difference is that the asparagine backbone amide exchanges protons with solvent 2 pH units higher in Ac-VNGA than in VNGA. This suggests that the asparagine backbone amide does not interact with the alanine carboxyl group as proposed for VNGA.

In conclusion, our results suggest that the deamidation of -NG- sequences in peptides is independent of general base catalysis by solvent buffer but is dependent on the conformation of the peptide backbone. This suggests that -NG- sequences in proteins will not all undergo deamidation at the same rate but will deamidate at rates which are controlled by the conformation of the segment of the protein containing the -NG- sequence. Our results also show that proteins which have a penultimate asparagine can undergo, at neutral pH, a deamidation reaction involving the amino terminus to form a cyclic amide. The driving force for this reaction to occur in VNGA appears to be the interaction of the negatively charged carboxyl group on the C-terminus with the positively charged N-terminus.

REFERENCES

Aswad, D. W. (1984) *J. Biol. Chem. 259*, 10714–10721.
Battersby, A. R., Robinson, J. C. (1955) *J. Chem. Soc.*, 259–269.
Breitmaier, E., & Voelter, W. (1987) *Carbon-13 NMR Spectroscopy*, 3rd ed., pp 415–420, VCH, Weinheim, FDR.
Bundi, A., & Wuthrich, K. (1979a) *Biopolymers 18*, 285–297.
Bundi, A., & Wuthrich, K. (1979b) *Biopolymers 18*, 299–311.
Bystrov, V. F., Ivanov, V. T., Portnova, S. L., Balashova, T. A., & Ovchinnikov, Yu. A. (1973) *Tetrahedron 29*, 873–877.
Geiger, T., & Clarke, S. (1987) *J. Biol. Chem. 262*, 785–794.
Haley, E. E., Corcoran, B. J., Dorer, F. E., & Buchanan, D. L. (1966) *Biochemistry 5*, 3229–3235.
Jornvall, H. (1974) *FEBS Lett. 38*, 329–333.
Kopple, K. D., Wiley, C. R., & Tauke, R. (1973) *Biopolymers 12*, 627–636.
Martini, F., Angelaccio, S., Pascarella, S., Barra, D., Bossa, F., & Schirch, V. (1987) *J. Biol. Chem. 262*, 5499–5509.
Meinwald, Y. C., Stimson, E., & Scheraga, H. A. (1986) *Int. J. Pept. Protein Res. 28*, 79–84.
Midelfort, C. F., & Mehler, A. H. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1816–1819.
Murray, E. D., Jr., & Clarke, S. (1984) *J. Biol. Chem. 259*, 10722–10732.
Robinson, A. B., & Rudd, C. J. (1974) *Curr. Top. Cell. Regul. 8*, 247–295.
Sandheimer, E., & Holley, R. W. (1954) *J. Am. Chem. Soc. 76*, 2467–2470.
Schirch, L., Gavilanes, F., Peterson, D., Bullis, B., Barra, D., & Bossa, F. (1984) *Chemical and Biological Aspects of Vitamin B6 Catalysis: Part A*, pp 301–388, Alan Liss, New York.
Schirch, V., Schirch, D., Martini, F., & Bossa, F. (1986) *Eur. J. Biochem. 161*, 45–49.
Stewart, J. M., & Young, J. D. (1969) *Solid Phase Peptide Synthesis*, W. H. Freeman, San Francisco.
Takio, K., Blumenthal, D. K., Walsh, K. A., Titani, K., & Krebs, E. G. (1986) *Biochemistry 25*, 8049–8057.
Yuksel, K. U., & Gracy, R. W. (1986) *Arch. Biochem. Biophys. 248*, 452–459.

Acceleration of Cleavage of the Carbon–Cobalt Bond of Sterically Hindered Alkylcobalamins by Binding to Apoprotein of Diol Dehydrase

Tetsuo Toraya* and Atsuhiko Ishida

Department of Chemistry, College of Liberal Arts and Sciences, Kyoto University, Sakyo-Ku, Kyoto 606, Japan

ABSTRACT: Cleavage of the C–Co bond of sterically hindered alkylcobalamins bearing neither an adenine moiety nor functional groups, such as isobutylcobalamin, neopentylcobalamin, and cyclohexylcobalamin, was markedly accelerated by their interaction with apoprotein of diol dehydrase, although these cobalamins do not function as coenzyme. Acceleration of the conversion of alkylcobalamins to enzyme-bound hydroxocobalamin was stoichiometric and obeyed first-order reaction kinetics. These results, together with strong competitive inhibition by these alkylcobalamins with respect to adenosylcobalamin, indicate that acceleration of the C–Co bond cleavage by the apoenzyme is due to labilization of their C–Co bond by binding to the active site of the enzyme. This labilization is considered to be caused by a steric distortion of the corrin ring which is induced by specific tight interaction of the cobalamin moiety with apoprotein. The importance of such a labilizing effect for activation of the C–Co bond of adenosylcobalamin in enzymatic reactions is discussed.

