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Similarity in the aldehyde oxidases from guinea-pig liver and polymorphonuclear leucocytes

C. BEEDHAM, *School of Studies in Pharmaceutical Chemistry, University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK*

Partially purified enzyme from guinea-pig leucocytes has been shown to have properties similar to both guinea-pig and rabbit liver aldehyde oxidase. The presence of molybdenum in the leucocyte enzyme has been demonstrated and substrate oxidation by either guinea-pig enzyme was found to be completely inhibited by menadione, a potent inhibitor of rabbit liver aldehyde oxidase. The leucocyte enzyme resembles the guinea-pig liver enzyme in terms of substrate specificity but there is considerable variation in substrate oxidation rates between the two species.

Aldehyde oxidase (E.C.1.2.3.1.) is a molybdenum containing enzyme which is involved in the oxidative metabolism of many aldehydes and nitrogen heterocycles (Kurth et al 1978; Krenitsky et al 1972; Beedham 1985). The enzyme is predominant in the liver and as rabbit liver appears to have the highest content of aldehyde oxidase (Kelsey & Oldham 1943) most workers prepare enzyme from this source. It also occurs in other tissues such as the lung and kidney although relatively little is known about the properties of enzyme from extrahepatic tissues (Holmes 1978).

Badwey et al (1981) isolated an enzyme from guinea-pig polymorphonuclear leucocytes which is similar to rabbit liver aldehyde oxidase but has a much narrower substrate specificity. The enzyme was active towards aldehydes and 2-hydroxypyrimidine; however no reaction occurred with typical substrates of aldehyde oxidase such as N^1-methylnicotinamide or 6-methylpurine. Furthermore, menadione, a potent inhibitor of rabbit liver aldehyde oxidase, did not decrease the oxidation rate of substrates. Although the closely related molybdenum hydroxylase, xanthine oxidase, has been identified in rat leucocytes (Tubaro et al 1980), Badwey et al (1981) found the guinea-pig leucocyte enzyme to be inactive towards xanthine.

There are two possible explanations for the difference in substrate specificity between rabbit liver aldehyde oxidase and the guinea-pig leucocyte enzyme. Firstly it may be due to variation between the two species. Thus human liver enzyme has a different substrate specificity to rabbit liver aldehyde oxidase (Johns 1967). Alternatively, the leucocyte enzyme may differ from hepatic enzyme within a particular species. Mouse aldehyde oxidase exists as two isoenzymes, one of which is located primarily in the liver and pancreas whereas the other is more widely distributed in other tissues (Holmes 1978). Evidence is presented in this communication that the guinea pig leucocyte enzyme is a molybdenum hydroxylase that has similar properties to guinea pig liver aldehyde oxidase.

Methods

Aldehyde oxidase was partially purified from livers of female, Dunkin Hartley guinea-pigs. Liver homogenates were heated at 55 °C for 10 min and the aldehyde oxidase subsequently precipitated with ammonium sulphate as was previously reported for rabbit liver enzyme (Stubley et al 1979). Polymorphonuclear leucocytes were obtained as described by Depierre & Karnovsky (1974); guinea-pigs were injected intraperitoneally with 30 ml sterile 12% sodium caseinate in isotonic (0·9%) saline and the leucocytes harvested from the peritoneal cavity 15–20 h later. The cells were homogenized (Baehner et al 1970) and treated as liver homogenates (see above) to isolate partially purified enzyme. Protein concentration was measured using the Bradford method (Bradford 1976).

The molybdenum content of the leucocyte enzyme was determined by electrothermal atomic absorption spectroscopy using a method of standard additions developed in our laboratories (Johnson et al 1984). Substrate oxidation was monitored spectrophotometrically at 420 nm as described by Stubley et al (1979). A slow rate of ferricyanide reduction was observed using leucocyte enzyme in absence of substrate which was compensated for by a reference cell containing all components except the substrate.

Results and discussion

The molybdenum concentration of the leucocyte enzyme was 0·40 ng Mo (mg protein)$^{-1}$ which is very similar to the value determined (Johnson et al 1984) for rabbit liver enzyme (0·38 ng Mo (mg protein)$^{-1}$). This would suggest that the aldehyde-oxidizing enzyme of the leucocytes is indeed a molybdenum hydroxylase.

Fig. 1 shows the Lineweaver–Burk plots calculated for the oxidation of phthalazine (2,3-diazanaphthalene) with enzyme from both sources. Similar values for the Michaelis-Menten affinity constants were obtained in each case, i.e. 40 μM for guinea-pig liver enzyme and 50 μM for leucocyte aldehyde oxidase. The maximum velocity for both enzymes was also of the same order, 0·3 μmol min^{-1} (mg protein)$^{-1}$. In contrast, under similar conditions rabbit liver aldehyde oxidase is less efficient towards phthalazine as a substrate, $K_m \approx$ 100 μM (Stubley et al 1979; Johnson et al 1984).

