradazine	-
Phenanthridine†	$<10^{-6}$	5,6-Benzoquinoline	-
8-Methylquinoline	*	7,8-Benzoquinoline	-

†Km determined by following reduction of substrate at 322 nm.

The identity of each oxidation product was confirmed as previously described (Stubley, 1979b). In each case oxidation occurred adjacent to a heterocyclic nitrogen atom.

Pyridine is not a substrate for aldehyde oxidase. However when a substituent lipophilic group is present in the molecule as in the case of 2,3- and 3,4-benzopyridine (i.e. quinoline and isoquinoline respectively) good substrates are produced. This is also the case with the diazines; pyrazine, pyrimidine and pyridazine are very inefficient substrates whereas the corresponding benzodiazines are rapidly oxidised by aldehyde oxidase (Stubley 1979a). Therefore it would appear that an increase in the lipophilic character of the molecule produces an efficient substrate and the optimum distance from the carbon undergoing nucleophilic attack to the edge of the lipophilic area is within 1½-2 times the width of a benzene ring. Thus neither 5,6- nor 7,8-benzoquinoline bind to the enzyme and in both cases the size of the lipophilic area is greater than the above optimum distance. By contrast 2,3,4,5-benzopyridine (phenanthridine) was found to be the most active substrate yet examined. Although it has similar lipophilic character to the benzoquinolines above, the 2 benzene rings are on either side of a heterocyclic ring and thus the geometry of the molecule is such that the distance between the α-carbon and the aromatic ring does not exceed that in either quinoline or isoquinoline.

In conclusion there are probably two important factors influencing the activity of this enzyme towards substrates; electronic factors which determine whether the molecule is susceptible towards nucleophilic attack and also the lipophilic character of the compound which facilitates binding to the enzyme. These lipophilic regions may be important in the binding of the phenolic oestrogens, oestradiol and diethylstilboestrol both of which are very potent inhibitors of the human liver enzyme (Johns 1967).

Stubley, C. et al (1979a) Xenobiotica 9 : 475-484
Stubley, C. et al (1979b) J. Chromatog. 177 : 313-322
Johns, D.G. (1967), J. Clin. Invest. 46 : 1492-1505