THE MECHANISM OF COBALAMIN BINDING TO HOG INTRINSIC FACTOR[+]

by

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Circular dichroism studies on the methylcobalamin-intrinsic factor complex, together with studies on the carbethoxylation of histidine residues in this complex, indicate that 5,6 dimethylbenzimidazole is probably displaced by a histidine residue of intrinsic factor which coordinates to the cobalt atom at the sixth coordination site. In addition, the stability of the Co-C σ bond for methylcobalamin bound to intrinsic factor is identical to that stability measured for the free methyl cobinamide-histidine complex, but considerably different from the stability of this σ bond in free methylcobalamin.

Former studies on the binding of cobalamins to intrinsic factor indicate that the substituent in the fifth coordination position is not important in the formation of the cobalamin-intrinsic factor complex (1-5).

However, the coordination of the benzimidazole base at the sixth coordination position is imperative since pseudovitamin B_{12} and cobinamides bind very weakly (1). Recent studies in our laboratory show that the corrin ring plays a minor role in cobalamin binding to intrinsic factor because the C-monocarboxylic acid derivative of cobalamins binds equally as well as the natural C-amide (6). Furthermore we have shown by sepharose affinity chromatography that the corrin ring is located no more than 5 A° from the surface of the protein (Scheme I), and that the 5,6 dimethylbenzimidazole binding site is located in a hydrophobic cleft of the intrinsic factor protein (6).

This communication provides the first evidence for displacement of 5,6 dimethylbenzimidazole from the coordination sphere of the cobalt atom followed by coordination of imidazole from the intrinsic factor protein in the formation of the cobalamin-intrinsic factor complex.

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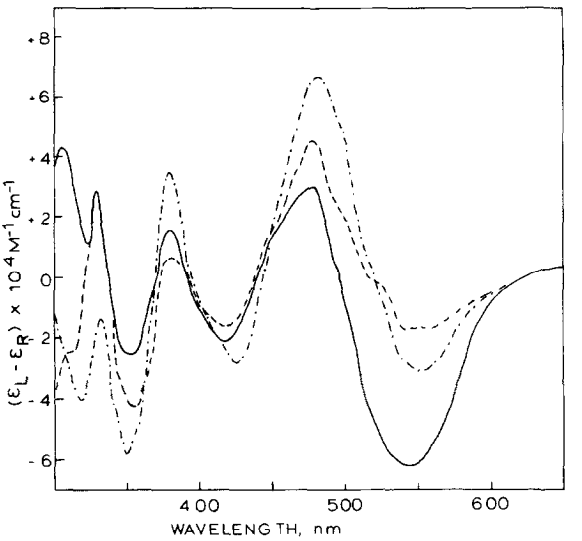


Figure 1

Circular dichroism spectra of:

(a) Methylcobalamin ($1 \cdot 3 \times 10^{-4}$M) (—— ·—— · ——).

(b) Methylcobalamin intrinsic factor complex ($1 \cdot 3 \times 10^{-4}$M) (——————).

(c) Methylcobinamide-histidine complex ($1 \cdot 3 \times 10^{-4}$M) (----------).

Results: The displacement of 5,6 dimethylbenzimidazole from the sixth coordination position of the cobalt atom causes a drastic change in the spectral properties of methylcobalamin (7) (8). "Base on" methylcobalamin has a λmax of 470 nm when 5,6 dimethylbenzimidazole is protonated and displaced so that water coordinates to the sixth coordination site. The UV-visible spectrum of the methylcobalamin-intrinsic factor complex is very similar to that of "base on" free methylcobalamin. The λmax of the methylcobalamin-intrinsic factor is 515 nm, and the only difference between this spectrum and "base on" methylcobalamin is a more distinct shoulder in the complex at 500 nm. The spectrum of the methylcobalamin-intrinsic factor complex indicates that either 5,6 dimethylbenzimidazole is still coordinated when methylcobalamin binds, or alternatively, 5,6 dimethylbenzimidazole may have been displaced by a histidine residue to give a complex of similar spectral properties.