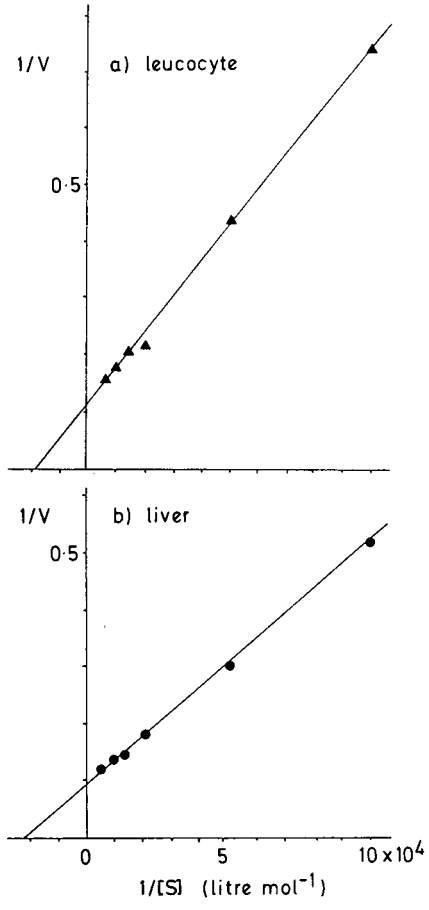

FIG. 1. Lineweaver–Burk plots for the oxidation of phthalazine at 37 °C by guinea-pig aldehyde oxidase. Cuvettes contained varying concentrations of phthalazine, 0·1 mM EDTA, 1 mM potassium ferricyanide in 0·067 M phosphate buffer pH 7 and (a) 0·1 ml leucocyte enzyme (1·5 mg ml^{-1} protein) or (b) 0·1 ml liver enzyme (2·2 mg ml^{-1} protein).

Although Badwey et al (1981) found the oxidation of 2-hydroxypyrimidine to be decreased by typical inhibitors of aldehyde oxidase such as methanol and arsenite, no such effect was noted with 2 mM menadione. In the present study phthalazine oxidation catalysed by either liver or leucocyte enzyme was completely inhibited by menadione (0·1 mM).

The relative oxidation rates of phthalazine and other substrates are shown in Table 1. These are also compared to the values obtained with rabbit liver aldehyde oxidase. There is considerable variation in the relative oxidation rates between the two species. However, the leucocyte enzyme appears to have similar catalytic properties to that of liver from the same species.

Furthermore, Badwey et al (1981) reported purine to be refractory towards oxidation by guinea-pig leucocyte enzyme whereas in the present study it was observed to be a substrate. This would be explained if purine has a relatively high Km value with leucocyte enzyme. If this is the case, the velocity of the reaction would be much slower in the 0·2–2 mM range (concentrations employed by Badwey) than it is with the concentrations used in this study (10 mM).

Table 1. Relative oxidation rates for substrates of aldehyde oxidase. The relative activities at 37 °C of the partially purified aldehyde oxidase were determined using the substrate concentrations listed in the Table in 0·067 M potassium phosphate buffer pH 7 containing 0·1 mM EDTA and 1 mM potassium ferricyanide; total cell volume = 3 ml. Reaction rates were monitored at 420 nm, with the exception of phenanthridine in which case the ferricyanide was omitted and product formation monitored directly at 322 nm.

Substrate	Relative activity* Rabbit liver	Guinea-pig liver	Guinea-pig leucocytes
Phthalazine (0·5 mM)	100	100	100
Phenanthridine (50 μM)	38	96	71
Purine (10 mM)	23	40	36
3-Methylisoquinoline (1 mM)	13	5	4

* Expressed as a percentage of the phthalazine oxidation rate. Actual activities measured with phthalazine were 0·59 μmol min^{-1} mg^{-1} (rabbit liver), 0·27 μmol min^{-1} mg^{-1} (guinea-pig liver) and 0·32 μmol min^{-1} mg^{-1} (guinea-pig leucocytes).

In conclusion, the inhibition of the guinea-pig leucocyte enzyme by menadione and the confirmed presence of molybdenum are consistent with the enzyme being molybdenum hydroxylase, probably aldehyde oxidase. The leucocyte enzyme appears to have similar properties to those of guinea-pig liver aldehyde oxidase, however both guinea-pig enzymes have a different substrate specificity from rabbit liver aldehyde oxidase. If aldehyde oxidase is also present in human leucocytes, as it is in human liver (Johns 1967), it may contribute significantly to the metabolism of heterocyclic drugs.

REFERENCES

Badwey, J. A., Robinson, J. M., Karnovsky, M. J., Karnovsky, M. L. (1981) J. Biol. Chem. 256: 3479–3486

Baehner, R. L., Gilman, N., Karnovsky, M. L. (1970) J. Clin. Invest. 49: 692–700

Beedham, C. (1985) Drug Metabolism Reviews, 16: 119–156

Bradford, M. (1976) Anal. Biochem. 72: 248–254

Depierre, J. W., Karnovsky, M. L. (1974) J. Biol. Chem. 249: 7111–7120

Holmes, R. S. (1978) Biochem. Physiol. 61B: 339–346

Johns, D. G. (1967) J. Clin. Invest. 46: 1492–1505

Johnson, C., Stubley-Beedham, C., Stell, J. G. P. (1984) Biochem. Pharmacol. 33: 3699–3705

Kelsey, F. E., Oldham, F. K. (1943) J. Pharmacol. Exp. Ther. 79: 77–80

Krenitsky, T. A., Neil, S. M., Elion, G. B., Hitchings, G. H. (1972) Arch. Biochem. Biophys. 150: 585–599

Kurth, J., Neumann, B., Aurich, H. (1978) Acta. Biol. Med. Ger. 37: 493–495

Stubley, C., Stell, J. G. P., Mathieson, D. W. (1979) Xenobiotica 9: 475–484

Tubaro, E., Lotti, B., Santiangeli, C., Cavallo, G. (1980) Biochem. Pharmacol. 29: 1945–1948