LIVER ALDEHYDE OXIDASE: A FORM OF XANTHINE OXIDASE

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SUMMARY

Pig liver aldehyde oxidase is not specific for aldehydes, but in addition catalyzes the oxidation at reduced rates of hypoxanthine, xanthine and DPNH. The sites of binding of acetaldehyde and hypoxanthine by the enzyme appear to be different. Flavin is a component common to the pathways of oxidation of acetaldehyde and hypoxanthine. Quinacrine inhibits the enzymic catalysis by preventing the reduction of the flavin. The heme component present in preparations of the enzyme does not appear to be involved in the enzymic catalysis.

INTRODUCTION

Previously GORDON *et al.*[1] described the isolation from pig liver of a purified enzyme preparation which catalyzed the oxidation of aldehydes, but was inactive with hypoxanthine or DPNH with methylene blue as electron acceptor. More recently MAHLER *et al.*[2] isolated the enzyme in highly purified form and showed it to be a molybdoflavoprotein. They confirmed the finding that the preparation did not catalyze the oxidation of hypoxanthine or DPNH by methylene blue under the conditions of assay used by GORDON *et al.*[1], but other assay conditions and electron acceptors were not tried by the authors.

In the present report data will be presented to show that pig liver aldehyde oxidase catalyzes the oxidation of hypoxanthine, xanthine and DPNH as well as aldehydes. Evidence for the non-participation of the heme component in the enzymic catalysis will be presented.

METHODS AND MATERIALS

Aldehyde oxidase was prepared from pig liver in highly purified form by modifications of the method of MAHLER *et al.*[2]. Several preparations were subjected to electrophoresis under the conditions described by MAHLER *et al.*[2], and the results were in agreement with those previously published[2] showing only a single maximum. The

Abbreviations: DPNH, reduced diphosphopyridine nucleotide; FAD, flavin adenine dinucleotide.

preparations were estimated to be over 90 % homogeneous from the symmetry of the peak and calculation of the apparent heterogeneity[3]. The electrophoretic measurements were performed by Mr. R. WADE of the Department of Biochemistry, University of Washington.

The assay systems which were used to determine enzymic activity were based on (a) the reduction of 2,6-dichloroindophenol and ferricytochrome *c* measured spectrophotometrically at 38° as described by MAHLER *et al.*[2], (the assays contained catalase but no alcohol), (b) the reduction of methylene blue measured colorimetrically in Thunberg tubes at 38° according to CORRAN *et al.*[4] and (c) the reaction with molecular oxygen determined by means of an oxygen polarograph as described by CHANCE AND WILLIAMS[5]. In the assay with oxygen the complete system contained 0.4 ml of 0.2 *M* phosphate buffer of pH 7.5, 2.0 μmoles of substrate, and 0.02 ml of 0.1 % catalase solution in a final volume of 1.0 ml. The reactions were started by addition of an appropriate amount of enzyme (0.2 to 0.4 mg protein) and were carried out at a temperature of 23°. Protein was determined by the biuret reaction[6]. FAD was estimated spectrophotometrically from the ΔE_{450} after reduction of the enzyme preparation with substrate or hydrosulfite using the molecular extinction coefficient of $11.3 \cdot 10^6$ cm²/mole[7].

Silicomolybdate solutions were prepared as described by GLENN AND CRANE[8]. Cytochrome *c*, DPNH and catalase were obtained from the Sigma Chemical Company.

RESULTS

Table I contains a summary of the activities of preparations of aldehyde oxidase with different acceptors and substrates. It is apparent on inspection of the table that the enzyme catalyzed the oxidation of hypoxanthine, xanthine and DPNH as well as acetaldehyde with a variety of electron acceptors. The rate of reaction with

TABLE I

ACTIVITY OF ALDEHYDE OXIDASE WITH DIFFERENT ACCEPTORS AND SUBSTRATES

The rates in parentheses were determined with silicomolybdate solution added to the assay system.

