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Synthesis and biological activity of a profluorescent analogue of coenzyme B_{12}

(adenosylcobalamin/*linear*-benzoadenosylcobalamin/binding/fluorescence/ribonucleotide reductase)

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ABSTRACT **We describe here the synthesis and chemical properties of *linear*(*lin*)-benzoadenosylcobalamin, a coenzyme B_{12} analogue that has a laterally extended nucleoside in the upper axial position. It is an effective competitive inhibitor of ribonucleotide reductase from *Lactobacillus leichmannii*. *lin*-Benzoadenosylcobalamin is nonfluorescent in solution but, on homolytic (light) or heterolytic (acid, cyanide) cleavage of the carbon-cobalt bond, forms fluorescent products. In addition, fluorescence is detectable on binding of the coenzyme analogue to ribonucleotide reductase, and the observed fluorescence polarization of the *lin*-benzoadenosyl moiety indicates that it is bound loosely to the enzyme when the coenzyme is partially dissociated.**

Fluorescence spectroscopy has found widespread application in examination of the mechanisms of enzyme catalysis (1). However, this useful technique has not yet been extended to the study of coenzyme B_{12}-dependent enzyme systems. This paper describes the synthesis and properties of a coenzyme B_{12} derivative (shown in abbreviated form in Fig. 1) possessing a fluorescent 5′-deoxy-*linear*(*lin*)-benzoadenosyl moiety in the upper axial ligand position in place of the 5′-deoxyadenosyl moiety. Since the 5′-deoxyribonucleoside is believed to be directly involved in the catalytic reaction (2), there is particular interest in replacing this group with an appropriate fluorophore.

lin-Benzoadenosine resembles adenosine structurally except for the formal insertion of a fused benzene ring between the imidazole and pyrimidine moieties, which results in a 2.4 Å linear extension of the *N*-heterocyclic ring system with retention of its hydrogen-bonding capabilities. This feature enables *lin*-benzoadenosine and its derivatives to serve as dimensional probes for estimating the geometric tolerance and flexibility of nucleoside binding sites (3–5). In addition, this laterally extended analogue of adenosine exhibits useful fluorescence properties such as a quantum yield of 0.44, a lifetime of 3.5 nsec, an absorbance maximum for its long wavelength band (331 nm) that is far removed from the absorbance range of proteins, and a sensitivity to environmental conditions (6).

The preparation of *lin*-benzoadenosylcobalamin (Fig. 1) enabled us to examine the effects of a "stretched-out" nucleoside on the interactions of coenzyme B_{12} with ribonucleotide reductase from *Lactobacillus leichmannii*. The chemical and physical properties of *lin*-benzoadenosylcobalamin are also reported.

MATERIALS AND METHODS

Chemicals. The following were obtained from commercial sources: adenosylcobalamin, dihydrolipoic acid, 2′-dGTP, and Purpald (Sigma); thionyl chloride (Aldrich). Ribonucleotide reductase (activity, 0.09 mmol of ATP reduced per hr per mg of protein) was a gift from Raymond L. Blakley, St. Jude Children's Research Hospital, Memphis, TN. Since the best preparation of enzyme (7) has a specific activity of 0.2 mmol of dATP formed per hr per mg, the apparent molar concentration of ribonucleotide reductase in the reaction mixtures was calculated from a molecular weight of 76,000 and corrected for the lower specific activity of our sample. *lin*-Benzoadenosine was prepared by the procedure of Leonard *et al.* (3). Aquacobalamin was synthesized by the method of Hogenkamp and Rush (8).

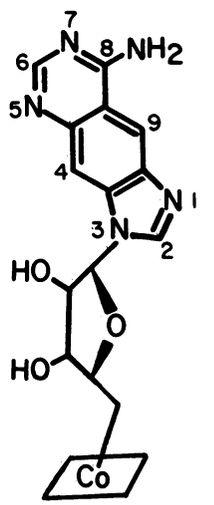

FIG. 1. *lin*-Benzoadenosylcobalamin. The corrin ring system is represented by the rhomboid.

