TERNARY COMPLEX FORMATION OF 1,2-PROPANEDIOL DEHYDRATASE, COBAMIDE COENZYME AND SUBSTRATE ANALOGUE

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Studies on chemical properties of the complex consisting of the apoprotein of 1,2-propanediol dehydratase (EC. 4.2.1.28) and 5,6-dimethylbenzimidazolylcobamide coenzyme (DBCC) have indicated that the apoprotein-DBCC complex (holoenzyme; E.DBCC) is inactivated by oxygen in the absence of the substrate. This inactivation process is considered to involve irreversible dissociation of the cobalt-carbon bond of DBCC resulting from the interaction of the coenzyme and apoprotein (Wagner, Lee, Frey and Abeles, 1966).

The present communication describes the protection of the holoenzyme with substrate analogues against the inactivation by oxygen. In the aerobic incubation of the apoenzyme and DBCC, styrene glycol or 1,2-butanediol prevented the holoenzyme from the inactivation. These analogues did not show a significant substrate activity but acted as weak competitive inhibitors. Displacement of the analogue with the substrate initiated the normal reaction. These observations suggest that a ternary complex is formed among the apoenzyme, coenzyme and substrate analogue, and that the complex is considerably more stable to oxygen and resembles structually an intermediate complex of the enzymatic reaction.

MATERIALS AND METHODS

DBCC was a gift of Yamanouchi Pharmaceutical Co., Ltd.. Styrene glycol and 1,2-butanediol were synthesized from DL-mandelic acid and 1,2-butylene oxide, respectively. Apoenzyme was prepared from Aerobacter aerogenes (ATCC 8724) according to the procedure of Lee and Abeles (1963). All other materials used were obtained from commercial sources.

Kinetic experiments were carried out in the usual manner to determine the type of the inhibition by substrate analogues against 1,2-propanediol. E•DBCC and substrate analogue-DBCC-apoprotein complexes were formed by preincubation at 37° in the absence of and in the presence of the analogue, respectively. The activities of these enzyme complexes were assayed in a similar way to that of Lee and Abeles (1963) except that enzyme reaction was started by addition of an excess of 1,2-propanediol (200 μmoles). Time-course changes in the activities were followed during incubations at various temperatures. In order to test the photo-stability of the apoenzyme and enzyme complexes, the preincubated mixtures were irradiated with 300 W tungsten lamp at a distance of 50 cm in an ice-water bath.

RESULTS AND DISCUSSION

Substrate Analogues as Competitive Inhibitor. Kinetic studies showed that styrene glycol and 1,2-butanediol acted as typical inhibitors in the

Table I. Kinetic Constants for Substrate and Substrate Analogues

	Km (10^{-3} M)	Ki* (10^{-3} M)	Ki** (10^{-3} M)	$-\Delta G$ (cal/mole)
1,2-Propanediol	0.0814			5800
1,2-Butanediol		1.97	2.08	3840
Styrene glycol		38.4	50.2	2010

* Values obtained from kinetic studies of the inhibition
** Values obtained from the holoenzyme-protecting effect

1,2-propanediol dehydratase system. As given in the first and second columns of Table I, Michaelis constant (Km) for the substrate, 1,2-propanediol, determined from the double reciprocal plots was 8.14×10^{-5} M, and inhibitor constants (Ki) were 3.84×10^{-2} M for styrene glycol and 1.97×10^{-3} M for 1,2-butanediol. These data show that both analogues are very weak competitive inhibitors.

The inhibition caused by 1,2-butanediol was less than 10 % when the analogue was used at a concentration equal to that of the substrate, in agreement with the result of Lee and Abeles (1963), and styrene glycol exerted almost negligible inhibition under the same condition. The affinity to the holoenzyme decreases in the order of 1,2-propanediol, 1,2-butanediol and styrene glycol as shown by $-\Delta G$ values in Table I. The fact that the substrate analogues were easily displaced in the ternary complex by addition of an excess of the substrate can be best interpreted by these data.