Acceptor	*Substrate*	*Activity (μmoles substrate/mg protein/min) of preparations of aldehyde oxidase*						
		1	*2*	*3*	*4*	*5*	*6*	*7*
Methylene blue 38°	Acetaldehyde	0.52	0.33	0.27	0.44			0.27
	Hypoxanthine	0.03	0.16	0.03	0.09			0.11
Indophenol 38°	Acetaldehyde	0.09	0.17	0.15	0.12	0.11	0.10	0.10
	Hypoxanthine	0.04	0.14	0.06	0.08	0.06	0.11	0.10
	Xanthine	0.02	0.05	0.02	0.03	0.01	0.04	
	DPNH	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Cytochrome *c* 38°	Acetaldehyde					0.02 (0.05)	0.03 (0.07)	
	Hypoxanthine					0.01 (0.01)	0.01 (0.01)	
Oxygen 23°	Acetaldehyde	0.20	0.18	0.28	0.19	0.18	0.19	0.14
	Hypoxanthine	0.03	0.05	0.03	0.03			
	Xanthine	0.02	0.02					

acetaldehyde was in most instances substantially faster than with other substrates. Oxygen and methylene blue appeared to be the most satisfactory acceptors as reaction rates were consistently higher than rates with the other acceptors. It should be noted that rates with oxygen were determined at 23° whereas the other rates were determined at 38°. DPNH was able to serve as a substrate for the enzyme but at a very slow rate. In accord with previous work[1,2] the preparations usually failed to exhibit activity with hypoxanthine when assayed with methylene blue (1.0 μmole) as described by CORRAN[4]. When the system was changed and less methylene blue (0.4 μmole) was used in the assay, rates of reaction were determined for acetaldehyde and hypoxanthine (as shown in Table I) which were in accord with the reaction rates observed with oxygen as acceptor. As previously described[2,8] silicomolybdate increased the rate of oxidation of acetaldehyde by the enzyme 2 to 3 fold when cytochrome *c* was used as acceptor. Rates of oxygen utilization by the enzyme preparations were unaffected by the presence of silicomolybdate, cytochrome *c* or both indicating that the increased rate of cytochrome *c* reduction in the presence of silicomolybdate was not secondary to a decreased rate of reaction of the enzyme with oxygen, and represented a real increase in the amount of acetaldehyde oxidized by the enzyme. However, the rate of reaction of hypoxanthine with the enzyme and cytochrome *c* was not increased by the addition of silicomolybdate.

Table II shows the rates of reaction of preparations of aldehyde oxidase with combinations of acetaldehyde and hypoxanthine as substrates. Sufficient amounts of acetaldehyde and hypoxanthine were used in the assays to saturate the system and give maximum rates of reaction. As shown in the table, reaction rates were significantly higher in all instances but one when both substrates were present in the assay system. In only one instance was the rate with both substrates equal to the sum of the rates with the individual substrates. Acceptors other than indophenol were not used in these studies since the rates of oxidation for acetaldehyde and hypoxanthine with other acceptors were not close enough in value to give significant results.

The difference spectrum (reduced minus oxidized) of a preparation of aldehyde oxidase reduced with acetaldehyde is shown in Fig. 1. An identical spectrum was obtained after reduction with hypoxanthine. No additional decrease in O.D. at

TABLE II

EFFECTS OF COMBINATIONS OF SUBSTRATES ON THE ACTIVITY OF ALDEHYDE OXIDASE WITH INDOPHENOL AS ACCEPTOR

Each value given is the average of duplicate determinations.

Preparation	*Activity (Δ O.D./mg protein/min)*		
	Acetaldehyde	*Hypoxanthine*	*Acetaldehyde plus hypoxanthine*
1	2.9	1.5	4.0
2	2.0	1.1	2.7
3	2.1	1.1	2.7
4	2.0	1.1	2.1
5	1.8	2.1	4.0
Average	2.2	1.4	3.1

450 mμ was noted after reduction by either substrate when hydrosulfite was added to the cuvette, indicating that the flavin was completely reduced by acetaldehyde or hypoxanthine. No flavin reduction was noted after addition of substrate until very low concentrations of oxygen were reached (followed by means of the oxygen polarograph) indicating that during the steady state phase of enzymic catalysis the flavin remained almost completely oxidized.