General Methods. UV and visible spectra were recorded on a Beckman ACTA MVI spectrophotometer. Mass spectra were run on a Finnigan MAT-731 mass spectrometer by J. Carter Cook and his staff. The purity of the cobalamin derivatives was determined by descending paper chromatography (Whatman 1) using solvent systems A [*n*-butanol/ethanol/H_2O, 50:15:35 (vol/vol)], B [2-butanol/NH_4OH/H_2O (50:14:36)], and C [*n*-butanol/2-propanol/H_2O (37:26:37)]. Purity of the 5′-chloro-5′-deoxynucleoside was determined by TLC on silica gel with fluorescent indicator (Macherey-Nagel) using solvent system D [*n*-butanol/H_2O (84:16)].

Because of the variable hydration of crystals of B_{12} derivatives, molar extinction coefficients are difficult to obtain

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Abbreviation: The prefix "*lin*-benzo" refers to the linear disposition of the pyrimidine, benzene, and imidazole rings in the "stretched-out" version of the adenine nucleus, as in *lin*-benzoadenine (8-aminoimidazo[4,5-g]quinazoline).

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through direct weighing of samples. Therefore, the extinction coefficients of *lin*-benzoadenosylcobalamin were determined by its quantitative conversion to the corresponding dicyano corrinoid [UV_{max}, 368 (ε_{mM} 36.8)]. This procedure was checked by calculating the extinction coefficients of the analogue on the assumption that UV_{max} = 523 nm (ε_{mM} 8.0) (9).

Chemical Properties. The heterolytic cleavage of the carbon-cobalt bond in *lin*-benzoadenosylcobalamin by cyanide and acid and the homolytic cleavage by light were investigated as follows. The procedures were repeated in each case with adenosylcobalamin for comparison. In decomposition by cyanide, *lin*-benzoadenosylcobalamin (final concentration, 0.01 mM) was added to 0.1 M KCN in 0.01 M sodium carbonate buffer (pH 10.0) in a 1-cm quartz cuvette (under dim red light). Release of the β-ligand was monitored by the increase in *lin*-benzoadenine fluorescence at 372 nm and by the appearance of dicyanocobalamin absorbance at 364 nm. In decomposition by acid, the analogue (0.01 mM) in 0.1 M HCl was heated at 100°C in the dark. Aliquots were removed from the reaction mixture and cooled to room temperature before the absorbance was determined. Formation of aquacobalamin was detected by absorbance at 350 nm, and release of a *lin*-benzoadenine derivative was measured by the appearance of fluorescence at 372 nm. For photolysis, the cobalamin derivative (0.01 mM) in 0.01 M potassium phosphate buffer (pH 7.5) was illuminated in the presence of air with a 100-W tungsten lamp at a distance of 10 cm. The extent of photolysis was measured by the increases in *lin*-benzoadenine fluorescence at 372 nm and in aquacobalamin absorbance at 350 nm. The photolyzed reaction mixture was also analyzed by descending chromatography (Whatman 3 MM) with solvent system E [*n*-butanol/acetic acid/H_2O, 4:1:5 (upper layer)]. Purpald spray (0.05 g/ml in 1 M NaOH) was used to detect aldehyde-containing compounds by the generation of dark purple zones on a yellow background.

5′-Chloro-5′-deoxy-*lin*-benzoadenosine. This compound was prepared by the following procedure based on the method of Kikugawa and Ichino (10). To a solution of thionyl chloride (0.14 ml, 1.96 mmol) in hexamethylphosphoramide (2.0 ml) was added 40 mg (0.13 mmol) of *lin*-benzoadenosine. The reaction mixture was stirred under anhydrous argon for 16 hr at room temperature. The mixture was then added to 5 ml of ice water and applied to a column (2 × 4 cm) of Dowex 50-X2 (H^+, 200–400 mesh). The column was washed with water, and the product was eluted with 2 M ammonium hydroxide. The appropriate fractions were evaporated to dryness. 5′-Chloro-5′-deoxy-*lin*-benzoadenosine was recovered as a white powder in 86% yield as determined by the absorbance of the sample [UV_{max} 331 nm (ε_{mM} 9.7)]. By TLC in solvent system D, the product appeared homogeneous and exhibited a R_F of 0.46 compared with *lin*-benzoadenosine at a R_F of 0.35. The UV and fluorescence spectra of the nucleoside derivative were in good agreement with published values for the parent nucleoside (3). An analytical sample was obtained by recrystallization from aqueous methanol. Fast atom bombardment mass spectrum, *m/e* 336 (M^+), 185 ($M^+ - C_5H_8ClO_3$). *Analysis.* Calculated for $C_{14}H_{14}ClN_5O_3 \cdot HCl \cdot 1.25\ H_2O$: C, 42.60; H, 4.15; Cl, 17.96; N, 17.74. Found: C, 42.32; H, 3.95; Cl, 18.17; N, 17.71.

