BBA 63296

The inhibition of yeast alcohol dehydrogenase by 2-bromo-2-phenylacetaldehyde

The inhibition of yeast alcohol dehydrogenase (EC 1.1.1.1) by 2-chloroethanol has been known for some years[1], and recently the stoichiometry of the reaction between the enzyme and 2-chloroacetaldehyde has been reported[2]. In this communication the potent substrate-directed inhibition of yeast alcohol dehydrogenase by 2-bromo-2-phenylacetaldehyde is reported. Phenylacetaldehyde is a substrate for yeast alcohol dehydrogenase, and K_m values for this substrate and acetaldehyde were determined from spectrophotometric measurements of NADH oxidation at 30° in 0.01 M phosphate buffer (pH 7.6) containing 0.1 mM NADH, to be 90 μM and 230 μM (*cf.* ref. 3), respectively. Since yeast alcohol dehydrogenase obeys an equilibrium kinetic mechanism[4], it follows that the enzyme has a higher affinity for phenylacetaldehyde than for acetaldehyde.

2-Bromo-2-phenylacetaldehyde was prepared by the reaction of dioxane dibromide[5] with phenylacetaldehyde by the method of YANOVSKAYA AND TERENT'EV[6], and the product was redistilled under reduced pressure in an atmosphere of nitrogen. Solutions of the product were made up in deionized water through which a stream of nitrogen had been passed for 20 min. The concentration of aldehyde groups in solutions was determined by titration with sodium bisulphite and iodine, and the bromine content of the product was determined, after fusion, by Volhardt's method[7]. The ratio of bromine atoms to aldehyde groups in the product was calculated to be 0.975 : 1.

Time courses of inhibition of crystalline yeast alcohol dehydrogenase (Boehringer) by 2-bromo-2-phenylacetaldehyde in the presence and absence of substrates are shown in Fig. 1. The presence of either acetaldehyde of NAD^+ protects the enzyme

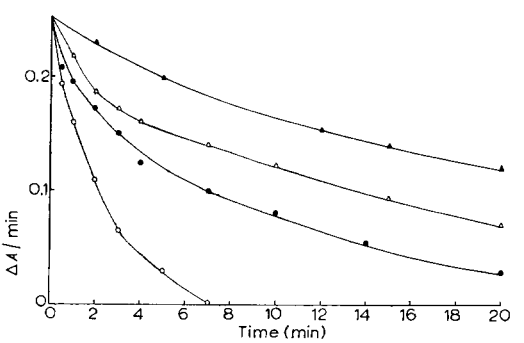

Fig. 1. Time courses of inhibition of yeast alcohol dehydrogenase by 2-bromo-2-phenylacetaldehyde. 5.0 ml of a crystalline yeast alcohol dehydrogenase solution in 0.01 M phosphate buffer (pH 7.6) containing 0.15 mg enzyme, was incubated at 20° with 9.6 mμmoles of 2-bromo-2-phenylacetaldehyde and 25 μl samples were removed for assay. Enzyme activity was assayed spectrophotometrically at 30° by following the decrease in absorbance at 340 mμ when NADH was oxidized to NAD^+. The assay mixture contained in a total volume of 2.0 ml, 20 μmoles of phosphate buffer (pH 7.6), 0.5 μmole of acetaldehyde, and 0.15 μmole of NADH. ●—●, enzyme preincubated as above; △—△, enzyme preincubated as above with 0.75 μmole NAD^+; ○—○, enzyme preincubated as above with 0.75 μmole NADH; ▲—▲, enzyme preincubated as above with 10 μmoles acetaldehyde.

from inhibition, whilst NADH accelerates inhibition. The inhibition was not reversed by dialysis.

