ON THE DUALITY OF MECHANISM OF RING CONTRACTION IN VITAMIN B_{12} BIOSYNTHESIS†

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Abstract - A proposal is made for the role of cobalt in the ring contraction and oxygen exchange mechanisms for vitamin B_{12} biosynthesis in anaerobic bacteria.

Nineteen enzymes are involved in the *aerobic* biosynthesis of vitamin B_{12} in the bacterium *Pseudomonas denitrificans*, a process which has now been defined in complete detail.[1-6] The transformation of 5-aminolevulinic acid (ALA) to hydrogenobyrinic acid is under control of the **first twelve enzymes** (Figure 1) while a further seven enzymes (some of them multi-functional) are necessary for *a,c*-amidation, cobalt insertion, four more amidations (*b, d, e, g*), successive addition of amino isopropanol, phosphorylation, addition of GDP and finally, insertion of dimethylbenzimidazole ribose-5'-phosphate to reach cobalamin-5'-phosphate.[4] By contrast, the *anaerobic* pathway, utilized by organisms such as *Salmonella typhimurium*, *Propionibacterium shermanii*, *Clostridia* spp. has been elucidated at the enzymatic level only as far as precorrin-3, since not until recently have the genetics of *S. typhimurium* allowed a logical approach to the anaerobic enzymology.[7] Although several of the genes appear homologous between the latter organism and *P. denitrificans* (Figure 2) only the *S*-adenosyl methionine (SAM) dependent methyl transferases *cbiL* and *F* from *Salmonella* have been shown to possess the same catalytic activity[8] as their homologs, CobI and CobM respectively.

The recent discovery[9] that the ring contraction mechanism in *P. denitrificans* is mediated by the insertion of a hydroxyl group from oxygen into the metal-free precorrin-3 (enzyme 6, CobG; Figure 1) serves to point out a major mechanistic difference for this fascinating process between the aerobic sequence where oxidation at C-20 of precorrin-3 and subsequent pinacolic like rearrangement proceeds in the absence of cobalt and without exchange

† Dedicated with respect to Dr. Arnold Brossi, a pioneer of alkaloid chemistry, on the occasion of his 70th birthday.