Homolytic cleavage of the C–Co bond of AdoCbl[1] is an essential early event in the AdoCbl-dependent enzymatic re-

actions involving diol dehydrase. Since the C–Co bond of AdoCbl is sufficiently weak (homolytic bond dissociation energy ca. 26 kcal/mol) (Halpern et al., 1984), only a modest additional labilization by interaction with apoprotein would be required for homolysis. That is, the apoenzyme elicits the function of the coenzyme by activating (labilizing) this organometallic bond.

During the course of our studies addressed to know the mechanism of activation of the C–Co bond of AdoCbl in

---

[1] Abbreviations: AdoCbl, adenosylcobalamin or vitamin B_{12} coenzyme; CN-Cbl, cyanocobalamin; aqCbl, aquacobalamin; OH-Cbl, hydroxocobalamin; MeCbl, methylcobalamin; neoPeCbl, neopentylcobalamin; iBuCbl, isobutylcobalamin; 2-MeBuCbl, [(*S*)-2-methylbutyl]cobalamin; cPrCbl, cyclopropylcobalamin; cHxCbl, cyclohexylcobalamin; Cbi, cobinamide.

enzymatic reactions, we have demonstrated with diol dehydrase that the interactions of the coenzyme with the apoprotein at the adenosyl group (Toraya et al., 1977, 1986, 1987; Toraya & Fukui, 1980, 1982; Ushio et al., 1984) as well as at the peripheral amide side chains of the corrin ring (Toraya & Abeles, 1980; Toraya et al., 1979) are essential for activation of the C–Co bond and therefore for catalysis. We have reported evidence for the presence of the adenosyl group binding site in diol dehydrase apoprotein (Toraya, 1985) and shown that the specific tight interaction of the adenine moiety of the coenzyme with its binding site in the enzyme (adenine-attracting effect) is essential for labilization of the C–Co bond. It seems likely that distortions, e.g., a kind of tensile force or angular strain, between the adenine part and the cobalt atom are produced by such interaction, which is at least one element of the force that weakens the C–Co bond (Toraya et al., 1986, 1987). $\Delta G°$ upon binding of the adenosyl group of the coenzyme to the enzyme at 37 °C, estimated from the K_D value for 5′-deoxyadenosine, was approximately –6.6 kcal/mol (Toraya et al., 1986), indicating that the interaction between the adenosyl group of AdoCbl and its binding site in the enzyme is not very strong. Therefore, basal labilization (decrease in bond dissociation energy) of the C–Co bond of the coenzyme through interaction with apoprotein at the cobalamin moiety seems to be needed in the enzymatic reactions. From spectroscopic and mechanistic evaluation of coenzyme analogues with modifications in peripheral amide side chains of the corrin ring, Toraya et al. (1979) have suggested that steric distortion of the corrin ring could be brought about by interactions of the side chains with the apoprotein, which would facilitate the C–Co bond cleavage. The distortion of the corrin ring of cobalamins upon binding to apoprotein is also suggested from changes in optical and CD spectra (Krouwer et al., 1980; Toraya et al., 1983). Considerable speculation has been accumulated from X-ray structural determinations (Brink-Shoemaker et al., 1964; Pett et al., 1987; Lenhert, 1968; Glusker, 1982) and nonenzymatic model studies (Bresciani-Pahor et al., 1982; Chemaly & Pratt, 1980a,b; Grate & Schrauzer, 1979; Halpern, 1982, 1983, 1985; Marzilli et al., 1979; Pratt, 1982, 1984, 1985; Randaccio et al., 1981; Schrauzer & Grate, 1981) that steric distortions are important for labilization of the C–Co bond.

In the present paper, we report that cleavage of the C–Co bond of intrinsically unstable alkylcobalamins bearing neither adenine part nor functional groups is accelerated by interaction with apoprotein. This suggests the validity of basal labilization mechanism through interaction at the cobalamin moiety in enzymatic processes.

