

COBALT - BIS(HISTIDINE METHYL ESTER) DEUTEROPORPHYRINE IX :

A COENZYME B₁₂ - RELATED MODEL

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The synthesis of cobalt - bis (histidine methyl ester) deuteroporphyrine IX in which histidine residues are covalently bound to the propionic acid side chains of the porphyrin ring is described. Spectroscopic evidences of a coordination of histidines to the cobaltic ion are given. Two monoelectronic waves in cathodic range are found by polarographic study of this compound in dimethylformamide. They are easily attributed to Co(III) - Co(II) and Co(II) - Co(I) reduction steps. The coordination state and the chemical reactivity of reduced species formed by electrolysis at each wave are characterized.

The coenzymes B₁₂ are Co(III)-tetrapyrrolic complexes further coordinated by the 5,6-dimethylbenzimidazole of the nucleotide chain of the corrinoid ring and an other ligand (5-deoxyadenosyl, CN⁻, OH⁻, CH₃) (1). A variety of cobalt complexes (cobalamins, cobaloximes, cobalt schiff bases, cobalt porphyrins) (2, 3, 4) have been prepared as coenzymes B₁₂ models. In such complexes the dependance of the oxidoreduction potential of the metallic ion on its coordination state is related to the stability constants in each redox state. In view of an better investigation of the chemical and stereochemical factors governing the redox behaviour of the cobalt ion, we prepared a model compound in which the two ligands (histidine) are covalently bound to the propionic acid side chains of the porphyrin ring.

The bis (histidine methyl ester) deuteroporphyrin IX was synthesized according to the carboxylic-carbonic acid anhydride method reported by A van der Heijden and al (5). The histidine methyl ester dichloride was coupled with deuteroporphyrin IX in dimethylformamide in the presence of

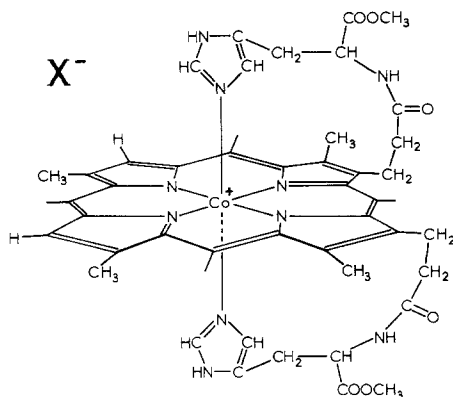


Figure 1 Cobalt (III) - bis(histidine methyl ester) deuterioporphyrine IX.

triethylamine and ethyl chlorocarbonate. The crude mixture was precipitated out upon addition of water and chromatographed on a column of Merckogel OR-PVA 2000 with dimethylformamide as the eluent. The fraction containing the desired bis (histidine methyl ester) derivative was transformed to the cobalt complex. The cobalt - bis (histidine methyl ester) deuterioporphyrine IX (PCo-His_2) was then purified by chromatography on a column of Merckogel OR-PVA 2000 using dimethylformamide. This compound is shown in Fig. 1.

Its absorption spectrum in dimethylformamide shows two peaks in the visible region (559 and 528 nm) and a peak in the Soret region (418 nm) (Fig. 2 A). This spectra is very similar to that of bis (imidazole) deuterioporphyrin IX dimethyl ester - Co(III) in organic solvents (6). The lack of electron paramagnetic resonance signal at 77 K indicates that the cobalt ion is in a diamagnetic +3 oxidation state (d^6). On platinum electrode, the polarogram of the model compound (in dimethylformamide at 20°C with dried tetraethylammonium perchlorate as electrolytic salt) is characterized by two mono-electronic cathodic waves. The potential values ($E_{1/2}$) of these waves (versus a saturated calomel electrode) are found at -0.65 v and -1.05 v.

