Dissociation of Diol Dehydrase into Two Different Protein Components

Tetsuo Toraya, Masaaki Uesaka, Masao Kondo and Saburo Fukui

Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Kyoto, Japan

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SUMMARY

Diol dehydrase from Aerobacter aerogenes was dissociated into two different protein components or subunits, designated Components F and S, by chromatography on DEAE-cellulose. Neither component alone possessed any appreciable catalytic activity. Diol dehydrase activity was restored when the two components were combined. Both components were also required for inactivation of coenzyme B_{12} by oxygen when incubation was carried out in the absence of substrate aerobically. The more acidic component, Component S, was a sulfhydryl protein sensitive to an alkylating agent, iodoacetamide. Coenzyme B_{12} was not bound by the individual components, F or S, both of which were necessary for the cobamide binding. The presence of substrate, 1,2-propanediol, in eluting buffer retarded the dissociation of the enzyme.

Diol dehydrase (DL-1,2-propanediol hydro-lyase, EC 4.2.1.28) is a B_{12} coenzyme-dependent enzyme which catalyzes the conversion of L- or D-1,2-propanediol to propionaldehyde and of 1,2-ethanediol to acetaldehyde (1). During the course of an investigation (2-6) on the ligand interaction with the apoprotein of diol dehydrase, we found that this enzyme can be dissociated into two protein fractions by chromatography on DEAE-cellulose. To obtain new information regarding the ligand interaction with this B_{12} enzyme, a detailed study on the apoenzyme components has been performed. This communication describes the separation of the apoenzyme into two dissimilar protein components.

MATERIALS AND METHODS

Materials. The crystalline coenzyme B_{12}, α-(5,6-dimethylbenzimidazolyl)-Co-5'-deoxyadenosylcobamide, was purchased from Glaxo Ltd., England. Other chemicals were obtained commercially. Diol dehydrase apoenzyme was prepared from Aerobacter aerogenes (ATCC 8724) by the same procedure as

described before (3), which is similar to that of Lee and Abeles (1).

Enzyme Assay. The activity of the diol dehydrase apoenzyme was assayed as described previously (3). The protein components (F and S) of diol dehydrase were estimated by the same assay by adding an excess of one component and making the other rate-limiting. Specific activities of the Components F and S were determined on the basis of the amount of protein in their fractions. Protein was determined according to the procedure of Lowry et al. (7).

RESULTS

Fractionation of Apoenzyme into Components F and S. About 100 units of partially purified preparation of apodiol dehydrase was dialyzed overnight against 2 liters of 0.01 M potassium phosphate buffer (pH 8.0), and then applied to a column (2.4 X 11 cm) of DEAE-cellulose. After being washed with the same buffer, the protein was eluted by a linear gradient in which 450 ml of 0.01 M potassium phosphate buffer (pH 8.0) were in the mixing chamber and 450 ml of 0.01 M potassium phosphate buffer (pH 8.0) with 0.40 M KCl were in the reservoir. As shown in Fig. 1, diol dehydrase apoenzyme was resolved by DEAE-cellulose chromatography into two protein components, neither of which, by itself, has any appreciable catalytic activity. But, when combined, they can be reconstituted into the active apoenzyme complex. The components eluted firstly and secondly from the column will be referred to as Components F and S, respectively. The presence of substrate, 1,2-propanediol, in the eluting buffer prevented the apoenzyme from dissociation into components (data not shown), suggesting that the substrate plays an important role in association of Components F and S.

The effect on enzyme activity of increasing amounts of one component in the presence and absence of a fixed level of the other component was examined. A typical enzyme saturation curve is shown in Fig. 2-B (Component F, fixed; Component S, varied). Figure 2-A shows that the amount of Component F was not sufficient to saturate Component S (18 μg). Since Component F is relatively unstable, the exact stoichiometric ratio has not yet been established.