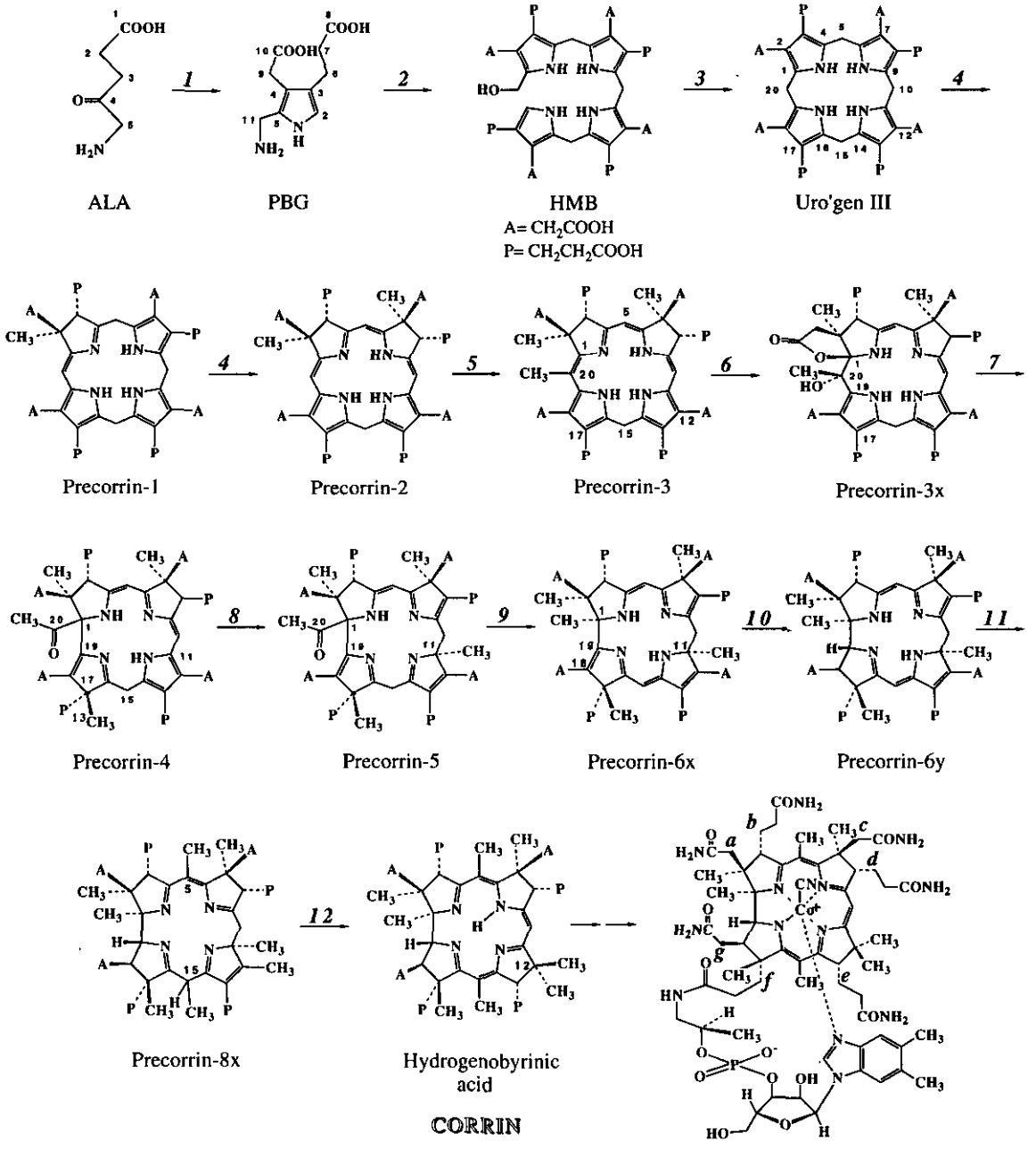


Figure 1

1. ALA dehydratase (*hemB*); 2. PBG deaminase (*hemC*); 3. Uro'gen III synthase [cosynthase] (*hemD*); 4. Uro'gen III methylase [M-1] (*cysG/cobA*); 5. M-2 (*cobI, cbiL*); 6. Precorrin-3x synthase (*cobG*); 7. Ring contractase/17 methyl transferase [M-3] (*cobJ*); 8. M-4 (*cobM*); 9. M-5 (*cobF*); 10. Reductase (*cobK*); 11. Precorrin-8x synthase (M-6/decarboxylase) (*cobL*); 12. [1,5]-sigmatropic shiftase [hydrogenobyrinic acid synthase] (*cobH*).

of oxygen functionality, whereas cell-free studies and pulse-labeling experiments have proved that, in the anaerobe *P. shermanii,* cobalt is already present in precorrin-3.[10] Thus the anaerobic ring contraction mechanism, which cannot involve O_2, operates on a cobalt complex and has been shown to feature exchange of at least one of the oxygen functions of the ring A acetate side chain.[11] In this memoir we present a mechanistic rationale for the metal dependent ring contraction process in the anaerobic pathway.