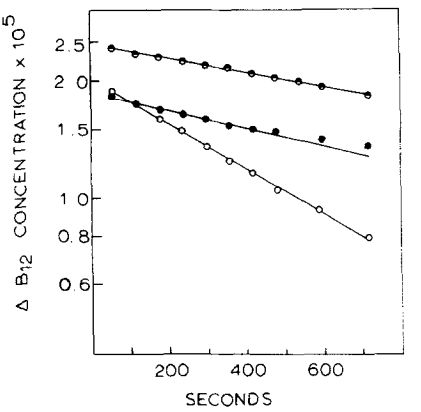


Figure 2

The photolysis kinetics of:

(a) Methylcobinamide-histidine complex (●—●)

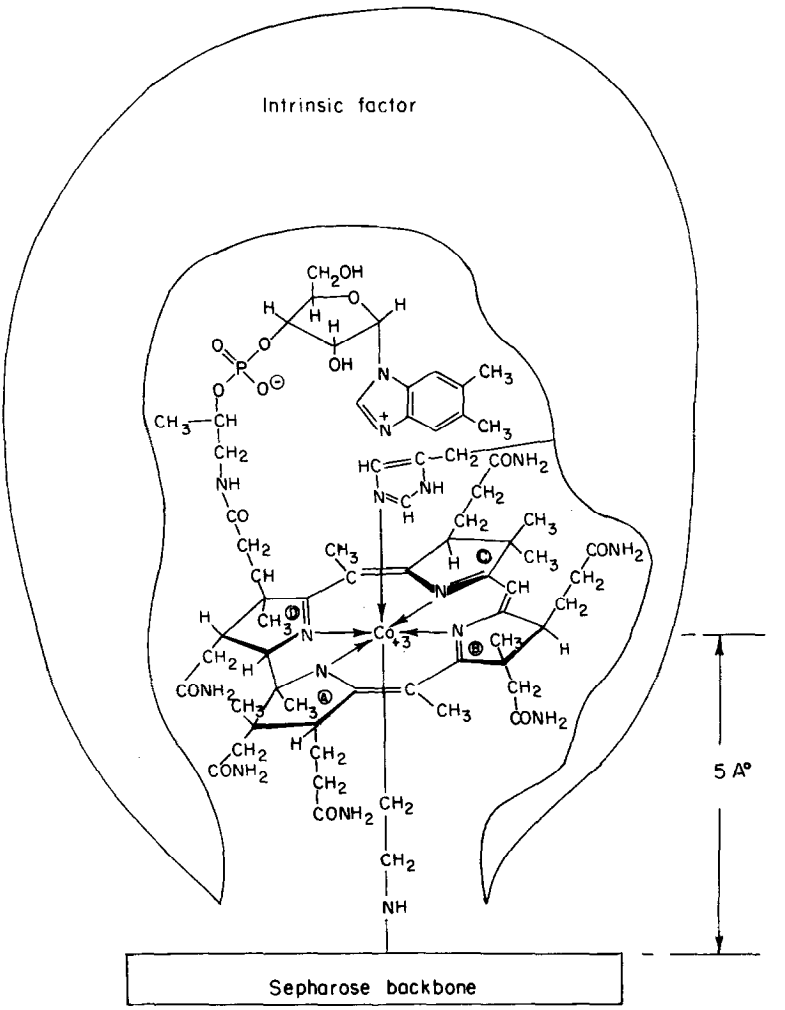
(b) Methylcobalamin-intrinsic factor complex (●—●)

(c) Methylcobalamin (○—○).

(Methylcorrinoid solutions were $1{\cdot}5 \times 10^{-5}$M).

The circular dichroism spectrum of the methylcobalamin-intrinsic factor complex ($1{\cdot}3 \times 10^{-4}$ M) was examined in 0.1 M KH_2PO4 buffer pH 7.4 (Figure 1). This complex has identical maxima and minima to the methylcobinamide-histidine complex. Free methylcobalamin has maxima at 428 nm and 553 nm, whereas the methylcobalamin-intrinsic factor and methylcobinamide-histidine complexes have maxima at 414 nm and 545 nm representing spectral shifts of 14 and 8 nm respectively. The circular dichroic spectrum of "base off" methylcobalamin differs markedly from its "base on" species, and also from the methylcobalamin-intrinsic factor complex.