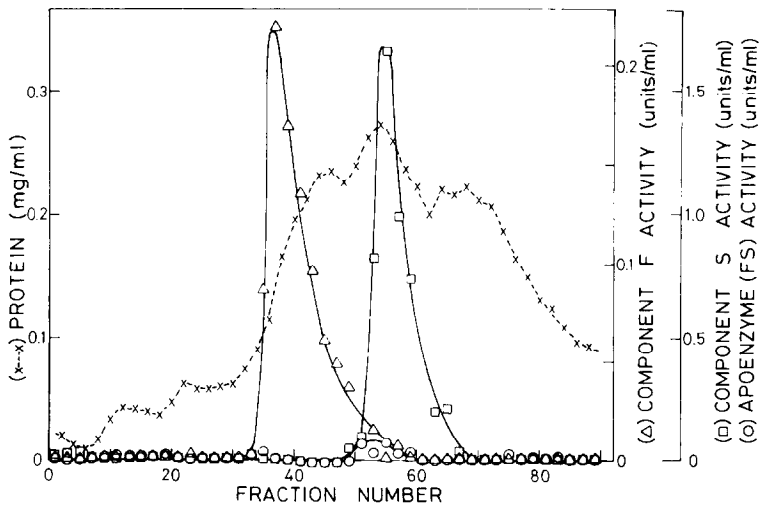
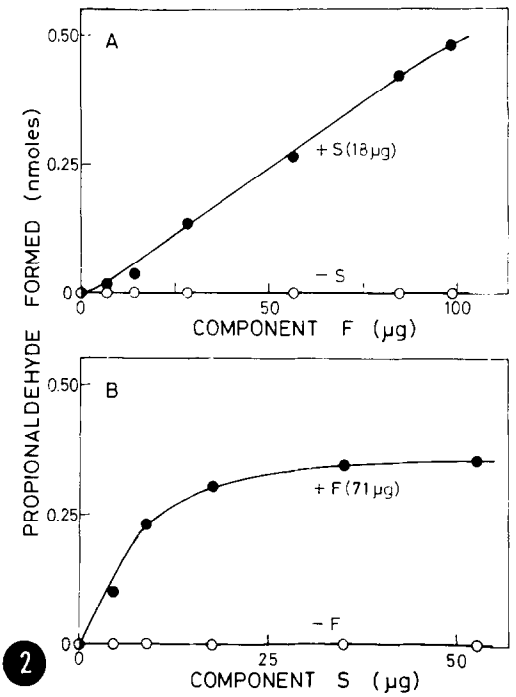


Figure 1. Dissociation of the diol dehydrase apoenzyme into Components F and S by chromatography on DEAE-cellulose. The activities of the apoenzyme (o) and Component F (△) were measured in 0.2 ml of each fraction. Component S (□) activity was determined in 0.02 ml of each fraction. Other experimental details are given in the text.

Requirement for Inactivation of Coenzyme B_{12} by Oxygen. When the diol dehydrase apoenzyme is incubated aerobically with coenzyme B_{12} in the absence of substrate, the enzyme-bound coenzyme reacts with oxygen, resulting in the rapid inactivation of the enzyme (1, 8). This inactivation process involves irreversible dissociation of the cobalt-carbon σ bond of the coenzyme B_{12} which is activated by the interaction with the apoenzyme. The ability of the enzyme to inactivate the coenzyme may be closely related to the catalytic process. Figure 3 depicts the effects of Component F and/or S on inactivation of the coenzyme by oxygen. Although neither component alone caused O_2-inactivation of the coenzyme, marked inactivation by oxygen took place when the two components were combined. This result suggests that both components are necessary for activation of the cobalt-carbon bond of the coenzyme moiety.

Coenzyme Binding. The holoenzyme of diol dehydrase is not resolved into apoenzyme and coenzyme B_{12} by gel filtration on Sephadex G-25 using potassium phosphate buffer containing both KCl and substrate (2, 3). This procedure was applied to test the ability of the individual components to