***lin*-Benzoadenosylcobalamin.** This compound was synthesized by the method of Hogenkamp (11). To ensure complete removal of oxygen, argon was bubbled vigorously through a solution of aquacobalamin (200 mg, 0.126 mmol) in 10 ml of water/methanol (4:1) in a small three-necked round-bottomed flask. After ≈15 min, 25 mg (0.65 mmol) of sodium borohydride and 5 mg of cobaltous chloride were added and the flask was sealed with serum stoppers. The solution changed from red to gray green, indicating the formation of cob(I)alamin. All remaining operations, including purification, were carried out in dim red light. A deoxygenated solution of 5′-chloro-5′-deoxy-*lin*-benzoadenosine (25 mg, 0.075 mmol) in 5 ml of water was added slowly by syringe, and the reaction mixture was stirred for ≈1.5 hr. Next, the reaction mixture was carefully neutralized with 1 M HCl and desalted by phenol/methylene chloride extraction (12). The aqueous layers were condensed to 10 ml and applied to a column (2.5 × 36 cm) of Dowex 50-X2 (H^+, 200–400 mesh). The column was washed with water and then *lin*-benzoadenosylcobalamin was eluted with 0.1 M Tris·HCl (pH 7.6). The appropriate fractions were combined and condensed to 50 ml. The cobalamin solution was desalted by the phenol/methylene chloride extraction procedure, and the recovered aqueous layers were evaporated to dryness. The desired product was isolated as a red glass. By descending paper chromatography, the cobalamin derivative appeared homogeneous and had the following $R_{cyanocobalamin}$ values: in system A, 0.85; in system B, 0.80; and in system C, 0.95. *lin*-Benzoadenosylcobalamin was obtained in 54% yield as determined by the UV absorbance of the sample at pH 7: λ_{max} 258 nm (ε_{mM} 43.7), 282 (26.2), 318 (21.6), 331 (24.3), 346 (20.7), 376 (11.0), 523 (8.0). *lin*-Benzoadenosylcobalamin appeared to be stable in powder form when stored at −20°C but decomposed slowly to fluorescent material when frozen in solution. To remove contaminating fluorescent impurities, solutions of the cobalamin analogue were reapplied to the Dowex 50-X2 (H^+, 200–400 mesh) column and eluted with Tris·HCl (pH 7.6) as described above.

Assay for Ribonucleotide Reductase. The assay mixture for ribonucleotide reductase (13) (total vol, 0.25 ml) was 1.0 mM GTP/20.0 mM dihydrolipoic acid/adenosylcobalamin/*lin*-benzoadenosylcobalamin (where indicated)/50 mM potassium phosphate buffer, pH 7.5/0.15 M KF/1.0 mM EDTA, containing 0.01 mg of ribonucleotide reductase. The mixture was incubated in the dark at 37°C for 10 min and the reaction was terminated by rapid cooling in an ice bath. Excess dihydrolipoate, which interferes with color development, was removed by heating for 20 min at 100°C in the presence of 0.20 ml of 0.5 M chloroacetamide in 0.25 M potassium phosphate buffer (pH 7.3). The solution was cooled and 1.0 ml of diphenylamine reagent was added. The contents were mixed well, covered, and incubated at room temperature for 20 hr. The blue adduct formed between deoxyribose and diphenylamine was determined spectrophotometrically at 595 nm with the mean reagent blank value subtracted from the absorbance values. dGTP formed was calculated from corrected values by comparison with the corrected absorbance of the standard curve.

Binding Studies. Absorbance changes as a result of binding (14) were measured in 0.25 ml of 50 mM potassium phosphate buffer, pH 7.5/5.0 mM dGTP/1.0 mM EDTA/4 μM *lin*-benzoadenosylcobalamin/0.05 mM ribonucleotide reductase/20 mM dihydrolipoic acid. The mixture was incubated at 37°C in the dark, and then the reaction was initiated by addition of dihydrolipoic acid and the absorption spectrum was recorded after 2 min. Photodecomposition from the light source in the spectrophotometer was checked and shown to be negligible.

