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THE MECHANISM OF ACTION OF ETHANOLAMINE AMMONIA-LYASE, AN ADENOSYLCOBALAMIN-DEPENDENT ENZYME

EVIDENCE THAT CARBON-COBALT BOND CLEAVAGE IS DRIVEN IN PART BY CONFORMATIONAL ALTERATIONS OF THE CORRIN RING

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Summary

Previous work has shown that the interaction between ethanolamine ammonia-lyase (ethanolamine ammonia-lyase, EC 4.3.1.7) and adenosylcobalamin weakens the C-Co bond of the cofactor with respect to homolytic cleavage. To obtain information concerning the mechanism by which this is accomplished, a study was conducted in which optical and circular dichroism spectroscopy were used to explore the interaction between ethanolamine ammonia-lyase and a series of adenosylcobalamin analogs composed of an adenyl residue attached to the cobalt atom of cobalamin by a methylene chain whose length varies from 2 to 6 carbons. These studies indicated that the binding of a cobalamin to the active site activates forces which tend to alter the conformation of the enzyme, and with it that of the corrin ring, but that these conformational changes are blocked by bulky Co-β substituents which restrict corrin ring flexibility. We postulate that at least one element of the force which weakens the C-Co bond of the enzyme-bound cofactor is the relief of conformational strain which occurs when C-Co bond cleavage, by releasing the interfering adenosyl group, permits the enzyme and the corrin ring to assume the energetically favored conformation.

Introduction

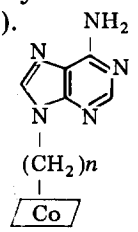
Ethanolamine ammonia-lyase (ethanolamine ammonia-lyase, EC 4.3.1.7) is an adenosylcobalamin-dependent enzyme from *Clostridium* sp. which catalyzes

the conversion of ethanolamine and propanolamine to NH_4^+ and the respective aldehydes [1,2]. An early step in the catalytic sequence is the homolysis of the carbon-cobalt bond of the cofactor [3,4]. The ability of ethanolamine ammonia-lyase to mediate this process indicates that the interaction between the enzyme and the cofactor reduces the energy of the C-Co bond, low to begin with [5], to the point where on the enzyme, the intact and homolyzed cofactor are in a near 1 to 1 equilibrium.

From the nature of the change induced in the cofactor by binding to the enzyme, it may be surmised that the enzyme applies a distorting force to the C-Co bond. From the results of experiments employing a series of adenosylcobalamin analogs in which the adenyl and corrin portions are separated by various distances, we have concluded that at least one element of this distorting force involves a change in the conformation of the enzyme induced by the binding of cobalamin to the active site, a change which forces a corresponding change in the conformation of the enzyme-bound corrin ring. In this paper, the evidence for these conclusions is presented, and a mechanism is proposed by which these conformational alterations (see Ref. 6) may facilitate C-Co cleavage.

Materials and Methods

Ethanolamine ammonia-lyase was purified and resolved of bound cobamides by the method of Kaplan and Stadtman [1]. Adenosylcobalamin was purchased from Sigma and purified by passage over carboxymethylcellulose as described elsewhere [7]. ω-Adeninylalkylcobalamins (I; n = 2–6).



(I)

were generously provided by Prof. H.P.C. Hogenkamp. Traces of contaminating adenosylcobalamin were eliminated by a 2 h incubation in 0.1 M NaCN, after which the analogs were reisolated by the usual phenol extraction procedure [8]. Visible spectra of the adeninylalkylcobalamins were unchanged by treatment with cyanide. Yeast alcohol dehydrogenase and NADH were obtained from Sigma. Other reagents were the best grade commercially available, and were used without further purification.

Absorption spectra were obtained on a Cary 118C recording spectrophotometer, using the microcell adapter when necessary. Circular dichroism spectroscopy was performed on a Cary 61 spectropolarimeter at room temperature. The slit widths were programmed to yield constant energy over the range of wavelengths examined. The instrument was calibrated using a standard optically active compound, (+)-10-camphorsulfonic acid, and an optically inactive compound, sodium dichromate. The latter assured that the instrument was free from absorbance artifacts at absorbances of 2.0 or less. A quartz cell with a pathlength of 1 cm was used. The data are expressed in terms of observed ellipticity (θ, degrees). Safelight conditions (Wratten 1A filter, 15 W bulb) were employed for all manipulation of samples prior to spectral studies to avoid

ambient light-catalyzed photolysis. Ethanolamine ammonia-lyase activity was measured spectrophotometrically as described by Kaplan and Stadtman [1]. Protein concentration was determined by the Lowry method [9] using bovine serum albumin as standard and applying the necessary correction [1]. Cobalamin concentrations were determined spectrophotometrically at 367 nm after photolysis and addition of NaCN, using 30.4 as the millimolar extinction coefficient for dicyanocobalamin [10].