MATERIALS AND METHODS

Materials. Alkylcobalamins used (Figure 1) were synthesized by reaction of the corresponding alkyl bromide with cob(I)alamin, which was formed by reduction of CN-Cbl or aqCbl with NaBH₄ for 20 min in the presence of equimolar $CoCl_2$ under a nitrogen atmosphere. Relatively stable alkylcobalamins, such as iBuCbl, 2-MeBuCbl, and cPrCbl (Chemaly & Pratt, 1980a; Grate & Schrauzer, 1979; Schrauzer & Grate, 1981), were synthesized from CN-Cbl and purified to homogeneity by phenol extraction, phosphocellulose (pH 3 and 6) column chromatography, and thin-layer chromatography on silica gel. Purified preparations of these cobalamins were stored in 0.001 N H_2SO_4 in the freezer, since they are stable in the protonated base-off form. neoPeCbl and cHxCbl (Chemaly & Pratt, 1980a,b; Grate & Schrauzer, 1979; Schrauzer & Grate, 1981), both of which are labile in the presence of oxygen at neutral pH, were synthesized in situ
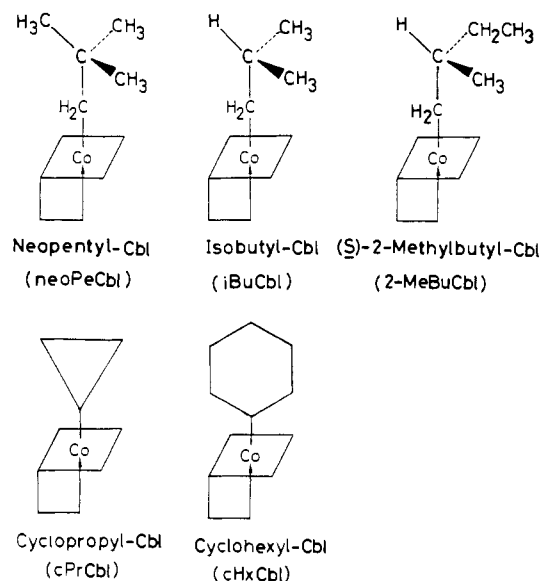

FIGURE 1: Partial structures of sterically hindered alkylcobalamins used in this study.

as follows: aqCbl was used as a starting material, and the alkylcobalamins formed were withdrawn by a syringe and used without purification (Chemaly & Pratt, 1980a,b). NaBH₄ was used as a reductant, since the conversion of aqCbl to neoPeCbl was better with NaBH₄ than with zinc/acetic acid (1 M) as a reducing system. AdoCbl and MeCbl were a gift from Eisai Co., Ltd., Tokyo, Japan. CN-Cbl was obtained from Glaxo Laboratories Ltd., Greenford, England. aqCbl was obtained by photolysis of MeCbl. All other chemicals were reagent-grade commercial products.

Diol Dehydrase. The apoprotein of diol dehydrase was purified as described before (Poznanskaja et al., 1979) from cells of *Klebsiella pneumoniae* ATCC 8724 (formerly *Aerobacter aerogenes*). The bacterium was grown without aeration in a complex glycerol-1,2-propanediol medium as described by Abeles (1966), except that 0.2% polypeptone (Daigo-Eiyou, Osaka, Japan) was used instead of 0.2% yeast extract. A highly purified preparation of the enzyme with a specific activity more than 40 units/mg of protein was used throughout this study. The molar concentration of the enzyme was calculated on the basis of a molecular weight of 230 000 (Essenberg et al., 1971; Poznanskaja et al., 1979).

Analytical Procedures. The diol dehydrase activity was measured by the 3-methyl-2-benzothiazolinone hydrazone method (Toraya et al., 1977). Protein concentration for purified diol dehydrase was determined by measurements of the absorbance at 278 nm. An extinction coefficient of 5.27 for 10 mg of diol dehydrase/mL and for a 1-cm light path was used (Poznanskaja et al., 1979).

The concentration of corrinoids was determined spectrophotometrically by measuring the absorbance at 367 nm after conversion into the dicyano form. Alkylcobalamins were converted into dicyanocobalamin by photolysis in the presence of 0.1 M KCN. ϵ_{367} for dicyanocobalamin is 30.4×10^3 M⁻¹ cm⁻¹ (Barker et al., 1960).

Optical spectra were measured on a Union Model SM-401 recording spectrophotometer.