Changes in the fluorescence properties of the analogue on reductase binding were monitored as follows. Samples were excited at 331 nm and photodecomposition from the lamps in the spectrofluorometers was minimized by decreasing the slit widths and limiting the exposure time of the cobalamin derivatives to the light source. Fluorescence polarization measurements, run in duplicate, were made with an SLM model 8000 polarization fluorometer with Corning glass 0-52 and 7-60 emission filters and a 7-54 excitation filter (excitation slit, 0.5 nm) using a 5-mm-square (internal dimensions) cuvette with a brass adaptor. The rate of photodecomposition of the cobalamin

derivative was determined to be <1% after 2 min of exposure to the light source. The final mixture (total vol, 0.25 ml) was potassium phosphate buffer, pH 7.5/5.0 mM dGTP/1.0 mM EDTA/0.01 mM *lin*-benzoadenosylcobalamin/0.01 mM ribonucleotide reductase/20 mM dihydrolipoic acid. The mixture was incubated in the dark at 37°C, and then the reaction was initiated by addition of dihydrolipoic acid. Polarization measurements were made after 1 min with total exposure to the light source not exceeding 1 min. Polarization values were corrected for background by subtracting the fluorescence intensity of a reaction mixture identical except for the omission of *lin*-benzoadenosylcobalamin. After the polarization measurements were completed on the enzyme/cobalamin mixture, the sample was extensively photolyzed, and a second polarization value was determined. The fluorescence polarization of a control sample that contained only photolyzed *lin*-benzoadenosylcobalamin (0.01 mM) in 50 mM potassium phosphate buffer (pH 7.5) was also recorded.

RESULTS AND DISCUSSION

Synthesis of *lin*-Benzoadenosylcobalamin. An upper-axial-ligand analogue of adenosylcobalamin (Fig. 1) was synthesized from cob(I)alamin and 5'-chloro-5'-deoxy-*lin*-benzoadenosine. Cob(I)alamin, which contains a cobalt atom in the +1 oxidation state, was prepared by the reduction of aquacobalamin with sodium borohydride. The 5'-chloro derivative of *lin*-benzoadenosine was recovered in good yield by direct hydroxyl displacement with thionyl chloride in hexamethylphosphoramide. The purity of the cobalamin analogue was verified by paper chromatography in solvent systems A, B, and C.

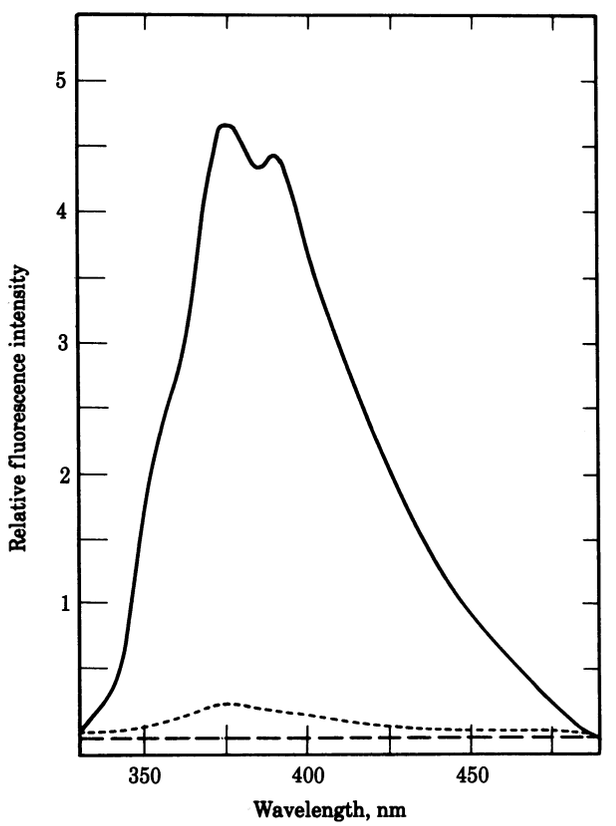

FIG. 2. Fluorescence spectra of *lin*-benzoadenosylcobalamin (0.01 mM) in 50 mM potassium phosphate buffer (pH 7.5) (———), after photolysis (——), and in the presence of ribonucleotide reductase/dGTP/dihydrolipoic acid at 37°C (------).