Results

Binding and deactivation

Hogenkamp and associates found by inhibition studies that adeninylalkyl cobamides could bind to ribonucleotide reductase [11] and diol dehydrase [12] Inhibition studies reported here show that each of the analogs I (n = 2—6) was able to bind to ethanolamine ammonia-lyase as well. It appears, however, that two distinct types of interaction between the analog and this enzyme are possible. Analogs whose methylene chains are three or more carbons long induced a rapid first-order transformation of the enzyme into a much less active species, a process which has been observed before with many other coenzyme analogs and which has been termed 'deactivation' [7,13]. Apparent dissociation constants for the deactivating analogs, calculated from the rates of deactivation by approx. 1 μM analog at various concentrations of adenosylcobalamin, are shown in Table I. It is seen that the propyl analog binds with substantially lower affinity than the longer chain analogs, all of which have similarly low dissociation constants.

Different behavior was observed with adeninylethylcobalamin, which contains a two-carbon methylene bridge. Deactivation with this analog was much slower than with the other analogs, so slow, in fact, that the possibility of deactivation by a contaminant present at a concentration otherwise too low to detect cannot be ruled out. Binding to the enzyme, however, was unequivocal, since this analog acted as a powerful (K_i = 1.3 μM) inhibitor competitive with adenosylcobalamin.

Although all of the analogs associated with the enzyme, none exhibited

TABLE I

KINETIC PARAMETERS FOR DEACTIVATING ADENINYLALKYLCOBALAMINS

Ethanolamine ammonia-lyase activity was determined spectrophotometrically using 0.2 mM NADH and enzyme concentrations of 4—16 μg/ml. Each analog was studied at a single concentration, using adenosylcobalamin concentrations of 1.5, 4.0, 8.0, and 15 μM. Analog concentrations (μM) were: (ω-AdePr)cobalamin, 1.4; (ω-AdeBu)cobalamin, 1.4; (ω-AdePe)cobalamin, 0.8; (ω-AdeHx)cobalamin, 1.5. The K_{deact} values were taken as the Michaelis constants for the deactivation reactions, determined by plotting the reciprocals of the deactivation rate constants against adenosylcobalamin concentrations. Deactivation rate constants were determined as described elsewhere [6]. The x-intercepts of such plots are $-(1 + K_m/[analog])$.

Analog	N	K_{deact} (μM)
(ω-AdePr)cobalamin	3	10.4
(ω-AdeBu)cobalamin	4	0.7
(ω-AdePe)cobalamin	5	1.0
(ω-AdeHx)cobalamin	6	0.6

detectable cofactor activity. Moreover, all were stable in the presence of the enzyme for periods up to 30 min as determined spectrophotometrically.

Photolysis

When free in solution, the adeninylalkylcobalamins were all rapidly converted to hydroxocobalamin (plus uncharacterized products derived from the adeninylalkyl fragment) on exposure to light. In this respect they reacted like other alkylcobalamins, whose susceptibility to photolysis is well known. When bound to the enzyme, however, their behavior upon illumination was drastically altered (Fig. 1). The ethyl and propyl analogs yielded cob(II) alamin (identified by optical spectroscopy), not hydroxocobalamin; enzyme-bound adenosylcobalamin behaved similarly. The longer chain analogs appeared to be completely stable to photolysis under the experimental conditions, although exposure to light did cause an unexplained increase in the absorbance of the enzyme-bound cobamides. The resistance of the long-chain analogs to photolysis when bound to the enzyme was confirmed by treatment of the irradiated enzyme-analog complexes with trichloroacetic acid, a procedure that released (spectrally characterized) alkyl cobamides which then rapidly yielded hydroxocobalamin on further irradiation.

Circular dichroism spectroscopy

Evidence concerning the basis for the foregoing observations was provided by circular dichroism (CD) spectroscopy. The long-chain analogs (n = 4—6) all