Effect of 1,2-Diol Concentration on Protection of Holoenzyme. The inactivation rate of the holoenzyme decreased markedly in the presence of styrene glycol or 1,2-butanediol. Fig. 1 illustrates the relationship between the residual activity after 5 minutes' preincubation at 37° and the concentration of the substrate analogue. Inhibitions by styrene glycol and 1,2-butanediol were almost negligible under these conditions. Intersection on the abscissa of the double reciprocal plots gives the value which seems to be apparent dissociation constant of the substrate analogue-holoenzyme complex. The apparent inhibitor constants thus obtained were 5.02×10^{-2} M for styrene glycol and 2.08×10^{-3} M for 1,2-butanediol (the third column of Table I). In view of the fact that these are almost in agreement with those obtained from kinetic studies of the inhibition, it seems reasonable to assume that the substrate analogues protect E·DBCC against the inactivation by oxygen, forming a ternary complex identical with that formed in the inhibition and resembling structually the Michaelis complex.

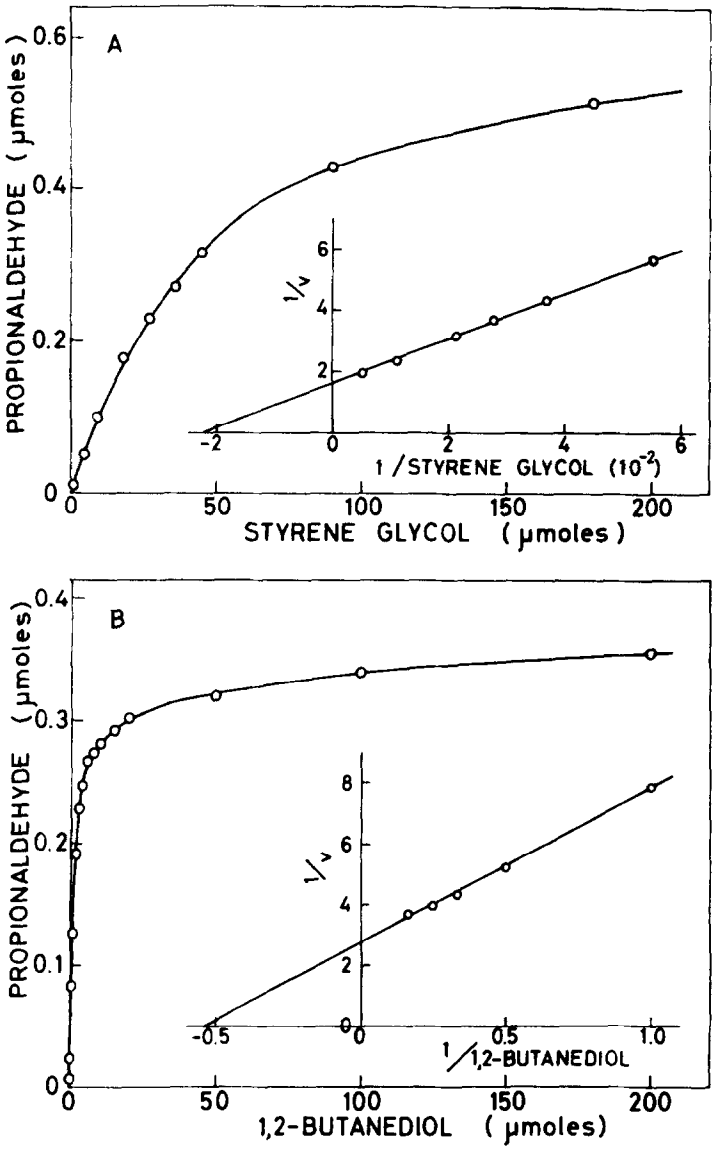

Figure 1. Effect of the concentration of styrene glycol (A) and 1,2-butanediol (B) on the protection of apopropanediol dehydratase-DBCC complex (holoenzyme).

A mixture (0.9ml) containing apoenzyme, 0.059 unit; DBCC 1.9 X 10^{-2} µmole; KCl, 50µmoles; K-phosphate buffer (pH 8.0), 25µmoles was incubated with the indicated amounts of styrene glycol (A) or 1,2-butanediol (B) for 5 minutes at 37°. After the preincubation, 200µmoles (0.1ml) of 1,2-propanediol were added and the mixtures were further incubated for 10 minutes at 37°. The amount of propionaldehyde formed was determined colorimetrically by conversion to its 2,4-dinitrophenylhydrazone.

