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NUCLEOTIDE INHIBITION OF UDPGLUCOSE 4-EPIMERASE FROM *SACCHAROMYCES FRAGILIS* AND FROM GOAT LIVER

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SUMMARY

UDPglucose 4-epimerase (EC 5.1.3.2) from both goat liver and the yeast *Saccharomyces fragilis* was strongly inhibited by uridine nucleotides. The yeast enzyme, unlike the liver one, showed substrate inhibition. UMP, in combination with various sugars, slowly but irreversibly inactivated the yeast enzyme. NADH became a strong inhibitor for the yeast enzyme once NAD^+ had been removed from the enzyme surface.

INTRODUCTION

UDPglucose 4-epimerase (EC 5.1.3.2) is a necessary enzyme in the metabolism of galactose in a variety of systems studied so far. The enzyme has been extensively purified from a wide variety of sources such as yeast[1], *Escherichia coli* and mammalian liver[3]. In all the cases studied so far, NAD^+ is an essential component for enzymic activity, though the mode of participation of NAD^+ is different in epimerases isolated from different sources. The liver enzyme, for example, needs NAD^+ from an outside source as a co-factor, whereas both the yeast and *E. coli* enzymes have NAD^+ bound tightly to the protein moiety[1,2]. We have observed some differences in nucleotide interaction between the yeast and the liver enzyme which are dependent on the mode of participation of NAD^+.

MATERIALS AND METHODS

The highly purified yeast epimerase was a gift from Dr. Kalckar's laboratory. The goat liver epimerase was purified 25-fold by protamine sulphate, ammonium sulphate and calcium phosphate gel treatment and was free from UDPglucose dehydrogenase. One unit of epimerase was defined as the amount which catalyzed the formation of 1 μmole of UDPglucose per min. The epimerase was assayed according to the coupled assay procedure of DARROW AND RODSTROM[1]. For certain experiments the assay was carried out in two steps, in which the pre-formed UDPglucose was

Abbreviation: PCMB, *p*-chloromercuribenzoate.

estimated with the dehydrogenase after inactivating the epimerase with hot alcohol. The *p*-chloromercuribenzoate (PCMB) treated inactive yeast epimerase was prepared according to the method of CREVELING *et al.*[4].

RESULTS

Inhibition by nucleotides

Fig. 1 shows that UMP acted as a strong, strictly competitive inhibitor for the liver enzyme, having a K_i of $4.1 \cdot 10^{-5}$ M. The yeast enzyme was equally strongly inhibited by UMP and the enzyme from both sources was inhibited by UDP and UTP though to a lesser extent. The yeast enzyme was found to be inhibited by a variety of nucleotides, *e.g.* CMP, CDP, TMP, TDP, GMP, GDP and AMP, whereas only the uridine nucleotides were inhibitory for the liver enzyme.

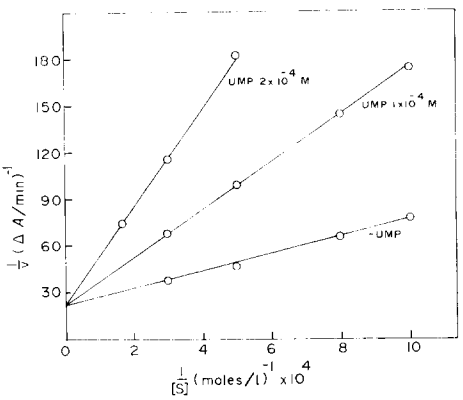

Fig. 1. Inhibition of liver epimerase with UMP.

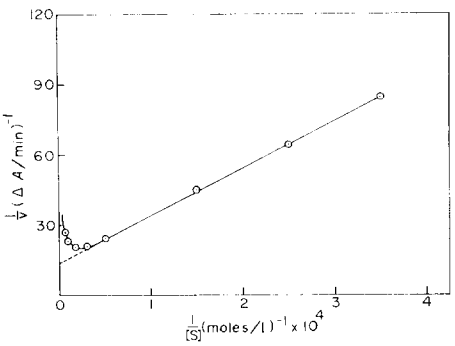

Fig. 2. Inhibition of yeast epimerase with UDPgalactose as substrate.

Inhibition by substrate

Fig. 2 shows that a typical substrate inhibition curve was obtained with the yeast enzyme at high concentrations of UDPgalactose. UDPglucose also inhibited the enzyme, as was shown by the 2-step assay. Neither of the substrates, however, had any inhibitory effect on the liver enzyme.