Yeast alcohol dehydrogenase obeys a random-order kinetic mechanism[4], and thus it would seem that the 2-bromo-2-phenylacetaldehyde acts most efficiently as an inhibitor when it is bound to the enzyme in a ternary complex with NADH. The partial protection by NAD^+ is to be expected since it has been suggested that its binding to yeast alcohol dehydrogenase will hinder the binding of aldehyde[8]. Although NADH markedly stimulates the inhibition of yeast alcohol dehydrogenase, no evidence for any oxidation of NADH could be detected using a highly sensitive fluorescence method[9] when 10 mμmoles of yeast alcohol dehydrogenase was allowed to react with 100 mμmoles of 2-bromo-2-phenylacetaldehyde in the presence of 150 mμmoles NADH.

The specificity of inhibition is shown by the fact that after a sample of 2-bromo-2-phenylacetaldehyde was oxidized to the corresponding acid[10], incubation of the enzyme for 15 min at 20° with a 0.07 mM solution of this inhibitor failed to produce detectable inhibition. Preincubation of crystalline liver alcohol dehydrogenase (Boehringer) with 0.5 mM 2-bromo-2-phenylacetaldehyde failed to produce detectable inhibition within 15 min.

It has been reported that substrate protects sulphydryl groups of yeast alcohol dehydrogenase from reaction with *p*-chloromercuribenzoate[11]. The sulphydryl group content of samples of yeast alcohol dehydrogenase were determined before and after inhibition by reaction with *p*-chloromercuribenzoate[12] both in the presence and absence of 8 M urea and by reaction with 5,5′-dithio-bis-(2-nitrobenzoic acid)[13] in the presence of 8 M urea. The results shown in Table I indicate that between 3 and 4 sulphydryl groups per molecule are protected after inhibition of the enzyme with 2-bromo-2-phenylacetaldehyde, even when the enzyme is denatured in 8 M urea.

TABLE I

THE EFFECT OF 2-BROMO-2-PHENYLACETALDEHYDE INHIBITION ON THE SULPHYDRYL GROUP CONTENT OF YEAST ALCOHOL DEHYDROGENASE

Determination method	*Sulphydryl groups per molecule*			
	Native enzyme	*Inhibited enzyme*	*Enzyme in 8 M urea*	*Inhibited enzyme in 8 M urea*
p-Chloromercuribenzoate[12]	19.8	16.5	20.0	16.8
5,5′-Dithio-bis-(2-nitrobenzoic acid)[13]	—	—	19.2	15.3

Estimates of the number of sulphydryl groups per molecule of yeast alcohol dehydrogenase vary widely and WALLENFELS AND SUND[14] have shown that there is a parallel between the specific activity of the enzyme preparation and its free sulphydryl group content. The results shown in Table I would suggest that 2-bromo-2-phenylacetaldehyde may act by reacting with the essential sulphydryl group present at each active centre, which RABIN AND WHITEHEAD[8] have shown to be a contact residue.

The binding of NADH by yeast alcohol dehydrogenase can be followed by the

increase in fluorescence at 443 mμ (ref. 15) and it was found that the increase in fluorescence which occurred when yeast alcohol dehydrogenase was added to a solution of NADH was unaffected by the prior incubation of the enzyme with 2-bromo-2-phenylacetaldehyde.

This compound would therefore appear to be a potent substrate-directed inhibitor of yeast alcohol dehydrogenase acting by reaction with a sulphydryl group at the active centre of the enzyme.

The skilled technical assistance of Mr. I. P. C. SPIRES is gratefully acknowledged.

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Received November 27th, 1967

Biochim. Biophys. Acta, 151 (1968) 692–694

BBA 63304

Studies on the thyroidal UDPG pyrophosphorylase: Partial purification and some of its properties

The existence of a UDPG pyrophosphorylase (EC 2.7.7.9) in thyroids was recently established[1]. It appears that this and other enzymes involved in the metabolism of sugar donors contribute to the biosynthesis of the carbohydrate moiety of thyroglobulin. In the present communication, a procedure for the partial purification of the enzyme, extracted from calf thyroids, is described. Furthermore, observations of influences exerted by magnesium, pyrophosphate and hydrogen ions on the reaction catalyzed by the purified enzyme will be reported.

All reagents and auxiliary enzymes were purchased from commercial source. Only the UDPG pyrophosphorylase, used as assay control, was prepared in this