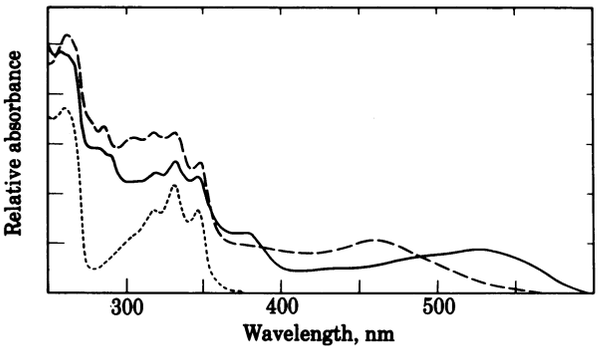

FIG. 3. Absorbance spectra of *lin*-benzoadenosylcobalamin (0.01 mM) at pH 7.0 in 50 mM potassium phosphate buffer (——) and at pH 1.0 in 0.1 M HCl (———). The absorbance spectrum of *lin*-benzoadenosine (0.02 mM) at pH 7.0 is also shown (------).

Spectroscopic and Chemical Properties. An interesting feature of *lin*-benzoadenosylcobalamin is that, although the nucleoside precursor is highly fluorescent, the coenzyme analogue is nonfluorescent in solution (Fig. 2). The term "profluorescent" describes this cobalamin derivative since physical and chemical treatments that cleave the carbon-cobalt bond result in a dramatic increase in the fluorescence intensity. X-ray crystallographic studies on coenzyme B_{12} have shown that the nucleoside base is close to the corrin ring (15). Thus, the quenching of the β-ligand *lin*-benzoadenosine fluorescence occurs as a result of an intramolecular energy transfer between the fluorophore and the nonfluorescent corrin ring system, favored by the overlap of the fluorescence emission and the absorbance of cobalamin. This finding is consistent with previous studies of Jacobsen *et al.* (16) who reported that upper-axial-ligand analogues of B_{12} with $1,N^6$-ethenoadenosine, formycin, 2-amino-9-ribosylpurine, and 2,6-diamino-9-ribosylpurine were all nonfluorescent when excited at wavelengths corresponding to absorbance maxima of the free nucleosides.

The absorbance spectra of *lin*-benzoadenosylcobalamin at pH 1 and pH 7 are shown in Fig. 3. In general, coenzyme B_{12} derivatives have similar absorbance features in the visible region of the spectrum, with a prominent broad band appearing at ≈525 nm at neutral pH (9). However, these compounds have dissimilar absorbance patterns in the UV region, where individual contributions from the nucleoside ligand are apparent. The visible absorbance maximum of the cobalamin analogue is shifted toward a shorter wavelength in acid solution. This is thought to be the result of protonation and uncoordination of the lower-axial dimethylbenzimidazole moiety (17).

The cyanolysis, photolysis, and acid hydrolysis reactions are convenient models for studying the effect of modification of the axial ligand on the stability of the cobalt-carbon bond in cobalamin derivatives (Table 1). The appearance of fluorescence on chemical or photolytic treatment of *lin*-benzoadenosylcobala-

Table 1. Reactivities of the cobalt-carbon bonds of adenosylcobalamin and *lin*-benzoadenosylcobalamin with light, acid, and cyanide

Cobalamin	Pseudo-first-order k for Co—C cleavage, min^{-1}		
	light	acid	cyanide
Ado-Cbl	0.75	0.029	0.045
lin-Ado-Cbl	1.02	0.036	0.041

Ado-Cbl, adenosylcobalamin; *lin*-Ado-Cbl, *lin*-benzoadenosylcobalamin.

min can be exploited as a simple and efficient measurement of release of the nucleoside ligand. Pseudo-first-order rate constants determined spectrofluorometrically were in excellent agreement with rate constants calculated from absorption changes.

