CHAPTER 18

Vitamin $B_{12}$: Biosynthesis of the Corrin Ring

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Abstract

Vitamin $B_{12}$ is a cobalt-containing modified tetrapyrrole, whose structural complexity and beguiling chemistry has fascinated scientists for over 80 years. As with all modified tetrapyrroles, its structure is derived from uroporphyrinogen III. This transformation requires a large number of enzyme-mediated steps that result in peripheral methylation, cobalt chelation, ring contraction, decarboxylation, amidation and adenosylation. There are two related though genetically distinct routes for cobalamin biosynthesis, which are referred to as the aerobic and anaerobic pathways. In this chapter the biosynthesis of the corrin ring component of vitamin $B_{12}$ along these two routes is described.

Introduction

Out of all the modified tetrapyrroles, perhaps the most aesthetic is vitamin $B_{12}$ (cobalamin), where the combination of its sheer utter complexity and the mesmerizing chemistry that it facilitates makes it a natural curiosity. Vitamin $B_{12}$ has the architecture of a molecular gyroscope, consisting of a corrin ring to which is bound a lower nucleotide and an upper ligand constituted by either a methyl or adenosyl group (Fig. 1). It is a vitamin, of course, because it is required by humans as an essential dietary supplement. Deficiency can result in pernicious anaemia, neurological disorders and diseases associated with disorders of methionine metabolism. These medical conditions relate to the fact that $B_{12}$ is required for two key metabolic enzymes, methionine synthase and methylmalonyl CoA mutase. What makes vitamin $B_{12}$ unique in comparison to other vitamins is that it is made only by certain bacteria - it is not made de novo by any eukaryote. At its simplest, cobalamin is a modified tetrapyrrole, belonging to the same family as, inter alia, haem and chlorophyll. For the synthesis of cobalamin, the basic tetrapyrrole primogenitor, uroporphyrinogen III (Fig. 1), is modified by the peripheral addition of methyl groups, amido groups, a nucleosyl side chain and an atom of cobalt, as well as by the loss of one of the integral macrocyclic framework carbon atoms in a ring contraction step.$^{4,6}$ Such modifications are reflected in a highly complex biosynthetic pathway, requiring a total of at least 19 enzyme-mediated reactions. In this chapter we shall deal with the biosynthesis of the corrin ring component of vitamin $B_{12}$. The synthesis and attachment of the nucleotide loop is dealt with in the subsequent chapter.

Cobalamin is synthesised in vivo by at least two independent pathways (Fig. 2). On the surface, these pathways differ in their requirement for oxygen. However, this requirement is not as simple as the substitution of a single oxygen-dependent reaction. The aerobic and anaerobic pathways exhibit different chemical approaches, using different sets of enzymes, to arrive at the same end product. However, some generalisations can be made about these pathways (Figs. 2, 3). They initiate from the dipyrrorocorphin precorrin-2, from which the routes diverge into aerobic and anaerobic pathways before converging with the synthesis of the intermediate adenosylcobyrinic acid $a,c$-diamide (Fig. 2). The final reactions concern the synthesis and attachment of the lower axial ligand, a modified purine nucleotide. This part is covered in detail in the subsequent chapter.

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The transformation of uroporphyrinogen III into vitamin \( B_12 \) requires a large number of enzyme-mediated steps to account for the peripheral methylation, ring contraction, decarboxylation, cobalt insertion, amidations, lower nucleotide loop assembly and attachment and upper ligand attachment. The numbering and lettering associated with cobalamin is also shown.

Figure 1. Uroporphyrinogen III and \( B_12 \). The transformation of uroporphyrinogen III into vitamin \( B_12 \) requires a large number of enzyme-mediated steps to account for the peripheral methylation, ring contraction, decarboxylation, cobalt insertion, amidations, lower nucleotide loop assembly and attachment and upper ligand attachment. The numbering and lettering associated with cobalamin is also shown.

genetic requirements and an outline of the two pathways are shown in Figure 3. In general, the genes encoding the enzyme of the anaerobic pathway are given the prefix \( \text{cbi} \), whereas the genes of the aerobic pathway are termed \( \text{cob} \).

The First Common Step: Production of Precorrin-2

As shown in Figure 2, the first reaction, common to both cobalamin biosynthetic pathways, involves the methylation of uroporphyrinogen III to produce precorrin-2. The nomenclature of the intermediates ("precorrin-\( n \)") has been standardised to reflect the number \( (n) \) of methyl groups attached to the basic ring structure of the tetrapyrrole.\(^9\) The first reaction is catalysed by \( S\)-adenosyl-L-methionine uroporphyrinogen III methyltransferase (SUMT, CobA).\(^10\) This homodimer catalyses the ordered transfer of two methyl groups, each donated by \( S\)-adenosylmethionine (SAM), to the C2 and C7 positions of uroporphyrinogen III, respectively. The reaction is strongly and competitively inhibited by \( S\)-adenosyl-L-homocysteine (SAH), the breakdown product of SAM.\(^11\) Interestingly, a second form of inhibition also acts on this reaction: uroporphyrinogen III, the substrate, inhibits the reaction at concentrations in excess of 0.5 to 2.0 \( \mu \text{M} \).\(^11,\,12\) Substrate inhibition of this type is likely to constitute a regulatory mechanism for the reaction and, hence, the pathways in which it is involved. However, this is not true of the reaction in all systems. For instance, the \( \text{cobA} \) orthologue from \( \text{Methanobacterium ivanovii} \), a methanogenic member of the archaeeae, does not exhibit substrate inhibition.\(^12\) Methanogenic organisms have a high requirement for coenzyme F\(_{430} \), also a product derived from uroporphyrinogen III (Fig. 2). Thus, the difference in inhibition may reflect differences in both the quantity and type of modified tetrapyrrole required by each cell type.

The crystal structure of SUMT has recently been solved.\(^13\) The enzyme is similar in structure to the methylase domain of CysG\(^14\) and to a number of other methyltransferases involved in cobalamin biosynthesis, including CobI, CobJ, CobM, CobF and CobL.\(^15\,\,18\) This structural similarity is likely to reflect the evolution of these enzymes from a common ancestral enzyme. Mechanistically, the enzyme is likely to employ the inherent chemistry of the two substrates to promote catalysis by