It was shown by Muhbrad et al (9) (10) and Ovadi et al (11) that diethylpyrocarbonate at pH 6.0 specifically carbethoxylates the histidyl residues of proteins without affecting any other amino acid residues. Therefore the involvement of histidyl residues in cobalamin binding to intrinsic factor can be established by studying the effect of diethylpyrocarbonate on binding.



Intrinsic factor (1 mg/ml) was dissolved in 0.05 M acetate (Na^+) buffer pH 6.0. To three 1 ml solutions, 20 µl aquocobalamin $(10)^{-3}$ M was added and the mixture was incubated at 4°C for 1 hour. Then, to the intrinsic factor solutions, with or without preincubation with aquocobalamin, diethylpyrocarbonate was added as a 10% ethanolic solution at different concentrations. The mixtures were incubated at 4°C for 12 hours. Controls were performed with cobalamin solutions containing no intrinsic factor. The number of carbethoxyhistidine groups formed

was evaluated by measuring changes in absorbancy at 240 mμ ($\varepsilon = 3.2 \times 10^3$) by the method of Ovadi et al (11). The results of these experiments showed that for intrinsic factor five histidines titrate, but for the intrinsic factor-cobalamin complex only four titrate. This result means that a single histidine residue is in a protected environment and cannot react with diethylpyrocarbonate.

When methylcobalamin is bound to intrinsic factor, the photolysis rate for the Co-C σ bond is observed to be 2.5 times slower than that of free methylcobalamin, but similar to the methylcobinamide-histidine photolysis rate (Figure 2). This increase in the stability of the Co-C bond could be indicative of more electron donation from the methyl group to the cobalt atom upon methylcobalamin binding to intrinsic factor. The increase in electron density on the cobalt atom is the result of a weaker interaction between cobalt and the base coordinated at the sixth position. Since histidine is a weaker base than 5,6 dimethylbenzimidazole, then a slower photolysis rate for the complex is consistent with our other observations.

Discussion: Grasbeck et al (12) reported some minor changes in the spectrum of cyanocobalamin upon binding to intrinsic factor, but this research group did not attempt to interpret this observation. The UV-visible spectra of methylcobalamin or aquocobalamin bound to intrinsic factor show minor changes from the respective free cobalamins. These small changes in spectra could be due to the loss of conformational freedom for the cobalamin, because once it is bound to intrinsic factor, then the cobalamin may be locked in one conformation. The spectrum of the methylcobalamin-intrinsic factor complex is very similar to the spectrum of "base on" methylcobalamin which suggests that 5,6 dimethylbenzimidazole or some other nitrogenous base must be coordinated to cobalt in the sixth coordination position.

The circular dichroic spectrum of the methylcobalamin-intrinsic factor complex is similar to the circular dichroic spectrum of the methylcobinamide-histidine complex (histidine coordinated to the sixth position of the cobalt). Several significant shifts in the wavelength of the maxima and minima were observed upon comparing the bound and free spectra of methylcobalamin. If histidine is coordinated

to the sixth site of methylcobalamin in the methylcobalamin-intrinsic factor complex, then several earlier observations may be explained. Cobalamin binding to intrinsic factor is greatly diminished when intrinsic factor is exposed to iodine or UV light. Since both iodine and UV light affect histidine residues, the blockage of these residues in intrinsic factor could cause a direct decrease in binding. When such blocking experiments were done with diethylpyrocarbonate, then one histidine residue per intrinsic factor molecule was found to be protected from reacting with this reagent in the presence of aquocobalamin.

An overall picture of the binding of cobalamins to intrinsic factor may now be considered (Scheme I). Complex formation involves corrin binding near the surface of the protein with no more than 5A° penetration (6). The photolysis, circular dichroism and diethyl pyrocarbonate data provide evidence for histidine displacing 5,6 dimethylbenzimidazole from the sixth coordination position of the cobalt atom. Finally, a strong interaction between benzimidazole and intrinsic factor must occur because of the specificity for this base upon binding.

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