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STEREOSPECIFICITY AND MECHANISM OF ADENOSYLCOBALAMIN-DEPENDENT DIOL DEHYDRATASE. CATALYSIS AND INACTIVATION WITH MESO- AND DL-2,3-BUTANEDIOLS AS SUBSTRATES

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ABSTRACT: A comparative study of the reaction of meso-, d-, and dl-2,3-butanediols with adenosylcobalamin-dependent dioldehydratase was carried out. While the meso isomer is both a substrate and inactivator of holoenzyme, the d and dl compounds act as purely competitive inhibitors, neither undergoing catalysis nor inactivating holoenzyme. Furthermore, d- and dl-2,3-butanediols protect holoenzyme from oxygen inactivation and enzyme-bound cofactor from photolysis, and do not induce detectable cleavage of the carbon-cobalt bond of cofactor. These results show that the stereospecificity of the inactivation reaction is the same as that of catalysis, suggest that hydrogen abstraction from C-1 of substrate may be concerted with cleavage of the carbon-cobalt bond of adenosylcobalamin, and further suggest that formation of a carbon-cobalt bond between coenzyme and substrate is not obligatory for catalysis.

INTRODUCTION

Diol dehydratase, an adenosylcobalamin-dependent enzyme, catalyzes the conversion of 1,2-propanediol to propionaldehyde. This enzyme also reacts with a variety of modified 1,2-propanediols (1,2). Like 1,2-propanediol, these substrates are converted to the corresponding carbonyl compounds; however, they also effect irreversible inactivation of holoenzyme. With glycerol as substrate (2,3), the mechanisms of catalysis are similar, while their stereospecificities are complementary: glycerol bound in the "R" conformation undergoes catalysis, while glycerol bound in the "S" conformation results in inactivation.

The observation that 2,3-butanediol is both a substrate and inactivator of diol dehydratase provides an opportunity to further characterize the stereospecificity of catalysis and inactivation. That 2,3-butanediol exists as the meso, d , and l isomers renders this molecule a useful tool for stereochemical and mechanistic studies of these reactions.

Abbreviations used: AdoCbl, adenosylcobalamin; OH-Cbl, hydroxycobalamin; Cbl^{II}, cob(II)alamin.

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MATERIALS AND METHODS

Enzyme preparations. Diol dehydratase was obtained from Klebsiella pneumoniae (ATCC 8724) as described (4). Fraction E-8 with specific activity between 25 and 58 was used for all determinations. 1,2-Propanediol-free enzyme was prepared by dialysis against several changes of 10 mM K_2HPO_4 buffer at 4° C. All assays were carried out as described (2).

Adenosylcobalamin (AdoCbl) was purchased from Sigma.

d-2,3-Butanediol was purchased from Burdick and Jackson and had $[\alpha]_D^{20}$ -11.25° (c = 1 M). This compound contained approximately 1.6 mole % 1,2-propanediol as an impurity, as determined by kinetic and chemical analysis with diol dehydratase holoenzyme. This impurity was removed enzymatically prior to use in kinetic experiments.

2,3-Butanediol was purchased from Aldrich as a mixture of the meso and d isomers and distilled before use. A 1 M solution of this substrate showed virtually no optical rotation, so the d isomer is present in very small amounts, if at all.

dl-2,3-Butanediol was prepared from cis-2-butene by performic acid oxidation as described (5).

UV-Visible spectra were obtained as described (2) at 37°. Sample and reference solutions contained: apoenzyme, 80-100 units; K_2HPO_4 buffer, 15 mM; substrate, 0.12 M; volume = 1 ml. A stoichiometric amount of AdoCbl was added in a 3-4 µl aliquot to the sample immediately before recording the spectrum. Beckman Acta III and Cary 118 spectrophotometers were used to record the spectra.