Our results show that introduction of a stretched-out nucleoside in place of adenosine has only a small effect on the strength of the organometallic bond. *lin*-Benzoadenosylcobalamin decomposed at slightly faster rates than did the normal coenzyme in the presence of acid and light and at a similar rate on cyanolysis (Table 1). The susceptibility of coenzyme B_{12} analogues to acid hydrolysis reflects the susceptibility of the glycosidic bond of the free nucleoside to acid. Purine-containing cobalamins are less stable to acid than are pyrimidine-containing cobalamins (18). In turn, *lin*-benzoadenosylcobalamin displays an even greater susceptibility to acid treatment as shown by the increase in the rate constant for Co—C cleavage compared with that of the normal coenzyme (Table 1).

One of the most distinctive properties of coenzyme B_{12} and analogues containing a carbon-cobalt bond is their sensitivity to light. When *lin*-benzoadenosylcobalamin was photolyzed under aerobic conditions, the appearance of fluorescent products was observed (Fig. 2). It is interesting to note that the fluorescence spectrum of the photolyzed reaction mixture was slightly different in shape compared with the reported spectrum of *lin*-benzoadenosine (3). This is not surprising since the photolytic cleavage of cobalamin derivatives results in the release of an organic free radical that is capable of undergoing secondary reactions such as the formation of cyclic nucleoside products.

The products from the photolysis of the coenzyme analogue were examined by descending paper chromatography in solvent system E. The principal fluorescent product (R_F, 0.48) gave a positive reaction with Purpald spray and was presumed to be the 5′-aldehyde of *lin*-benzoadenosine. Aquacobalamin, produced by oxidation of cob(II)alamin, was identified by the similarity of its chromatographic behavior (R_F, 0.25) to that of an authentic sample. A second fluorescent product also present on the chromatogram was identified tentatively as 2,5′-cyclic-*lin*-benzoadenosine (R_F, 0.35) (note the numbering system in Fig. 1).

Coenzyme Activity in the Ribonucleotide Reductase System. Previous studies with coenzyme B_{12} analogues have shown that ribonucleotide reductase exhibits a high degree of specificity at the coenzyme site. Any alterations in the adenosyl moiety usually diminish or abolish catalytic activity. Consistent with these findings, *lin*-benzoadenosylcobalamin was unable to function as a cofactor in the enzyme reaction. However, it did act as an effective competitive inhibitor (K_i = 5.2 μM versus K_m = 0.6 μM for adenosylcobalamin). This finding indicates that ribonucleotide reductase is able to accommodate the nucleoside analogue in the active site but stretching out the purine ring system has a profound effect on coenzyme activity.

Binding Study. At 37°C in the presence of dGTP and dihydrolipoic acid, coenzyme B_{12} reacts rapidly with equimolar ribonucleotide reductase to give an absorbance spectral change that corresponds to partial conversion of coenzyme B_{12} to cob(II)alamin and 5′-deoxyadenosyl radical. Cooling the reaction mixture to 5°C causes the spectrum to revert to that of adenosylcobalamin (14). These findings indicate a situation in which the enzyme-bound coenzyme can exist in two forms, one form having an intact carbon-cobalt bond and the other being a loose radical pair protected within the active site. Increasing the temperature shifts the equilibrium toward the dissociated coenzyme. The stabilized radical pair, also detectable by EPR in the case of adenosylcobalamin (19–21), is considered to be the first intermediate in the ribonucleotide reductase reaction. Previous studies with a variety of coenzyme B_{12} analogues have shown that the preliminary degradation step is prerequisite for catalysis but does not guarantee that ribonucleotide reduction will be completed (22).

In the presence of ribonucleotide reductase and the appropriate cofactors, the absorbance spectrum of *lin*-benzoadenosylcobalamin underwent changes that are consistent with the transient formation of cob(II)alamin (14), as shown in Fig. 4. In addition, at 37°C, a weak fluorescence signal was detected from the enzyme/coenzyme mixture that corresponded in intensity to ≈4% photolyzed cobalamin analogue (Fig. 2). The fluorescence disappeared on cooling the mixture to 5°C.