RESULTS

Acceleration of the C–Co Bond Cleavage by Diol Dehydrase. From the spectral changes illustrated in Figure 2C,D, it was demonstrated that iBuCbl was gradually converted to enzyme-bound OH-Cbl in the presence of apodiol dehyrase,
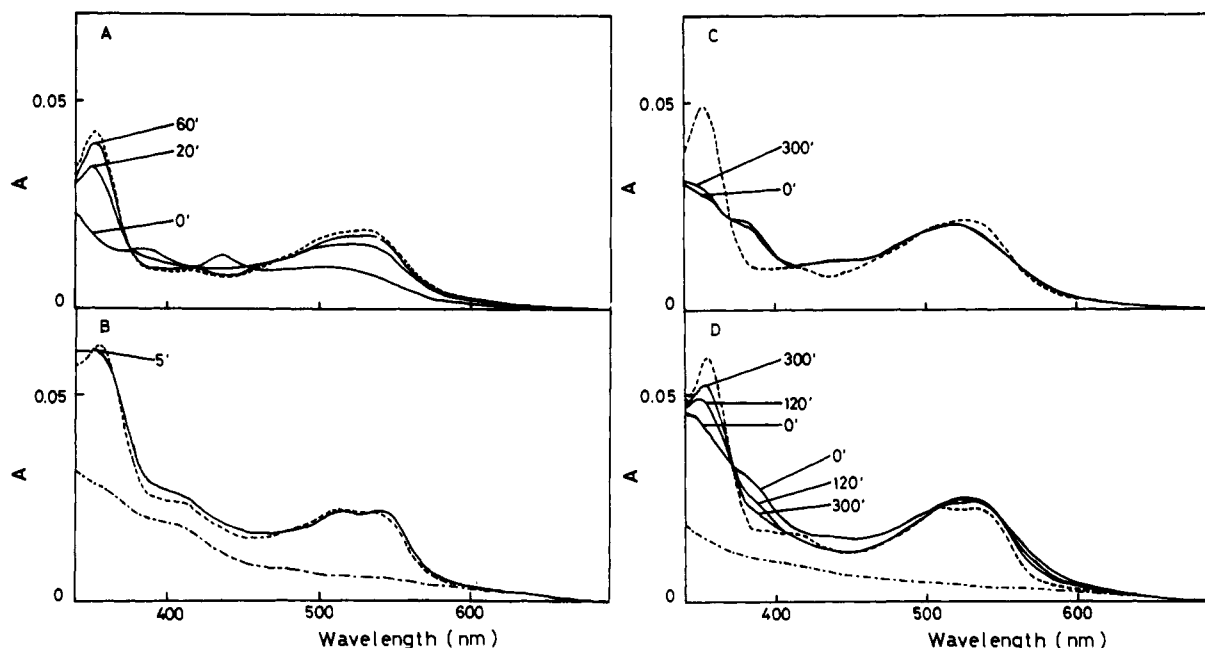
FIGURE 2: Spectral changes observed during incubation of neoPeCbl and iBuCbl with apodiol dehydrase. Spectra are corrected for dilution. neoPeCbl (2.1 μM) (A, B) or iBuCbl (2.1 μM) (C, D) was incubated at 37 °C for the indicated time periods with (B, D) or without (A, C) apoenzyme (49 units/mL, 3.1 μM) in 0.045 M potassium phosphate buffer (pH 8.0) containing 0.14 M 1,2-propanediol (—). Photoirradiation was then carried out until the spectrum no longer changed (---). Bottom spectrum shows an apoenzyme control (–·–).

Table I: Effect of Apoenzyme on the Rate of the C–Co Bond Cleavage of Sterically Hindered Alkylcobalamins[a]

	$t_{1/2}$ at 37 °C		acceleration
alkylcobalamin	–apoenzyme	+apoenzyme	(x-fold)
neoPeCbl	14 min	1.5 min	9.3
iBuCbl	34 h	3.7 h	9.2
cHxCbl	22 min	11 min	2.0
2-MeBuCbl	30 h	26 h	1.1
cPrCbl	b	b	

[a] Each alkylcobalamin was incubated at 37 °C for appropriate time periods in the presence and absence of apoenzyme under the conditions described in Figure 2. The percentage of alkylcobalamins remaining was determined from the change of absorbance at 356 nm. $t_{1/2}$ was calculated by a plot of the logarithm of the percentage of alkylcobalamins remaining against time of incubation. [b] No decomposition for at least 5 h. $t_{1/2}$ without enzyme has been reported to be ca. 6 months (Grate & Schrauzer, 1979).

although it was essentially stable in the absence of the enzyme ($t_{1/2}$ 34 h). This cobalamin was totally inactive as a coenzyme (data not shown). When the extent of cleavage of the C–Co bond was determined from the increase of absorbance at 356 nm, the conversion of iBuCbl to OH-Cbl obeyed first-order reaction kinetics with a half-life ($t_{1/2}$) of 3.7 h at 37 °C. Thus, it is clear that C–Co bond cleavage of this coenzymically inactive cobalamin was 9.3 times accelerated by the interaction with apoprotein. neoPeCbl, a sterically more hindered primary alkylcobalamin, decomposed spontaneously to aqCbl under the same conditions even in the absence of apoenzyme ($t_{1/2}$ 14 min), but the C–Co bond cleavage was at least 9.2 times accelerated by the presence of apoenzyme (Figure 2A,B). Such acceleration of the C–Co bond cleavage was not observed at all when bovine serum albumin (2 mg/mL) was used instead of apoprotein (data not shown).