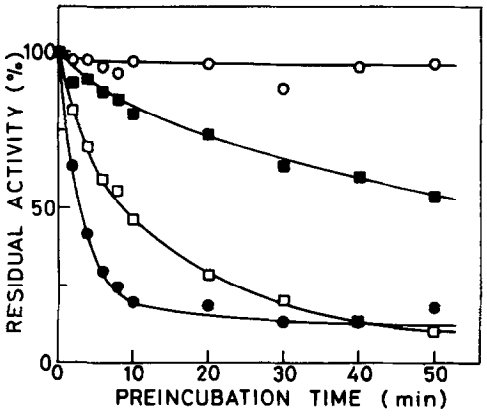

Figure 2. Comparison of the inactivation rates of the apoenzyme and enzyme complexes. The preincubation mixture contained the following. (1) o—o : apoenzyme, 0.074 unit, KCl, 50μmoles; K-phosphate buffer (pH 8.0), 25μmoles in a total volume of 0.8 ml. (2) ●—● : the same constituents of (1) plus DBCC, 1.5 X 10^{-2} μmoles in a total volume of 0.9 ml. (3) ■—■ : (2) plus styrene glycol, 255μmoles. (4) □—□ : (2) plus 1,2-butanediol, 50μmoles. At the end of the indicated time of preincubation at 37°, 1.5 X 10^{-2} μmoles (0.1 ml) of DBCC was added to 0.8ml aliquots. After addition of 200μmoles (0.1ml) of 1,2-propanediol to each test tube, the reaction mixtures were treated as described in Fig. 1. Residual activities (%) are expressed as relative values to that of a control tube which was not preincubated.

Comparison of Inactivation Rates of Apoenzyme and Enzyme Complexes.

Fig. 2 shows the time-course changes in the activities of apoenzyme, holoenzyme and holoenzyme-analogue complexes during the preincubation at 37°. Although the apoenzyme was stable at 37°, the holoenzyme was inactivated rapidly by oxygen. Substrate analogue-holoenzyme complexes, especially that of styrene glycol, were considerably stable.

Stability on Heating. The dependence of the residual activities of the apoenzyme and enzyme complexes on the preincubation temperature was studied. As shown in Fig. 3, the apoenzyme was inactivated rapidly at 40° to 50°, on the other hand the styrene glycol-holoenzyme complex resisted denaturation by heat and retained about 60 % of the initial activity even after 5 minutes at 50°.

Photo-sensitivity. Experiments with reference to stabilities of the apoenzyme and enzyme complexes to light revealed that the holoenzyme-

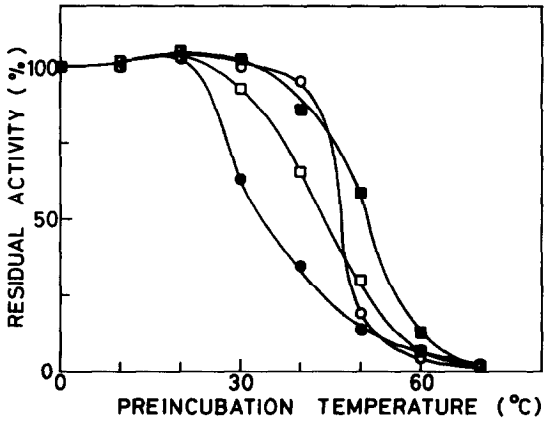

Figure 3. Effect of the preincubation temperature on the activities of the apoenzyme and enzyme complexes.

The preincubation mixtures were identical in composition with those in Figure 2. After preincubation for 5 minutes at the indicated temperature, the residual activities were assayed as described in Fig. 2.

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substrate analogue complexes were somewhat sensitive to light and inactivated gradually by irradiation, while the apoenzyme was photo-stable. Evidently the enzyme-coenzyme-substrate (product) complex is not affected by irradiation (Yamane, Shimizu and Fukui, 1965). Further work is in progress to explain fully the behavior of the enzyme complexes to light.

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