The corrected fluorescence polarization value for *lin*-benzoadenosylcobalamin in the presence of ribonucleotide reductase was 0.09 ± 0.01. The polarization values for the photolyzed coenzyme B_{12} analogue in the absence and presence of ribonucleotide reductase were 0.011 and 0.015 ± 0.001, respectively. The polarization of the fluorophore rigidly attached to an enzyme can be calculated from the following equation:

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{RT\tau}{\eta V}\right),$$

where P_0 is assumed to be equal to 0.42 (6), R is the gas constant, T = temperature, η = viscosity of water at 37°C, and V = volume of a protein. V can be calculated from the ribonucleotide reductase molecular weight by assuming a specific volume for proteins of 0.73 cm^3/g and that these values are constant for the bound and photolyzed fluorophore. However, since the fluorescence of the bound *lin*-benzoadenosylcobalamin is quenched, it is likely that the fluorescence lifetime, τ, is less for the enzyme-bound fluorophore. We can estimate the lifetime range of this bound fluorophore by assuming the quenching is dynamic such that $\frac{\tau}{\tau_0} = \frac{F}{F_0}$. Since the intensity of the bound *lin*-benzoadenosylcobalamin is 4% of the intensity of the photolyzed product, the lifetime of the bound *lin*-benzoadenosylcobalamin would be ≈4% of 3.5 nsec or ≈0.14 nsec. It is likely that static quenching is also occurring. However, if static quenching occurs, $\frac{\tau}{\tau_0} > \frac{F}{F_0}$. Thus, 0.14 nsec is a reasonable lower limit for τ. Given a lifetime range of 3.5–0.14 nsec, the polarization calculated for the fluorophore rigidly attached to the enzyme is then 0.349–0.417. Accordingly, the observed polarization value of 0.09 indicates that the *lin*-benzoadenosyl moiety is *loosely* bound to the enzyme.

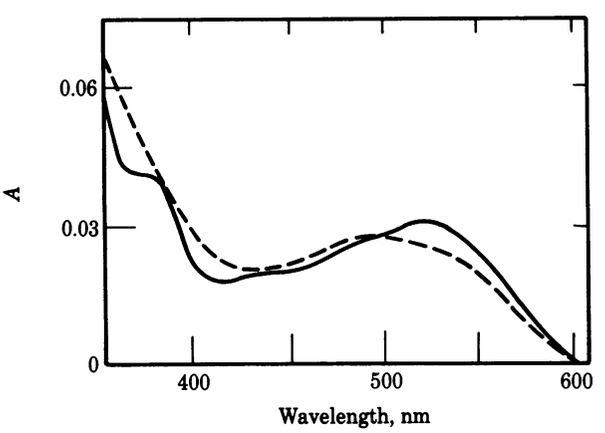

FIG. 4. Comparison of absorption spectra of ribonucleotide reductase/*lin*-benzoadenosylcobalamin in the absence (——) and presence (– – –) of dihydrolipoic acid.

Even though the stretched-out analogue of coenzyme B_{12} is inactive with ribonucleotide reductase, it is still able to undergo rapid enzymatic degradation to cob(II)alamin and the 5′-deoxy-*lin*-benzoadenosyl radical. As with adenosylcobalamin, the initial dissociation is reversed by decreasing the temperature of the reaction mixture. An intriguing discovery is the appearance of a weak fluorescence emission that presumably originates from the reductase-bound coenzyme B_{12} analogue. Theoretically, the cob(II)alamin derivative and the 5′-deoxynucleosyl radical must be far enough apart at the active site to prevent recombination and allow for ribonucleotide reduction. EPR measurements on the radical intermediates have previously indicated that the interaction between the unpaired electrons of cob(II)alamin and the adenosyl radical from adenosylcobalamin span a distance of ≈10 Å (19–21). A similar spatial separation may result in decreased quenching interaction between the fluorescent nucleoside and the nonfluorescent corrin ring system, thereby permitting detection of the fluorescence signal.

In summary, we have described the synthesis and chemical properties of a coenzyme B_{12} analogue that has a laterally extended nucleoside in the upper axial position. *lin*-Benzoadenosylcobalamin is an effective competitive inhibitor of ribonucleotide reductase from *Lactobacillus leichmannii*. It is nonfluorescent in solution but, on homolytic (light) or heterolytic (acid, cyanide) cleavage of the carbon-cobalt bond, forms fluorescent products. In addition, fluorescence is detectable on binding (loosely) to ribonucleotide reductase. The profluorescent property may be of further use in examining the mechanisms of coenzyme B_{12}-dependent reactions that involve scission of the organometallic bond.

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