Similar experiments were performed with the other three cobalamins shown in Figure 1. The results are given in Table I. cHxCbl, an unstable secondary alkylcobalamin, also underwent acceleration of C–Co bond cleavage by the apoenzyme, although the degree of acceleration was rather small. Apparently, no labilizing effect of the apoenzyme was observed with cPrCbl and 2-MeBuCbl. The former seems too stable
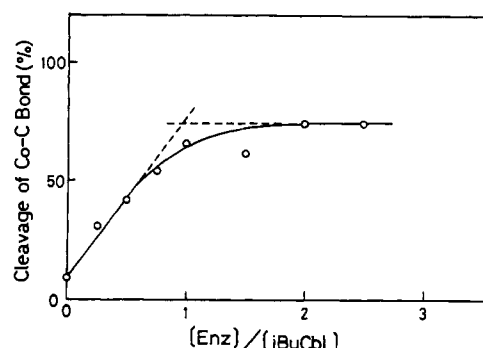

FIGURE 3: Effect of enzyme concentration on the extent of the C–Co bond cleavage of iBuCbl. The experimental conditions are the same as those described in Figure 2D, except that the indicated amounts of apoenzyme were used. The extent of cleavage of the C–Co bond was determined from the increase of absorbance at 356 nm after 5 h of incubation at 37 °C. Enz, apoenzyme.

to detect its decomposition by the enzyme.

Stoichiometry of the C–Co Bond Cleavage. As shown in Figure 3, the extent of apoenzyme-accelerated cleavage of the C–Co bond of iBuCbl, determined after incubation with apoprotein at 37 °C for 5 h, was proportional to the [enzyme]/[iBuCbl] ratio in the range from 0 to 0.75 and reached a maximum when the concentration of apoenzyme was equal to that of iBuCbl. Therefore, it is evident that the conversion of this alkylcobalamin to OH-Cbl by the apoenzyme is not catalytic but stoichiometric. Since diol dehydrase possesses one coenzyme binding site per molecule (Essenberg et al., 1971; Toraya, 1985), this result suggests strongly that acceleration of the C–Co bond cleavage by apodiol dehydrase is due to labilization of the C–Co bond of alkylcobalamins by binding to the active site of the enzyme.

Competition of Alkylcobalamins with AdoCbl for Binding at the Active Site of the Enzyme. All the alkylcobalamins used in this study were quite inactive as coenzymes in the diol dehydrase system. So far as tested with purified preparations of iBuCbl, 2-MeBuCbl, and cPrCbl, these alkylcobalamins behaved as strong competitive inhibitors with respect to AdoCbl. Apparent K_i values for iBuCbl, 2-MeBuCbl, and
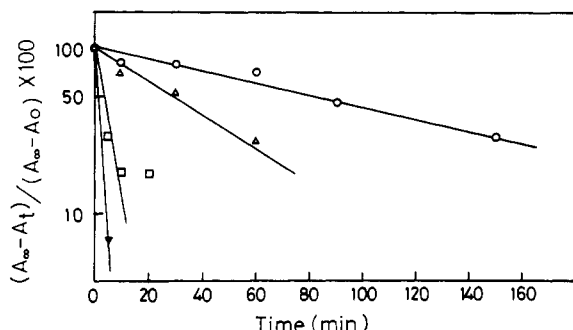
FIGURE 4: Photosensitivity of the C–Co bond of enzyme-bound alkylcobalamins. The enzyme–alkylcobalamin complexes were formed by incubation of apoenzyme (50 units/mL, 3.1 μM) with 2.0 μM each alkylcobalamin at 37 °C for 30 min in 0.040 M potassium phosphate buffer (pH 8.0) containing 0.14 M 1,2-propanediol. Photoirradiation was carried out for the indicated time periods in the ice–water bath with a 200-W tungsten light bulb from the distance of 20 cm. A_0, A_t, and A_∞ are absorbance at 356 nm at the time points of 0, t, and ∞ (irradiated until the spectrum no longer changed). (O) Enzyme-2-MeBuCbl complex (86 min); ($\triangle$) enzyme–iBuCbl complex (30 min); ($\square$) enzyme–cPrCbl complex (<4 min); ($\blacktriangledown$) enzyme–MeCbl complex (<2 min). The $t_{1/2}$ values of enzyme-bound alkylcobalamins upon photolysis are given in parentheses.

cPrCbl, determined by the conventional method, were 0.41, 0.36, and 0.30 μM, respectively. Compared with a K_m of 0.7 μM for AdoCbl, the data indicate that these alkylcobalamins are accommodated in the coenzyme binding site of the enzyme with very high affinity.

