PREPARATION AND SOME PROPERTIES OF FERRIBALAMIN, THE Fe(III)-ANALOGUE OF VITAMIN B_{12}

Rainer BIEGANOWSKI and Wilhelm FRIEDRICH
Physiologisch-Chemisches Institut der Universität, 2000 Hamburg 13, Grindelallee 117, 4. OG, FRG

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1. Introduction

In contrast to heme and chlorophyll the central atom of vitamin B_{12} can neither be removed nor exchanged with other metals [1]. Studies of the function of the cobalt in the B_{12}-coenzyme have been hindered by the limited availability of the relevant substituted corrinoids. In order to prepare such compounds cobalt-free corrinoids have been isolated from phototrophic bacteria [2] and this material has been utilized in the synthesis of metal corrinoids which contain copper or rhodium instead of cobalt as the central atom [3].

We describe here the first successful attempt to incorporate iron into the corrin ring wherein ferribalamin was formed from hydrogenobalamin. Ferribalamin is an especially interesting compound because it would be expected that iron as the central atom of the corrin ring might be more readily analogous to cobalt than other neighbouring atoms in the periodic system. Iron-substituted corrinoids may be useful in clarifying whether cobalt is essential for the B_{12}-coenzyme function.

2. Materials and methods

Iron(III)acetate, iron powder, water-free sodium acetate and acetic acid were purchased from Fluka. Hydrogenobalamin was purified from the bacterium *Chromatium vinosum* as in [3]. For the synthesis of ferribalamin, 27 mg hydrogenobalamin were dissolved in 15 ml acetic acid under N_2 at room temperature. A mixture containing 200 mg alkaline iron(III)acetate, 100 mg iron powder, and 750 mg water-free sodium acetate was added and the reaction mixture was heated for 2 h at 80°C in the dark. A colour change from red-brown to dark-green occurred and at the same time a reduction of the fluorescence at 360 nm was observed. The solution, still under N_2, was diluted to 100 ml with water, filtered and applied to a column (15 × 200 mm) of Amberlite XAD-2, 100–120 mesh. The iron-containing corrinoid was retained on the column as indicated by a green zone. Salts and acetic acid were eluted with water. From this point on in the procedure we ceased using nitrogen protection. Iron-containing corrinoid was eluted with a 10% aqueous solution of tertiary butanol and the eluate was freed from butanol under vacuum. Chromatography on CM-cellulose yielded 19 mg of a uniform red-brown complex, containing 4.2% iron (determined by atomic absorption). The theoretical iron content of ferribalamin ($C_{64}H_{88}O_{14}N_{14}PFe$) is calculated as 4.13%.

3. Results and discussion

In analogy to Cob(III)alamin the axial ligands of ferribalamin can be readily substituted; aqua-, cyano- and dicyanoferribalamin were readily prepared by ligand exchange in aqueous solution. The respective absorption spectra of aqua-, cyano- and dicyanoferribalamin are shown in fig.1, while the absorption maxima are listed in table 1. Adjustment of dicyanoferribalamin solution with acetic acid to pH 3 yielded cyanoferribalamin. The binding of the CN^- and the electrophoretic mobility of aqua- and cyanoferribalamin is consistent with the formulation as Fe(III)-complexes. The iron-containing corrinoids migrate

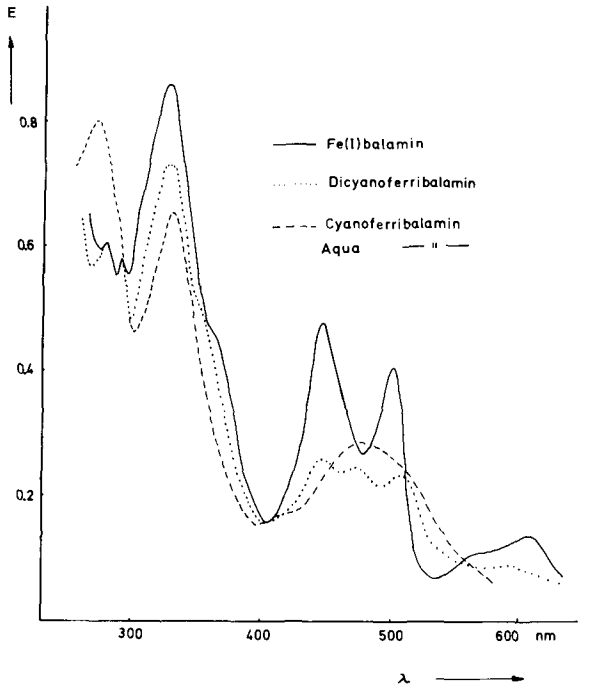

Fig.1. Absorption spectra of Fe(I)balamin (———), dicyanoferribalamin (· · · · ·), cyanoferribalamin (– – –) and aquaferribalamin (– – –) in water.

electrophoretically within pH 2.7–10.5 as do the homologous Cob(III)alamins. Reduction of ferribalamin by either zinc powder in NH_4Cl solution or aqueous $NaBH_4$ solution yields a green, oxygen-sensitive corrinoid, which probably contains Fe(I) (cf. absorption spectra fig.1 and table 1). Similar to vitamin B_{12s} [4] the absorption maxima of 5,6-dimethylbenzimidazole at 278 nm and 290 nm (which is due to lack of coordination) can be observed in Fe(I)balamin preparations.

Evidence that iron is the central atom in ferribalamin and not otherwise bound (e.g., by amide groups) comes from the following observations:

(i) The 5,6-dimethylbenzimidazole residue is bound coordinately in cyanoferribalamin, as indicated by the absence of characteristic peaks of the base at 278 nm and 290 nm in absorption spectra (the same is true for cyanocobalamin). These peaks are easily visible in dicyanoferribalamin (fig.1) and dicyanocobalamin (the base in both complexes is not coordinated). If the iron was bound differently (not as central atom) in ferribalamin, then such coordination would be highly improbable. This interpretation is consistent with observations made with cobalt-corrinoids, in which very small changes in the region of the cobalt-nucleotide–isopropanolamine-corrin loop (e.g., binding of phosphate residue to C′-2 instead to C′-3 of the ribose) result in a reduction of the coordination [5].

(ii) After purification of the complex the iron content of the ferribalamin, as determined by atomic absorption spectroscopy, was in good agreement with the theoretical value for a corrinoid-containing 1 iron atom. If the iron was bound to one of the side chains, one would expect an iron-mediated connection of 2 corrin rings and a different (other than 1:1) ratio of Fe and corrin ring.

Table 1
Absorption maxima of the iron-corrinoids

Corrinoid	Wavelength (nm)					
Aquaferribalamin	271	331	471			
Cyanoferribalamin	271	332	472			
Dicyanoferribalamin	278	290	329	445	470	505
Fe(I)balamin	278	290	327	447	502	605

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