Inactivation by UMP and sugars

Table I shows that in the presence of 10^{-2} M glucose, UMP at a concentration of 10^{-3} M brings about a slow but completely irreversible loss of activity in the case of the yeast enzyme only. The system is without any effect on the liver enzyme. The irreversible nature of the change is apparent since removal of UMP and glucose did not restore the activity. The interaction is specific for UMP as it could not be replaced by any other nucleotide. In case of sugars, however, the specificity is completely lost and glucose could be completely or partially replaced by a number of sugars, such as galactose, mannose, rhamnose, arabinose, *etc.* Galactose could completely replace glucose for this interaction.

TABLE I

IRREVERSIBLE INACTIVATION OF YEAST EPIMERASE IN THE PRESENCE OF UMP AND GLUCOSE

For each of the individual experiments several tubes were used, each containing 100 μmoles potassium phosphate buffer (pH 7.4), 2 mg albumin and 0.16 unit epimerase in a total volume of 1 ml. UMP and glucose were added as indicated. Incubation temperature was maintained at 25°. After a particular time of incubation, the enzyme was precipitated with solid $(NH_4)_2SO_4$, redissolved in 1 ml of 0.1 M potassium phosphate buffer and assayed for the total activity recovered. The range of error for the activity is $\pm$ 5%.

Expt. No.	*System*	*Activity (%)*			
		0 h	*1 h*	*3 h*	*5 h*
1	Yeast epimerase (Y)	100	100	98	96
2	(Y) + UMP (10^{-3} M)	100	100	98	97
3	(Y) + glucose (10^{-2} M)	100	100	97	96
4	(Y) + UMP (10^{-3} M) + glucose (10^{-2} M)	100	77	28	3
5	(Y) + UMP (10^{-4} M) + glucose (10^{-2} M)	100	88	66	45
6	(Y) + UMP (10^{-3} M) + glucose (10^{-3} M)	100	97	94	90
7	Liver epimerase (L)	100	100	98	97
8	(L) + UMP (10^{-3} M) + glucose (10^{-2} M)	100	100	98	96
9	(L) + UMP (10^{-2} M) + glucose (10^{-2} M)	100	100	97	96

TABLE II

INHIBITION OF THE DARK EPIMERASE FROM YEAST WITH NADH

The liver and the yeast enzymes were incubated in a medium containing 100 μmoles Tris buffer, (pH 8.1), 0.2 μmole UDPgalactose and an amount of enzyme that gave a rate of 0.04–0.05 absorbance unit per min in a total volume of 1 ml. Amount of NAD^+ and NADH present are indicated separately. For the Dark epimerase, the PCMB-treated enzyme was preincubated in a medium containing 100 μmoles Tris buffer (pH 8.1), 60 μmoles β-mercaptoethanol and varying amouts of NAD^+ and NADH in a total volume of 1 ml. After preincubation for 60 min at 30°, the epimerase reaction was started by addition of 0.2 μmole of UDPgalactose. After 8 min incubation, UDPglucose formed was assayed with UDPglucose dehydrogenase.

Enzyme	$[NAD^+] \times 10^4$ *(M)*	$[NADH] \times 10^4$ *(M)*	*UDPglucose formed (nmoles)*
Liver	—	—	1.0
	2	—	35.3
	5	—	36.1
	2	1	27.0
	2	5	8.7
Yeast	—	—	30.9
	5	—	30.1
	—	5	31.3
Dark epimerase	—	—	1.0
	1	—	27.5
	2	—	28.3
	—	5	1.0
	2	5	6.1
	2	10	2.7

Inhibition of Dark epimerase with NADH

MAXWELL[5] had shown that the bound NAD^+ of yeast enzyme is released into the medium on treatment with PCMB. Partial activity (40%), but not the native fluorescence, could be recovered by preincubation with β-mercaptoethanol. For activity NAD^+ had to be added from outside. This enzyme, designated as the Dark epimerase by CREVELING *et al.*[5], was inhibited strongly by NADH and thus resembled the liver enzyme, as is shown in Table II.

DISCUSSION

The metabolic significance of inhibition by UMP and other nucleotides is not clear. The total inactivation of yeast epimerase in the presence of UMP and high concentrations of galactose may, however, be significant for the understanding of stasis when the kinase or the transferase is missing[6].

The involvement of NAD^+ in two different ways for the yeast and liver enzymes results in certain differences in properties. In the case of the yeast enzyme, when UMP and glucose are added in conjunction, the catalytic process probably proceeds only half-way as is shown by the reduction of bound NAD^+ on the enzyme surface[7]. Since all the available NAD^+ is converted to the reduced form gradually and no more bound NAD^+ is available for the initial oxidation of the sugar moiety on the enzyme surface, the enzyme becomes inactive. In the case of Dark epimerase, NAD^+ is supplied from the medium for activity. This modified yeast enzyme therefore resembles the liver enzyme more closely and accordingly is strongly inhibited by NADH.

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