Resistance to Photolysis. Although the C–Co bond of 2-MeBuCbl is intrinsically more labile than that of iBuCbl, its cleavage was apparently not accelerated by the apoenzyme. This exception may be due to rapid recombination of the initial products formed from cobalamin and alkyl moieties upon cleavage of the C–Co bond. If this is the case, an alkyl group derived product must be retained by the enzyme near the cobalt atom of the cobalamin moiety. In order to test this possibility, the photostability of the enzyme-bound alkylcobalamins was compared. As shown in Figure 4, the enzyme-bound 2-MeBuCbl and iBuCbl were markedly resistant to photolysis, although the free counterparts were as photosensitive as other alkylcobalamins. As expected, the enzyme-bound 2-MeBuCbl was more resistant to photolysis than iBuCbl. In contrast, the enzyme-bound MeCbl and cPrCbl were photosensitive like free counterparts. These results suggest that the alkyl group derived radicals formed from 2-MeBuCbl and iBuCbl upon photolysis are kept near the cobalamin moiety and rapidly recombine with cob(II)alamin to regenerate the C–Co bond (Endicott & Ferraudi, 1977; Endicott & Netzel, 1979). Therefore, by analogy, it is likely that 2-MeBuCbl also undergoes labilization and cleavage of its C–Co bond by binding to the apoenzyme, but acceleration of the cleavage is apparently not observed because of the rapid recombination of the cobalamin and the alkyl group derived products formed. In this context, the actual degree of acceleration of the C–Co bond cleavage of alkylcobalamins by the apoenzyme is at present very difficult to estimate quantitatively but is probably much larger than observed (Table I).

DISCUSSION

It is first demonstrated here that through interaction with the cobalamin moiety apoprotein labilizes the C–Co bond of alkylcobalamins that contain neither an adenine moiety nor functional groups in the Coβ ligand. This labilizing effect results in acceleration of the C–Co bond cleavage of intrinsically unstable, sterically hindered alkylcobalamins, such as

iBuCbl, neoPeCbl, and cHxCbl. 2-MeBuCbl is exceptional, but its apparent lack of acceleration may be accounted for by rapid recombination of the cobalamin and the alkyl group derived products formed (Endicott & Ferraudi, 1977; Endicott & Netzel, 1979). By taking the recombination into consideration, the actual degree of acceleration of the C–Co bond cleavage of these alkylcobalamins may be larger than observed.

From the oxygen sensitivity of neoPeCbl, the equilibrium Co–R $\rightleftharpoons$ Co^{II} + R$^\bullet$ has been postulated with free neoPeCbl by other investigators (Chemaly & Pratt, 1980b; Schrauzer & Grate, 1981). There is a possibility that the apoenzyme accelerates dealkylation simply by shifting the equilibrium toward decomposition through trapping cob(II)alamin. However, the spectrum obtained here was that of OH-Cbl, although the enzyme-bound cob(II)alamin is stable to atmospheric oxygen (Toraya, 1985). Thus, the above-mentioned possibility seems unlikely, and the observed acceleration of the decomposition of neoPeCbl should result from labilization of the C–Co bond of the enzyme-bound one.