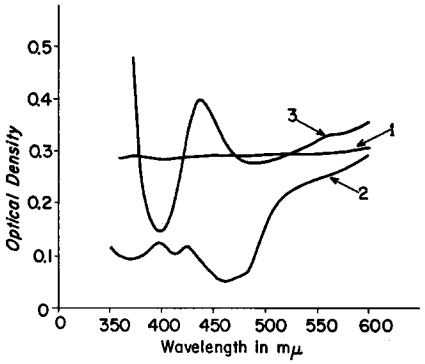

Fig. 1. Difference spectra of aldehyde oxidase: (1) Baseline, (2) enzyme reduced with excess acetaldehyde minus oxidized enzyme, (3) enzyme reduced with acetaldehyde and hydrosulfite minus enzyme reduced with acetaldehyde only. All cuvettes contained 10 mg of enzyme protein, 0.4 ml of 0.2 *M* phosphate buffer of pH 7.5 and 0.02 ml of 0.1 % catalase solution in final volume of 1.1 ml. To reduce the enzyme 0.02 ml of 1 *M* acetaldehyde or a small amount (less than 1 mg) of solid hydrosulfite was added to the cuvette.

Quinacrine has previously been shown to be an inhibitor for flavin nucleotide containing enzymes[9] and for aldehyde oxidase[2]. When added to the assay system in concentration 0.001 *M*, quinacrine inhibited the enzyme catalyzed oxidation of acetaldehyde and hypoxanthine by oxygen to the same degree (approx. 80 %) indicating again that the flavin is a component common to the pathways of oxidation of both hypoxanthine and acetaldehyde. Difference spectra (reduced minus oxidized) of preparations of enzyme reduced by either acetaldehyde or hydrosulfite in the presence of 0.001 *M* quinacrine showed that only 14 % of the flavin present could be reduced. As stated earlier, 100 % of the flavin was reduced by substrate or hydrosulfite in the absence of quinacrine. It would appear that quinacrine interferes with enzymic catalysis by blocking the reduction of the flavin and thus interfering with the chain of electron transport.

The difference spectrum of aldehyde oxidase reduced by hydrosulfite minus reduced by acetaldehyde is shown in Fig. 1. The spectrum has maxima at 435 and 560 mμ indicating that most of the heme which is present in preparations of aldehyde oxidase[2] is reduced by hydrosulfite but not by acetaldehyde. The finding that the heme is not reduced by substrate suggests strongly that the heme does not participate directly in the enzymic catalysis.

DISCUSSION

Results of the experiments described above suggest that pig liver aldehyde oxidase is a form of xanthine oxidase. The enzyme is not specific for aldehydes, reacting with

hypoxanthine and xanthine although at rates appreciably slower than with acetaldehyde. DPNH can also serve as substrate for the enzyme, but at a very slow rate. The finding that hypoxanthine as well as acetaldehyde can completely reduce the enzyme bound flavin indicates that the same enzyme catalyses the oxidation of both substrates and that the oxidation of hypoxanthine is not due to the presence in the preparation of a small amount of a second contaminating enzyme. This is confirmed by the finding that quinacrine inhibits the oxidation of acetaldehyde and hypoxanthine to the same degree. Although flavin appears to be a component common to the paths of reaction for acetaldehyde and hypoxanthine, results of studies using combinations of substrates demonstrate that the sites of reaction with the enzyme are different for acetaldehyde and hypoxanthine, and that the flavin is not the rate limiting component in the catalysis. The findings that the relative rates of reaction for acetaldehyde and hypoxanthine differ for different acceptors and that silicomolybdate stimulates the reduction of cytochrome *c* by acetaldehyde but not by hypoxanthine strongly suggest the possibility of multiple pathways of reaction. Failure of substrate to reduce the heme which is present in preparations of the enzyme indicate that the heme is not involved in the enzymic catalysis.

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