On the biosynthesis of the porphyrin-like moiety of vitamin B_{12}: the mode of utilization of δ-aminolevulinic acid*

We previously reported[1] that δ-aminolevulinic acid is directly utilized for the biosynthesis of the porphyrin-like moiety of vitamin B_{12}. The vitamin B_{12} synthesized in a medium containing δ-aminolevulinic acid-1,4-^{14}C was highly radioactive. On the assumption that the biosynthetic pathway for the tetraheterocyclic ring system was similar to that known for porphyrins[2,3], we concluded that fifteen carbon atoms of the vitamin should be equally radioactive (see Fig. 1).

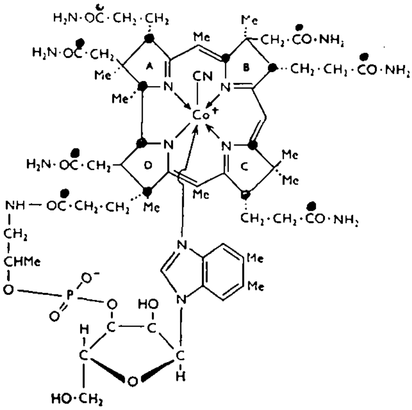

Fig. 1. Structure of vitamin B_{12}. The closed circles (●) indicate the radioactive carbon atoms derived from δ-aminolevulinic acid-1,4-^{14}C.

This assumption appeared valid even though there are some striking structural differences between vitamin B_{12}[4,5,6,7] and the porphyrins (*e.g.*, the vitamin has many extra methyl groups, pyrroline and pyrrolidine rings instead of pyrroles, and lacks one bridge carbon atom). The ^{14}C activity of each of the fifteen carbon atoms was calculated to be one-fourth that of each radioactive carbon atom in the δ-aminolevulinic acid.

In order to support our conclusions, we undertook a series of degradations aimed at determining the ^{14}C activity of those fifteen carbon atoms in the vitamin B_{12} which should contain all of its radioactivity. We have obtained the six primary amide carbon atoms of the vitamin, as carbon dioxide, and have determined their ^{14}C activity. These carbon atoms should, according to our postulation, contain six-fifteenths of the total activity of the vitamin if no side reactions had occurred during biogenesis.

A sample of vitamin B_{12} was oxidized to CO_2 by the wet combustion method of VAN SLYKE AND FOLCH[8] in order to obtain the total radioactivity or the average activity of each of the sixty-three carbon atoms (Table I). Another sample of vitamin B_{12} (10 mg in 2.25 ml water) was treated at room temperature with 7 equivalents of aqueous alkaline NaOCl (0.75 ml of a 0.07 M solution in 2 M NaOH). The solution containing the polychloroamide, which formed during the 50 min the reaction mixture was stirred at room temperature, was boiled gently for 10 min to effect the Hofmann haloamide rearrangement[9,10], and to hydrolyze the resulting polyisocyanate to a polyamine and CO_2. After acidification of the reaction mixture, the CO_2 (theory: 6 moles) was collected and its ^{14}C activity determined (Table I). Approximately theoretical yields of CO_2 were obtained with the addition of 7 moles of NaOCl. An increase in yield of CO_2 of only about 10% was observed after the addition of 14 moles of NaOCl. The latter finding, coupled with our observation that the absorption spectrum of the intense blue residue was similar to that of chlorinated vitamin B_{12}[11,12,7], indicates that no extensive destruction of the vitamin's chromophoric system took place under our conditions, and that in all likelihood the CO_2 was derived from the primary amide carbon atoms. We have observed, however, that large excesses of NaOCl or, particularly, NaOBr cause extensive destruction of the vitamin.

The data obtained are given in Table I. The specific activity of the CO_2 obtained by each of the procedures was calculated from the measured activity of thin $BaCO_3$ plates. The specific

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TABLE I

DISTRIBUTION OF ^{14}C ACTIVITY AMONG THE CARBON ATOMS OF VITAMIN B_{12} SYNTHESIZED FROM δ-AMINOLEVULINIC ACID-1,4-^{14}C

Positions analyzed for radioactivity	*Specific* activity (counts/min/mmole C)*	*Molar** specific activity (counts/min)*
a. Total carbon (wet combustion)	990	62,500
b. Total carbon (wet combustion)	1140	72,000
c. Primary amide carbon atoms	3100	18,500
d. Primary amide carbon atoms	3060	18,500
e. Fifteen carbon atoms starred in Fig. 1	—	46,000***

* As $BaCO_3$ calculated to infinite thinness.

** (Specific activity) × (the number of moles of the carbon position analyzed in each parent molecule).

*** Calculated: (specific activity of the primary amide carbon atoms) × (15).

activity of the CO_2 obtained on combustion can be used to calculate a molar specific activity (Table I) of about 67,000 c.p.m. for the vitamin B_{12} which we degraded. If no extraneous reactions occurred and if our postulation is correct, all of this activity should reside in the designated 15 carbon atoms (Fig. 1). Since the activity of these 15 carbon atoms should be equally divided, a sample of any of these carbon atoms (*i.e.*, the primary amide carbons) can be utilized to calculate their total contribution. It can be seen from Table I that the radioactivity of the CO_2 obtained by the Hofmann haloamide reaction is about 3 times that of the CO_2 from total oxidation. This finding, of unequal ^{14}C distribution among the carbon atoms of vitamin B_{12}, supports in part our previous conclusion. Further, calculating the molar specific activity of these 6 carbon atoms, it can be seen that they contain about 30% of the radioactivity found originally in the intact vitamin (18,500 c.p.m./67,000 c.p.m. × 100%). This is in rather good agreement with the theoretical value of 40% (6/15 × 100%). If all the activity resided in the postulated 15 carbon atoms, then the molar specific activity of these carbons would be 46,000 c.p.m. The discrepancy between this value and that based on the total oxidation may be attributed to some indirect utilization of the labeled substrate, or to our experimental procedures, or to both. Nevertheless the data warrant the conclusion that δ-aminolevulinic acid is an intermediate in the synthesis of the porphyrin-like moiety of vitamin B_{12}, and that most, if not all, of the radioactivity of vitamin B_{12} synthesized from δ-aminolevulinic acid-1,4-^{14}C resides in the predicted 15 carbon atoms.

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