How does the enzyme labilize the C–Co bond of alkylcobalamins through interaction with cobalamin moiety? There is no answer yet obtained from enzymatic studies. Therefore, we have to consult the results obtained by the X-ray structural analysis of corrinoids as well as by nonenzymatic model studies. X-ray diffraction studies have revealed that in AdoCbl the C–Co bond is quite long (2.05 Å) and the Co–C–C bond angle (125°) is much larger than the tetrahedral angle (Brink-Shoemaker et al., 1964; Lenhert, 1968), apparently reflecting steric crowding and repulsions between the adenosyl group and the corrin ring with its substituents. Furthermore, it has been demonstrated that the flexible corrin ring in cobalamins is upward bent by coordination of the 5,6-dimethylbenzimidazole moiety in the nucleotide loop, a very bulky base, to the cobalt atom (Glusker, 1982; Pett et al., 1987). When a bulky Coβ ligand, such as the adenosyl group, binds to the cobalt atom, the upward bending of the corrin ring becomes less, probably because of balance of the two opposing steric demands. The importance of the sterically unfavorable upward distortion in labilization of the C–Co bond has been pointed out from model studies by several groups (Bresciani-Pahor et al., 1982; Grate & Schrauzer, 1979; Halpern, 1982, 1983, 1985; Marzilli et al., 1979; Randaccio et al., 1981; Schrauzer & Grate, 1981). Sterically hindered primary and secondary alkylcobalamins in neutral solution are reported to decompose rapidly under aerobic conditions, although they are fairly stable in acidic solution (Chemaly & Pratt, 1980a,b; Grate & Schrauzer, 1979; Schrauzer & Grate, 1981). Corresponding alkylcobinamides are as stable as the base-off alkylcobalamins in both acidic and neutral solutions, and the addition of heterocyclic bases markedly increases the rate of decomposition of neoPeCbi (Chemaly & Pratt, 1980b) and iPrCbi (Grate & Schrauzer, 1979). Therefore, upward distortion of the corrin ring is believed to be induced by the attachment of the axial base, 5,6-dimethylbenzimidazole, to the cobalt atom, and increased steric repulsion between a bulky Coβ ligand and the substituents on the corrin ring could bring about cleavage of the C–Co bond. Pratt and his co-worker have described that steric compression around C$_\alpha$ in the base-on, six-coordinate complex leads to distortion of the Co–C–C bond angle and lengthening of the C–Co bond and that this is the major factor which increases the lability of the C–Co bond (Chemaly & Pratt, 1980a,b; Pratt, 1982, 1984, 1985). He has speculated that such steric distortion is induced by a substrate-induced conformational change of the enzyme and ascribed the absorption peak at ~440 nm to such a highly distorted, unstable

state. He has explained that the stimulating effect of heterocyclic bases on decomposition of sterically hindered alkylcobinamides is due to the change from five- to six-coordination and not to any changes in the conformation of the corrin ring. He has suggested that the coordination of a heterocyclic base is less important for the mechanism of action of this coenzyme.

From the enzymological study, Toraya et al. (1979) have demonstrated that modification of the propionamide side chains on the periphery of the corrin ring leads to considerable variation in coenzymic activity, depending upon the types and positions of modification. Since all of the side chain analogues tested still bind tightly to the apoenzyme, the variation in activity suggests that, in addition to the adenine-attracting effect (Toraya et al., 1986, 1987), there is another labilizing effect of apoprotein on the C–Co bond that is based on the specific interactions between enzyme and peripheral amide side chains. We believe that this labilization is caused by the upward distortion of the corrin ring. Changes in optical and CD spectra observed with several cobalamins upon binding to apodiol dehydrase (Toraya et al., 1983) and apoethanolamine ammonia-lyase (Krouwer et al., 1980) may reflect the distortion-induced changes in the electronic structure of the corrin ring. The results reported in this paper provide evidence for such labilization mechanism. With neoPeCbl or iBuCbl, this effect is presumably strong enough to break the C–Co bond, whereas the effect alone is not enough strong to cleave the C–Co bond of stable alkylcobalamins, such as MeCbl and cPrCbl.

In the case of AdoCbl, in addition to such basal labilization mechanism, the adenine-attracting effect of apoprotein would produce a kind of tensile force or angular strain between the adenine part and the cobalt atom (Toraya et al., 1986, 1987). This may include lengthening of the C–Co bond and distortion of the Co–C–C bond angle, as proposed by Pratt (1982, 1984, 1985). Thus, we conclude that two kinds of labilizing effects are exerted by apoprotein through the specific tight interactions at the cobalamin moiety and at the adenosyl group and that they operate in a concerted manner on activation and cleavage of the C–Co bond of this coenzyme during enzymatic reactions.

It is currently being investigated whether the 5,6-dimethylbenzimidazole moiety of the nucleotide loop contributes to the upward distortion of the corrin ring at the active site of the enzyme or not.

REFERENCES

Abeles, R. H. (1966) *Methods Enzymol. 9*, 686–689.

Barker, H. A., Smyth, R. D., Weissbach, H., Munch-Petersen, A., Toohey, J. I., Ladd, J. N., Volcani, B. E., & Wilson, M. R. (1960) *J. Biol. Chem. 235*, 181–190.