RESULTS AND DISCUSSION

The kinetic parameters of meso-2,3-butanediol have been reported (2). The kinetics observed with this substrate indicate that it binds at the same site on the enzyme as 1,2-propanediol, whereupon either catalysis or inactivation may occur. The kinetic data for the d and dl isomers have now been determined; values of K_I, k_{cat}, and k_i for both substrates are given in the Table. K_I values were determined by inhibition of propionaldehyde production from 1,2-propanediol and (for the d isomer) by inhibition of inactivation of holoenzyme by glycerol. K_I for l-2,3-butanediol was calculated from the K_I measured for the d and dl compounds. (The relative magnitudes of the K_I values are consistent with those observed for other diol dehydratase substrates (2,6); in all other respects the d and dl compounds behaved identically.) Unlike the meso substrate, the dl isomer neither reacts with nor inactivates holoenzyme; it acts as a purely competitive inhibitor of catalysis and inactivation by other substrates.

Table

Kinetic parameters of 2,3-butanediols.

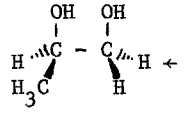
2,3-Butanediol	K_I, M x 10^4	k_{cat}, sec^{-1}	k_i, min^{-1}
meso	2.0 ± 0.3	4 ± 2	0.068 ± 0.005
d	5.5 ± 0.5	0	0
dl	1.07 ± 0.05	0	0
l*	0.59	0	0

K_I was measured as described in text. k_i, the inactivation rate constant, and k_{cat} were measured as described (2).

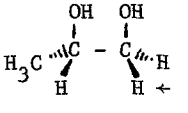
*Values for the l isomer were calculated from data obtained for the d and dl substrates (6).

UV-Visible spectra of holoenzyme-2,3-butanediol complexes are shown in the figure. The meso substrate induces conversion of an AdoCbl spectrum to one resembling that of OH-Cbl at a rate comparable to that of inactivation (2). dl-2,3-Butanediol, in contrast, elicits no change in the spectrum. Moreover, the dl compound protects enzyme-bound AdoCbl from inactivation by oxygen and photolysis. Exposure of a solution containing holoenzyme and dl-2,3-butanediol to a 60 watt bulb at 10 cm for 15 minutes caused less than a 15 % increase in A_{360} due to formation of OH-Cbl, in contrast to a ∿ 40 % increase observed in the absence of substrate. Addition of 1,2-propanediol to a holoenzyme/dl-2,3-butanediol solution produced a peak at 480 nm characteristic of Cbl^{II}; the spectrum reverted to its original appearance upon depletion of added substrate. In contrast, addition of 1,2-propanediol to a similar solution containing the meso substrate did not visibly alter the observed OH-Cbl spectrum.

These data illustrate the stereospecificity of the diol dehydratase reaction. The hydrogen abstracted from C-1 of 1,2-propanediol is that located "opposite" the C-3 methyl group in the following diagram (7) (arrows):



(R)-1,2-propanediol



(S)-1,2-propanediol

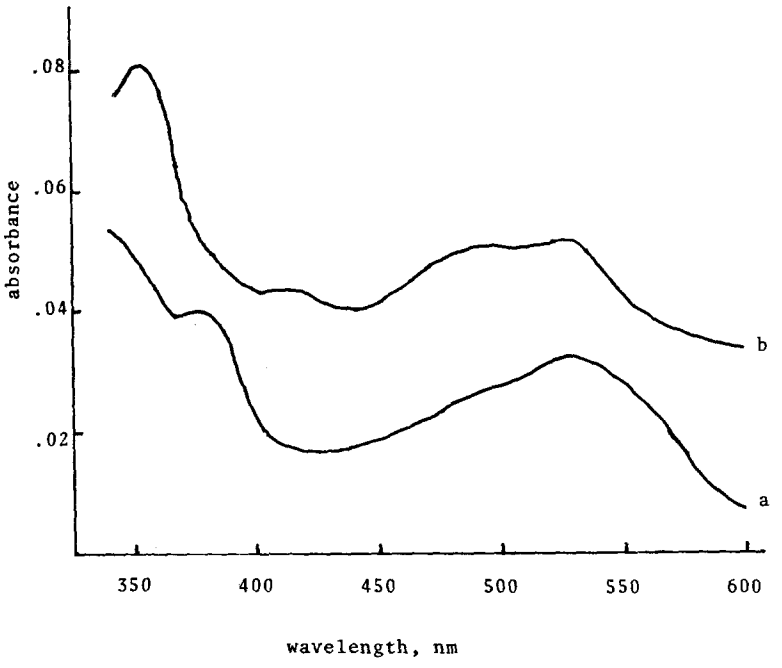
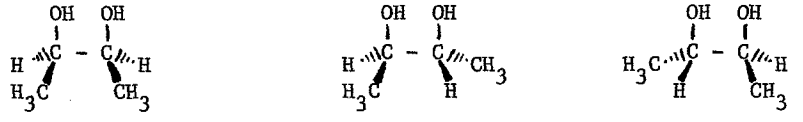