The absorbance changes have been followed during the course of an electrolytic reduction at -0.85 v and -1.10 v, well defined isobestic points are observed (Fig. 2 A - B). They demonstrate that each reduction involves

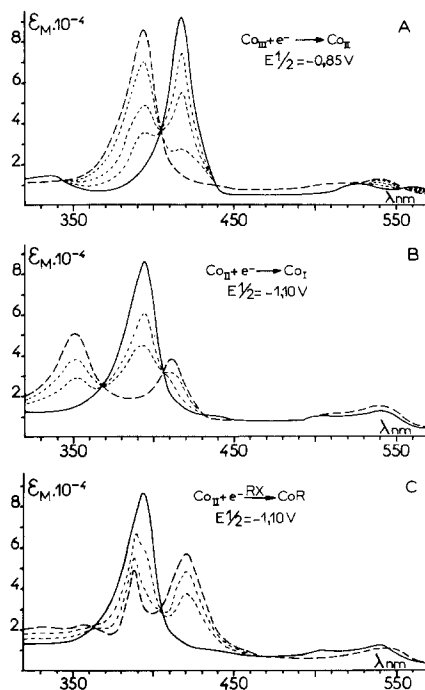


Figure 2 Optical evidence of PCo-His₂ redox reactions in dimethylformamide (TEAP 0,1 M) on platinum grid : A) réduction on the first plateau ; B) réduction on the second plateau ; C) similar reduction in the presence of RX(n-butyl bromide). ——— initial product, - - - final product, intermediate spectra.

only two spectral species. At -0.85 v, the new compound formed shows a absorption spectrum with two bands in visible region (540 and 510 nm) and a Soret band shifted towards the shorter wavelengths (394 nm) (Fig. 2 A). It is identical with that obtained with the deuteroporphyrin IX dimethyl ester -Co(II) in various solvents in the presence of imidazole, as ligand, in anaerobic conditions (7). When the EPR spectrum of the complex at 77 K was examined, it was shown to be a typical "one-base coordinated" spectrum of Co(II)(d⁷) with a super-hyperfine structure of $g_{//}$ multiplets (8) ($g_{\perp} = 2.315 \pm 0.010$, $g_{//} = 2.034 \pm 0.003$, $a_{//}^{Co} = 82.5 \text{ G} \pm 2.5$, $a_{//}^N = 17.5 \text{ G} \pm 2.5$).

Spectral changes during the reduction electrolysis at -1.10 v of the cobaltous form of PCo-His₂ are similar to those we assign to Co(I) complex (d⁸) with deuteroporphyrin dimethyl ester -Co(I) (6). The optical spectrum

shows that two coordinating nitrogenous bases are released whatever an electrochemical or chemical reduction procedure is employed. The spectrum of the Co(I) compound is similar to that of deuteroporphyrine dimethyl ester -Co(I) : 542, 510, 412 and 351 nm. By analogy we can say that the Co(I) ion in the model is no more axially liganded as it was already observed in B_{12r} reduction compound (4, 6). This complex would have a square-planer like structure.

Upon addition of a ten-fold excess of histidine (in substituted soluble dipeptide, N_α formyl-S(triphenylmethyl) cysteinyl histidine methyl ester) to a solution of the PCo-His₂, no modification of the electrochemical potential value ($E_{1/2}$) of Co(II) - Co(I) wave of the model compound is found. This fact confirms the presence of one histidine side chain coordinated to cobaltous ion and the relative stability of this covalently bound complex.

Addition of n-butyl bromide in excess to a dimethylformamide solution of Co(II) - model in anaerobic conditions and the reduction at -1.10 v do not result in the formation of Co(I). The absorption spectrum is composed of three peaks at 548, 422 and 388 nm and no isobestic point is observed during the electrolysis (Fig. 2 C). Momenteau and al (6) reported the absorption spectra of methyl derivatives of deuteroporphyrin dimethyl ester cobalt in dimethylacetamide and pyridine. They show that the Soret band positions at 389 and 417 nm respectively are assigned to pentacoordinated and hexacoordinated complexes. In the hexacoordinated complex, a molecule of pyridine is liganded to the central metallic ion in sixth position of coordination. The same spectral changes in the presence of imidazole has been observed in dimethylformamide with alkylated tetraphenylporphin - cobalt complex (9,10). By comparison with these studies, the spectrum recorded for the n-butyl derivative must correspond to one pentacoordinated and an hexacoordinated compounds mixture. Thus a partial coordination of histidine after the formation of the Co-C bond is observed.

The electrochemical behaviour of the bis(histidine methyl ester)

deuteroporphyrin IX - Co(III) reported in the present communication shows the reactivity of the metallic ion in different oxidation states, principally under the +1 redox state which is necessary to the formation of the Co-C bond. Further studies of this symmetrical complex depending upon various ligands by linear sweep voltametry are undertaken and should allowed us a better understanding of the redox behaviour of the coenzymes B₁₂.

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