Bresciani-Pahor, N., Calligaris, M., Nardin, G., & Randaccio, L. (1982) *J. Chem. Soc., Dalton Trans.*, 2549–2551.

Brink-Shoemaker, C., Cruikshank, D. W. J., Hodgkin, D. C., Kamper, M. J., & Pilling, D. (1964) *Proc. R. Soc. London, A 278*, 1–26.

Chemaly, S. M., & Pratt, J. M. (1980a) *J. Chem. Soc., Dalton Trans.*, 2259–2266.

Chemaly, S. M., & Pratt, J. M. (1980b) *J. Chem. Soc., Dalton Trans.*, 2274–2281.

Endicott, J. F., & Ferraudi, G. J. (1977) *J. Am. Chem. Soc. 99*, 243–245.

Endicott, J. F., & Netzel, T. L. (1979) *J. Am. Chem. Soc. 101*, 4000–4002.

Essenberg, M. K., Frey, P. A., & Abeles, R. H. (1971) *J. Am. Chem. Soc. 93*, 1242–1251.

Glusker, J. P. (1982) in B_{12} (Dolphin, D., Ed.) Vol. 1, pp 23–106, Wiley, New York.

Grate, J. H., & Schrauzer, G. N. (1979) *J. Am. Chem. Soc. 101*, 4601–4611.

Halpern, J. (1982) in B_{12} (Dolphin, D., Ed.) Vol. 1, pp 501–541, Wiley, New York.

Halpern, J. (1983) *Pure Appl. Chem. 55*, 1059–1068.

Halpern, J. (1985) *Science (Washington, D.C.) 227*, 869–875.

Halpern, J., Kim, S.-H., & Leung, T. W. (1984) *J. Am. Chem. Soc. 106*, 8317–8319.

Krouwer, J. S., Holmquist, B., Kipnes, R. S., & Babior, B. M. (1980) *Biochim. Biophys. Acta 612*, 153–159.

Lenhert, P. G. (1968) *Proc. R. Soc. London, A 303*, 45–84.

Marzilli, L. G., Toscano, P. J., Randaccio, L., Bresciani-Pahor, N., & Calligaris, M. (1979) *J. Am. Chem. Soc. 101*, 6754–6756.

Pett, V. B., Liebman, M. N., Murray-Rust, P., Prasad, K., & Glusker, J. P. (1987) *J. Am. Chem. Soc. 109*, 3207–3215.

Poznanskaja, A. A., Tanizawa, K., Soda, K., Toraya, T., & Fukui, S. (1979) *Arch. Biochem. Biophys. 194*, 379–386.

Pratt, J. M. (1982) in B_{12} (Dolphin, D., Ed.) Vol. 1, pp 325–392, Wiley, New York.

Pratt, J. M. (1984) *J. Mol. Catal. 23*, 187–193.

Pratt, J. M. (1985) *Chem. Soc. Rev. 14*, 161–170.

Randaccio, L., Bresciani-Pahor, N., Toscano, P. J., & Marzilli, L. G. (1981) *J. Am. Chem. Soc. 103*, 6347–6351.

Schrauzer, G. N., & Grate, J. H. (1981) *J. Am. Chem. Soc. 103*, 541–546.

Toraya, T. (1985) *Arch. Biochem. Biophys. 242*, 470–477.

Toraya, T., & Abeles, R. H. (1980) *Arch. Biochem. Biophys. 203*, 174–180.

Toraya, T., & Fukui, S. (1980) *Adv. Chem. Ser. No. 191*, 139–164.

Toraya, T., & Fukui, S. (1982) in B_{12} (Dolphin, D., Ed.) Vol. 2, pp 233–262, Wiley, New York.

Toraya, T., Ushio, K., Fukui, S., & Hogenkamp, H. P. C. (1977) *J. Biol. Chem. 252*, 963–970.

Toraya, T., Krodel, E., Mildvan, A. S., & Abeles, R. H. (1979) *Biochemistry 18*, 417–426.

Toraya, T., Watanabe, N., Ushio, K., Matsumoto, T., & Fukui, S. (1983) *J. Biol. Chem. 258*, 9296–9301.

Toraya, T., Matsumoto, T., Ichikawa, M., Itoh, T., Sugawara, T., & Mizuno, Y. (1986) *J. Biol. Chem. 261*, 9289–9293.

Toraya, T., Watanabe, N., Ichikawa, M., Matsumoto, T., Ushio, K., & Fukui, S. (1987) *J. Biol. Chem. 262*, 8544–8550.

Ushio, K., Fukui, S., & Toraya, T. (1984) *Biochim. Biophys. Acta 788*, 318–326.