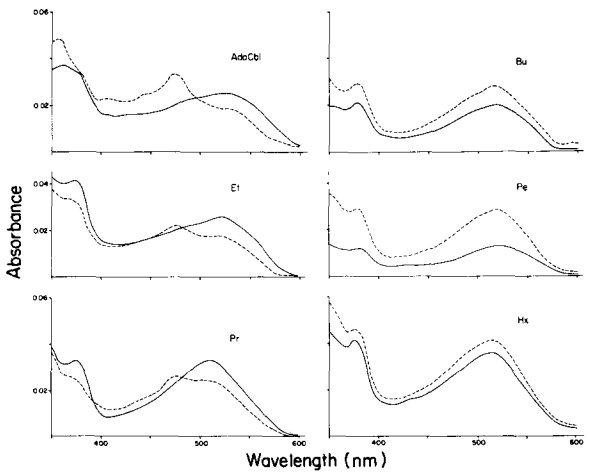


Fig. 1. Absorption spectra of enzyme-bound cobalamins before and after photolysis. Enzyme (0.78 nmol of active sites) and 1.45 μmol of potassium phosphate buffer (pH 7.4) in a total volume of 45 μl were placed in a microcell, and a spectrum was taken. Cobalamin (0.5 nmol of adeninylalkyl cobalamin or 0.4 nmol of adenosylcobalamin) in 5 μl of water was then added to the microcell, and the spectrum repeated immediately and after 5 min. No change was noted in this interval. The samples were then irradiated for 25 min (55 min, in the case of (ω-AdePr)cobalamin) with a 40 W blue-white fluorescent lamp at a distance of 8 cm, after which the spectra were repeated. The spectra shown were constructed by subtracting the spectrum of the enzyme alone from that of enzyme plus cobalamin. In this calculation, no correction was made for the slight fall in enzyme concentration due to dilution by the added cobalamin. ———, before photolysis; - - - - -, after photolysis.

showed very similar CD spectra, all of which underwent the same pronounced changes on binding of the analogs to the enzyme (Fig. 2). These changes were characterized by large shifts in amplitudes and peak positions throughout the region between 350 and 600 nm. Since circular dichroism reflects the optical asymmetry of the transitions responsible for light absorption, which in this region of the spectrum are transitions involving the π-electrons of the corrin ring, and since optical asymmetry reflects the stereochemical asymmetry of the absorbing species, the changes in circular dichroism noted in these experiments strongly suggest a change in the conformation of the corrin ring on binding to the enzyme. Conversely, the similarity between the spectra of free and enzyme-bound (ω-AdeEt)cobalamin suggests that rather little change in the conformation of the corrin ring accompanies the binding of this analog to the enzyme.

The present studies also showed that the CD spectrum of adenosylcobalamin underwent little change on binding to ethanolamine ammonia-lyase, a finding in agreement with previously reported work [14]. In this respect, the binding of (ω-AdeEt)cobalamin to the enzyme is similar to that of adenosylcobalamin. There is in addition a remarkable resemblance between enzyme-bound (ω-AdeEt)cobalamin and adenosylcobalamin with regard to the CD spectra themselves. Both show peaks of the same relative size in the vicinity of 385 and 485 nm, and a trough at 430 nm which is deeper in the spectrum of adenosylcobalamin than in that of (ω-AdeEt)cobalamin. In contrast, the CD spectra of enzyme-bound long-chain analogs are rather different from that of adenosylcobalamin (Fig. 2). In particular, the bands between 400 and 500 nm which are

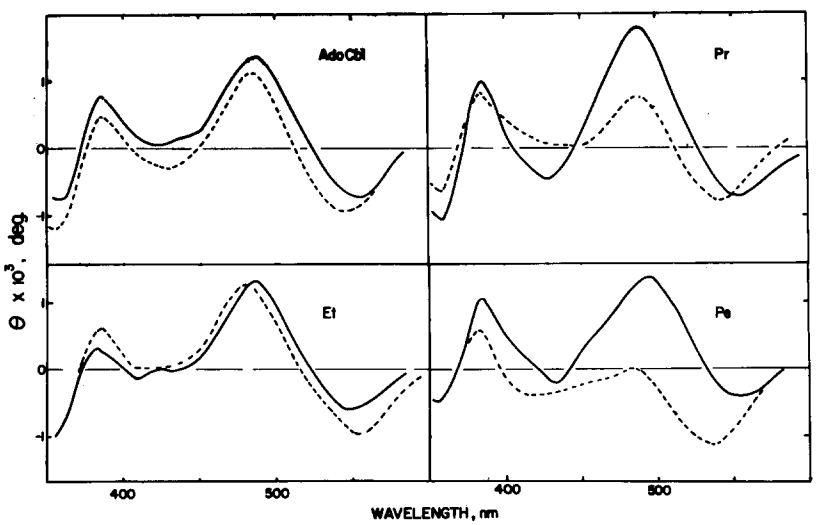


Fig. 2. Circular dichroism spectra of free and enzyme-bound cobalamins. Cobalamin (8.0 nmol), 8.0 μmol potassium phosphate buffer (pH 7.4) and where indicated, 8.6 nmol of enzyme (active sites) in a total volume of 1.0 ml were placed in a cylindrical quartz cuvette with a 1 cm pathlength. Spectroscopy required 10 min per sample; photolysis was negligible during this time. Reference CD spectra were obtained with 10 mM potassium phosphate buffer (pH 7.4). The spectra shown represent the difference between the sample and reference spectra. The long-chain analog spectrum is that of (ω-AdePe)cobalamin; the spectra of the other two long-chain analogs were indistinguishable from this spectrum. ———, no enzyme; - - - - - -, enzyme present.