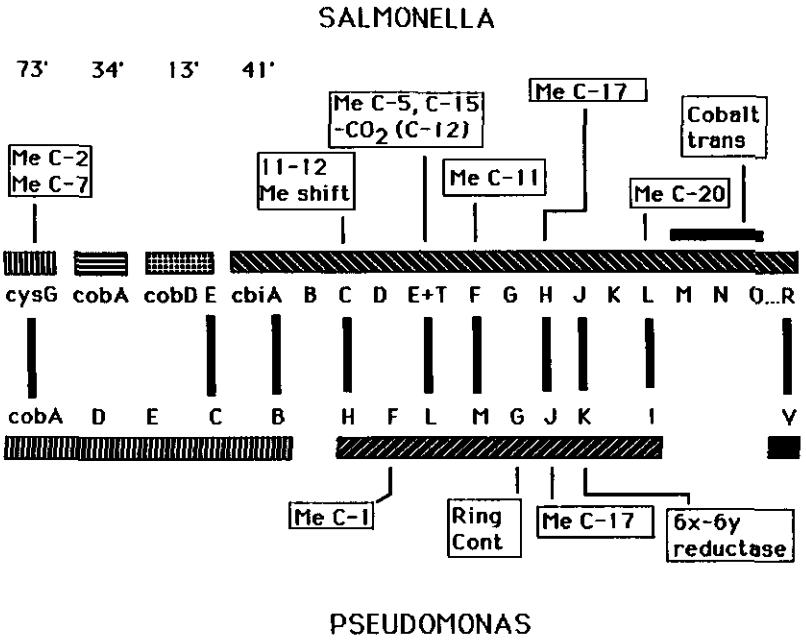
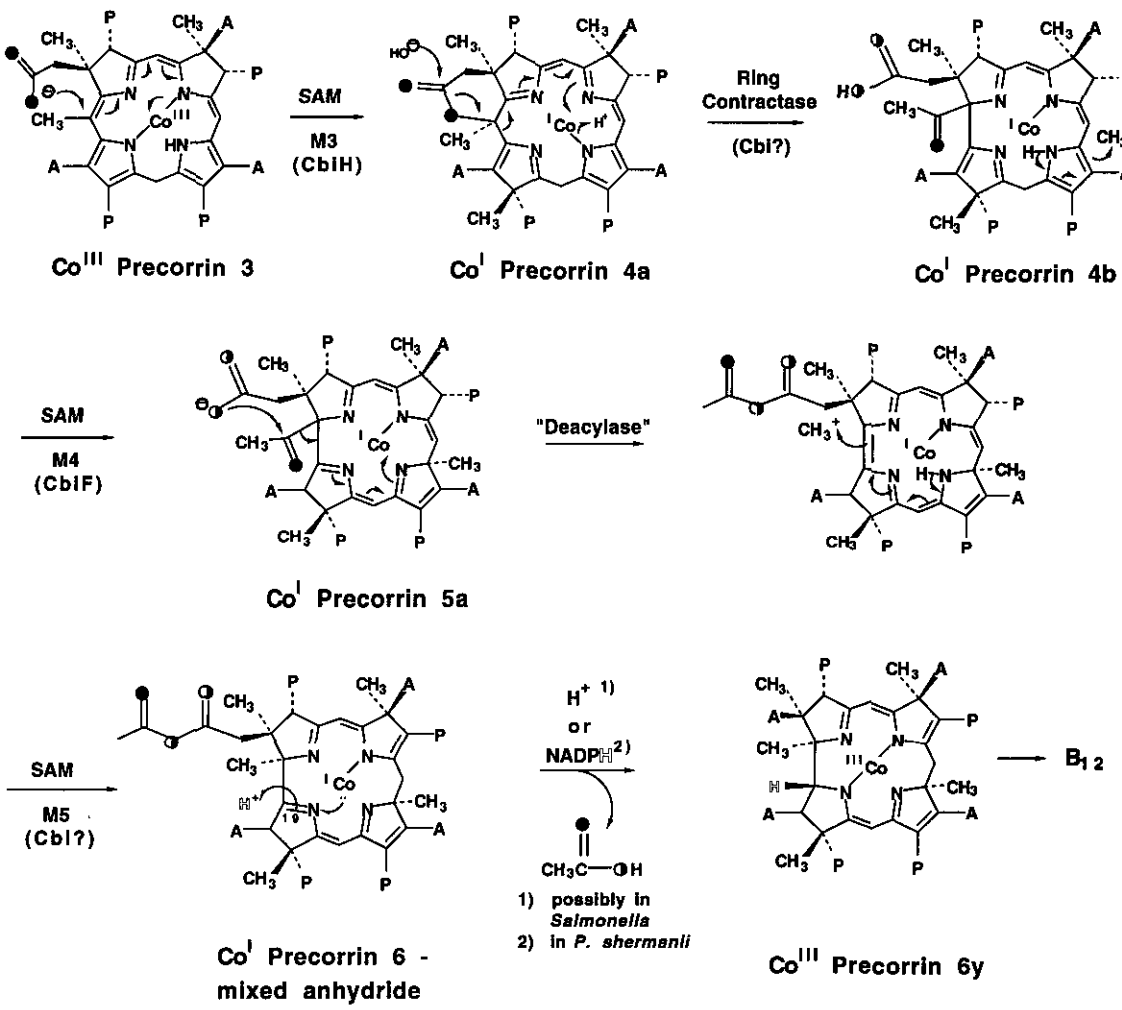