Fig. 1. UV-Visible absorption spectra of holoenzyme-2,3-butanediol complexes. (a) dl-2,3-Butanediol; apoenzyme, 100 units. (b) meso-2,3-Butanediol; apoenzyme, 80 units. Spectrum (b) has been displaced vertically for the sake of clarity. The d isomer gave the same results as the dl.

Meso-, d-, and l-2,3-butanediols have the following configurations (8).

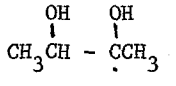


The configurations of the d and l isomers correspond to replacement of reactive hydrogen at C-1 of (R)- and (S)-1,2-propanediol, respectively, by a methyl group. The kinetic data reported here indicate that this modification precludes both catalysis and inactivation, which are known (2,3) to require abstraction of hydrogen from this carbon atom. Furthermore, the reactivity of the meso substrate indicates that the mere steric bulk of a methyl group does not prevent catalysis. Accordingly, we conclude that the catalysis and inactivation reactions with the 2,3-butanediols, and presumably other substrate ana-

logs, both exhibit a high degree of stereochemical discrimination with respect to hydrogen attached to C-1 of substrate. These results illustrate the remarkable stereospecificity of the diol dehydratase reaction, and constitute additional evidence for the previous assertion (2,3) that the catalysis and inactivation mechanisms are very similar.

d- and dl-2,3-butanediols form Michaelis-Menten complexes with holoenzyme, but do not alter the uv-visible spectrum of enzyme-bound AdoCbl, while binding of 1,2-propanediol and other substrate analogs to diol dehydratase holoenzyme is commonly associated with homolysis of the carbon-cobalt bond of AdoCbl to generate a species with an absorption spectrum characteristic of Cbl^{II} (in a few cases, this spectrum rapidly converts to one characteristic of OH-Cbl) (2,6,9). Other substances less structurally similar to 1,2-propanediol (glycolaldehyde (9) and chloroacetaldehyde (10)) behave similarly. In this respect, the behavior of dl-2,3-butanediol, which has no hydrogen stereochemically available for abstraction from C-1, is a notable departure from that of other diol dehydratase substrates, all of which present hydrogen for transfer to C-5' of AdoCbl. These results imply that simple binding of substrate to holoenzyme is not sufficient to effect cleavage of the carbon-cobalt bond. On this basis, we suggest that hydrogen abstraction from C-1 of substrate may be concerted with homolysis of the carbon-cobalt bond of AdoCbl.

During catalysis with meso-2,3-butanediol as substrate, initial transfer of hydrogen from substrate to C-5' of AdoCbl would generate a tertiary radical.



Subsequent formation of a cobalamin adduct from this species and Cbl^{II}, as described (11,12) and supported by model studies (13), would result in a tertiary alkylcobalamin, an exceedingly unstable molecule whose formation has never been observed (14). Thus, the ability of meso-2,3-butanediol to function as a substrate for dioldehydratase suggests that an intermediate with a covalent carbon-cobalt bond between Cbl and substrate is not obligatory for catalysis. Some

results for the ethanolamine deaminase reaction have been similarly interpreted (15,16).

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