seen in the CD spectrum of adenosylcobalamin are missing from the long-chain spectra. To the extent that CD spectroscopy provides stereochemical information about cobalamin derivatives, these observations suggest the following simplified interpretation. The corrin ring seems to assume one of two different conformations on binding to the enzyme: that characteristic of the long-chain analogs, or that characteristic of adenosylcobalamin and (ω-AdeEt)cobalamin. The conformation of the corrin ring of enzyme-bound long-chain analogs is different from that of the free analogs. However, the free and enzyme-bound conformations of adenosylcobalamin and (ω-AdeEt)cobalamin are substantially the same.

Discussion

The present findings suggest that the binding of cobalamin to the active site of ethanolamine ammonia-lyase activates forces which tend to induce a change in the conformation of the enzyme. With most cobamides, binding results in deactivation of the enzyme [12]. With the long-chain (deactivating) adeninylalkyl cobalamins, and presumably other deactivating cobalamins as well, binding also results in an adjustment in the conformation of the corrin ring. However, there are certain cobamides, including coenzymically active cobalamins (adenosylcobalamin and 2′-deoxyadenosylcobalamin) as well as uridinylcobalamin and (ω-AdeEt)cobalamin, which have been found to cause little or no deactivation when they associate with the enzyme [13]. Spectra presented here shows that at least two of these non-deactivating cobamides, adenosylcobalamin and (ω-AdeEt)cobalamin, undergo little alteration in corrin ring conformation when they bind to the enzyme, not a surprising feature in view of the steric constraints which are likely to be imposed on the ring by the β-alkyl groups in these two compounds. These findings suggest that deactivation is a consequence of a corrin-induced change in the conformation of the enzyme, a conformational alteration permitted by the flexibility of the corrin ring, which can undergo conformational alterations of its own to accomodate those taking place at the corrin binding site. Cobamides whose rings have little conformational flexibility are also able to associate with the corrin binding site, presumably activating forces which drive the conformational change. However, the enzyme's own mobility would be restricted by the rigid structure attached to the corrin binding site, so that it could not achieve the induced conformation and consequently retains its own original activity.

With the conversion of the enzyme to the induced conformation blocked by an inflexible ligand, the forces which under other circumstances would induce this conformational change may be exerting their effects along other lines. Evidence for this is found in the results of the photolysis experiments. The long-chain analogs, in which the conformational alteration of the corrin ring can reach completion, are stable to light when bound to the enzyme, while complexes containing cobamides whose corrin ring flexibility is hindered all produce cob(II)alamin when illuminated. Our interpretation of this finding is that in the case of the long-chain analogs, there is no force to separate the fragments produced by the light-induced homolysis of the C-Co bond because the enzyme has already undergone a conformational transition and is, in a sense,

relaxed. These fragments therefore always recombine and photolysis is a virtual process. Observable photolysis would take place when the factors which prevent the enzyme from relaxing, i.e., the steric constraints on the conformational flexibility of the corrin ring, are eliminated by the dissociation of the C-Co bond. With the loss of these constraints the enzyme can change conformation in such a way as to separate the photolyzed fragments so they cannot recombine. Presumably, this displacement could involve the physical separation of the alkyl fragment from the cob(II)alamin, a conformational change involving the corrin ring, or both. In any case, these findings suggest that the enzyme exerts a force on conformationally rigid adenyl cobamides which tends to separate the corrin ring from the β-alkyl group.

These same considerations may apply to the thermal (i.e., dark) dissociation of the C-Co bond by adenosylcobamide-requiring enzymes. Homolysis of this bond is an essential step in catalysis by these enzymes [3,4,15—17]. In addition, they can split the C-Co bond of a variety of alkyl cobamides, most of which have no cofactor activity, even in the absence of substrate [17]. The common structural feature of these enzyme-dissociable cobamides is a Co-β substituent consisting of an aldopentofuranosyl nucleoside, a bulky group which almost certainly imposes constraints on the flexibility of the corrin ring, and consequently on the ability of the enzyme-cobamide complex to relax to the induced conformation. It is likely that this structural splinting produces strains on the complex which are relieved when the nucleosidylcobalamin dissociates to the flexible cob(II)alamin. It is possible that the relief of this conformational strain constitutes one of the driving forces for the cleavage of the C-Co bond of enzyme-bound nucleosidylcobalamins.

Acknowledgements

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