Figure 2 Genes required for cobinamide biosynthesis

The oxidative formation of precorrin-3x prepares the macrocycle for ring contraction catalyzed by enzyme 7, CobJ (which also inserts a C-methyl group at C-17, Figure 1) while the anaerobic mechanism must reach a **similar but non-identical reactive intermediate by a completely different process** (Figure 3). Based on the experimental analogy of metal-assisted ring contraction of the dihydrocorphinol model system,[12] we propose that the Cobalt(III) complex of precorrin-3 undergoes SAM dependent C-methylation at C-17 catalyzed by CbiH (homologous to CobJ in the aerobic series), concomitantly with (or preceded by) δ-lactone formation, which by the redox chemistry of cobalt ($Co^{III} \rightarrow Co^{I}$) formally reaches Co^{I} precorrin-4a, the metallo-equivalent of precorrin-3x. The next step which could be spontaneous(!) involves the hydrolysis of the δ-lactone followed by an acyloin-like ring contraction on precorrin-4a to reach the cobalt (I) complex-4b which corresponds to precorrin-4 in the *P. denitrificans* series (Figure 1). Further methylation by M-4 (CbiF) at C-11 and deacylation catalyzed



Co^{III} Precorrin 3 — Co^{I} Precorrin 4a — Co^{I} Precorrin 4b

Co^{I} Precorrin 5a

Co^{I} Precorrin 6 - mixed anhydride — Co^{III} Precorrin 6y

Figure 3 Hypothetical cobalt pathway to precorrin 6y in anaerobes (*Salmonella, P. shermanii*) showing fate of ALA derived ^{18}● in the extruded acetic acid

by M-5 leads to Co^{I} precorrin-6. Then hydrolysis and reduction to Co^{III} precorrin-6y restores the original valency of cobalt (Co^{III}) at the same time inserting a proton at C-19. Alternatively, reduction by NAD(P)H, for which evidence has been adduced in *P. shermanii*, can catalyze this reductive step.[13] In either case, the rest of the pathway would then proceed as in Figure 1, except that all the intermediates are cobalt complexes. The proposed mechanism in Figure 3 also takes account of the fate of ^{18}O in the ring A carboxylate (●) which is exchanged on the way to vitamin B_{12} in *P. shermanii* via hydrolysis of the δ-lactone in Co-precorrin-4a. This sequence contrasts with the aerobic pathway where the 20-hydroxyl is introduced from dioxygen by the enzyme CobG,

there being no homology for CobG in the *Salmonella* gene repertoire (Figure 2). For the mechanism of deacetylation it is proposed that a mixed anhydride is formed as part of the process leading to intramolecular deacylation of Co^{I}-precorrin-5a and thence by C-methylation to precorrin-6 - mixed anhydride, hydrolysis of which releases acetic acid bearing some of the original ^{18}O label (●) derived from the ring A acetate.[14]

The above scenario rationalizes the following experimental findings:

(a) the insertion of cobalt at an early stage (into precorrin-2),[10] originally as Co^{II}, followed by one-electron oxidation to Co^{III}

(b) the loss of at least one oxygen from the original ring A acetate[11] by hydrolysis of the δ-lactone formed as a result of the $Co^{III} \rightarrow Co^{I}$ change

(c) the insertion of labeled oxygen from the ring A side chain acetate into the acetic acid extruded from precorrin-5a via the mixed anhydride mechanism[14]

(d) the requirement for cobalt as part of the ring contraction mechanism in anaerobic organisms[10]

(e) the finding that one-electron redox changes in cobalt are required for the attachment of the adenosyl ligand in the synthesis of coenzyme B_{12}.[15]

We suggest that in the evolution of B_{12} biosynthesis[7] the "early" anaerobes (archaebacteria) depended on a metallo complex to effect hydration at C-20 thereby allowing the ring contraction mechanism to operate. In the more recent, aerobic bacteria metal free precorrin-3 receives its 20 hydroxyl function, not from water, but from one atom of dioxygen.

It is not clear why nature has retained both mechanisms over the last 2×10^9 years[7] but the comparative stabilities of the metal free intermediates in the aerobic pathway contast with those of the cobalt complexes of precorrins-2 and -3 (and presumably the later precorrins) whose extreme lability to oxygen, has so far precluded their isolation and identification.

The hypothesis that the redox chemistry of cobalt mediates the central part of the B_{12} pathway in anaerobic bacteria can be tested in the future using the biosynthetic enzymes of *S. typhimurium* which have been overexpressed in soluble form.[8] These studies are in progress.

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