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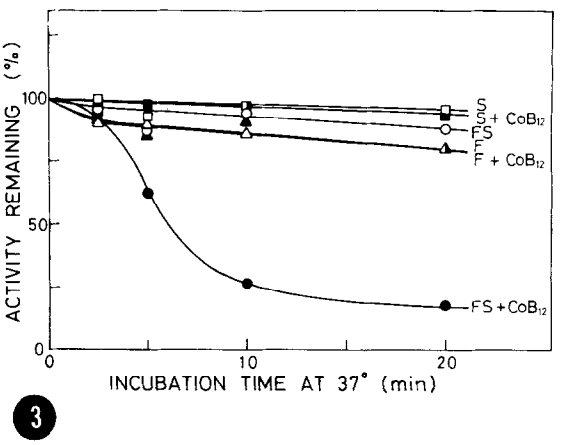

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Figure 2. Effect of concentrations of Components F (A) and S (B) on diol dehydrase activity in the presence and absence of a fixed level of the other component. The enzyme activity of the indicated amount of Component F or S (O) alone and (●) together with 18 μg of Component S or 71 μg of Component F was assayed as described in the text. The enzyme reaction was carried out at 37° for 10 min.

Figure 3. Effects of Components F and/or S on inactivation of coenzyme B_{12} by oxygen. Components F (85 μg) and/or S (35 μg) were aerobically incubated with or without 15 nmoles of coenzyme B_{12} at 37° for the indicated time. The remaining reactants of the usual assay mixture were then added, and the activity was assayed.

bind coenzyme B_{12}. Neither component alone bound coenzyme B_{12} in such a manner that coenzyme could not be removed by Sephadex G-25 gel filtration. This fact indicates that both Components F and S are absolutely required for the cobamide binding.

Effect of Sulfhydryl Inhibitor. It has been reported that diol dehydrase is a sulfhydryl enzyme, and that the apoenzyme is highly sensitive to various mercurial (1, 6) and alkylating (6) reagents. It is of interest to know which component of the two, or both, are sensitive to sulfhydryl inhibitors. Table I summarizes the effect of treatment of the components

TABLE I : Effect of Iodoacetamide Treatment on Activities of Component F, Component S, and the Apoenzyme Complex (FS).[a]

Component(s)	Propionaldehyde Formed (μmoles)		Inhibition (%)
	Not Pretreated (Control)[b]	Pretreated with Iodoacetamide	
Component F	0.44	0.26	40
Component S	0.83	0.04	95
Apoenzyme complex (FS)	0.47	0.00	100

[a] Component F (85 μg) and/or S (35 μg) were pretreated with 1 mM iodoacetamide at 37° for 15 min. Then the residual iodoacetamide was destroyed by reaction with a 10-fold excess of mercaptoethanol (10 mMO at 37° for 15 min. Finally the remaining reactants of the usual assay mixture were added, and the activity was assayed. The enzyme reaction was carried out at 37° for 20 min. [b] Samples preincubated without iodoacetamide were treated in a similar manner as controls.

with an alkylating agent, iodoacetamide, on catalytic activity. Component S and the apoenzyme complex (FS) were completely inactivated by this treatment, whereas Component F was rather insensitive to iodoacetamide (1 mM). These data suggest that Component S has sulfhydryl group(s) which is essential for catalytic activity.

DISCUSSION

The coenzyme B_{12}-requiring enzymes known at present are of two general types (9). There are those that appear to consist of similar subunits having sulfhydryl groups and cobamide binding sites on the same subunit (10-14), and there are others that are made up of two dissimilar protein moieties (15-20). In the latter group, one of the two protein components binds the cobamide and the other is a sulfhydryl protein; both are required for catalysis of the overall reactions. Diol dehydrase has been generally considered

to belong to the former group (1, 9). Starch gel electrophoresis indicates that about 90 % of the protein of the best preparation is present in a single component that contains all of the activity (1). The resolution of the enzyme into protein fractions might be retarded during starch gel electrophoresis, since the buffers used contained the substrate (2 % 1,2-propanediol).

Although one protein moiety of other enzymes except for glycerol dehydrase (17) binds cobamide and is usually obtained as a pink protein, both components of diol dehydrase are necessary for the cobamide binding. Similar results have been obtained with glycerol dehydrase (17, 21). Dissociation and reassociation of the components, influenced by the substrate, may control both the enzyme activity and the level of coenzyme B_{12} available for other B_{12}-dependent systems in this microorganism.

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