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Effect of ascorbic acid on the photolysis of cyanocobalamin and aquocobalamin/hydroxocobalamin in aqueous solution: A kinetic study

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ABSTRACT

The photolysis of cyanocobalamin (B_{12}) and aquocobalamin (B_{12a})/hydroxocobalamin (B_{12b}) in the presence of ascorbic acid (AH_2) at pH 2.0–12.0, under aerobic conditions, has been studied. It follows first–order kinetics and the values of apparent first–order rate constants (k_{obs}) at pH 2.0–12.0 range from 0.37 to $2.63 \times 10^{-4}\,s^{-1}$ and 0.21–$6.35 \times 10^{-4}\,s^{-1}$, for B_{12} and B_{12a}/B_{12b}, respectively. The second–order rate constants (k_2) for the photochemical interaction of AH_2 and B_{12} and AH_2 and B_{12a}/B_{12b} range from 0.20 (pH 2.0) to $1.09 \times 10^{-2}\,M^{-1}\,s^{-1}$ (pH 5.0) and 5.88 (pH 2.0) to $91.08 \times 10^{-2}\,M^{-1}\,s^{-1}$ (pH 5.0), respectively. The values of the k_2 for AH_2–B_{12a}/B_{12b} interaction are 30–80 times greater than those of AH_2–B_{12} suggesting a greater susceptibility of B_{12a}/B_{12b} to photodegradation compared to that of B_{12} in this pH range. The k_2–pH profiles for both B_{12} and B_{12a}/B_{12b} are bell–shaped curves indicating the effect of AH_2 ionization on the rates of interaction. The complete discoloration of B_{12} and B_{12a}/B_{12b} solutions on prolonged photolysis indicates the formation of corrin ring cleavage (oxidation) products in acid and alkaline solutions. These oxidation products do not absorb in the visible region. Reaction schemes for the mode of photodegradation of B_{12} and B_{12a}/B_{12b} in the presence of AH_2 have been presented.

1. Introduction

Cyanocobalamin [α–(5,6–dimethylbenzimidazol–1–yl) cobamide cyanide] (vitamin B_{12}) (B_{12}) (1) is sensitive to light [1–3] and is degraded to hydroxocobalamin [Coα–[α–(5,6–dimethylbenzimidazolyl)]–Coβ–hydroxocobamide] (vitamin B_{12b}) (B_{12b}) (2) (Fig. 1) and further products in aqueous solution [4–6]. The photochemical behavior of B_{12} has been under study since it isolation in 1948 [7]. The earlier studies on the photolysis of B_{12} [8–18] indicated its loss on exposure to light, formation of B_{12b} and its further degradation to oxidation products by corrin ring cleavage of the molecule. Further studies of the effect of nicotinamide [19] and riboflavin [20,21] on the photolysis of B_{12} confirmed the formation of B_{12b} in the reaction. B_{12b} is also susceptible to photolysis [4,6,14,16,17], giving rise to unknown oxidation products. Hydroxocobalamin (B_{12b}) exists in equilibrium with aquocobalamin (B_{12a}) in aqueous solution [18]. The notation B_{12a} and B_{12b} for these compounds has been used in the literature [22–24].

$$[\underset{B_{12b}}{Co^{3+}OH}] \underset{OH^-;pKa=7.8}{\overset{H^+}{\rightleftharpoons}} [\underset{B_{12a}}{Co^{3+}OH_2}]^+ \quad (1)$$

A recent study of the effect of ascorbic acid on the chemical degradation of B_{12} [25] showed the formation of a colorless solution that may result from the compounds formed by the cleavage of corrin ring nucleus [26], and absorbing in the UV region.

In the absence of oxygen the photolysis of an aqueous solution of B_{12} coenzyme (5′–deoxyadenosylcobalamin) yields reduced cobalamin [B_{12r}] containing bivalent [Co^{2+}] and $CN^{\bullet}$ radical by homolytic cleavage [27].

$$\underset{B_{12}\text{coenzyme}}{[Co^{3+}CN]} \xrightarrow{h\nu} \underset{B_{12r}}{[Co^{2+}]} + CN^{\bullet} \quad (2)$$

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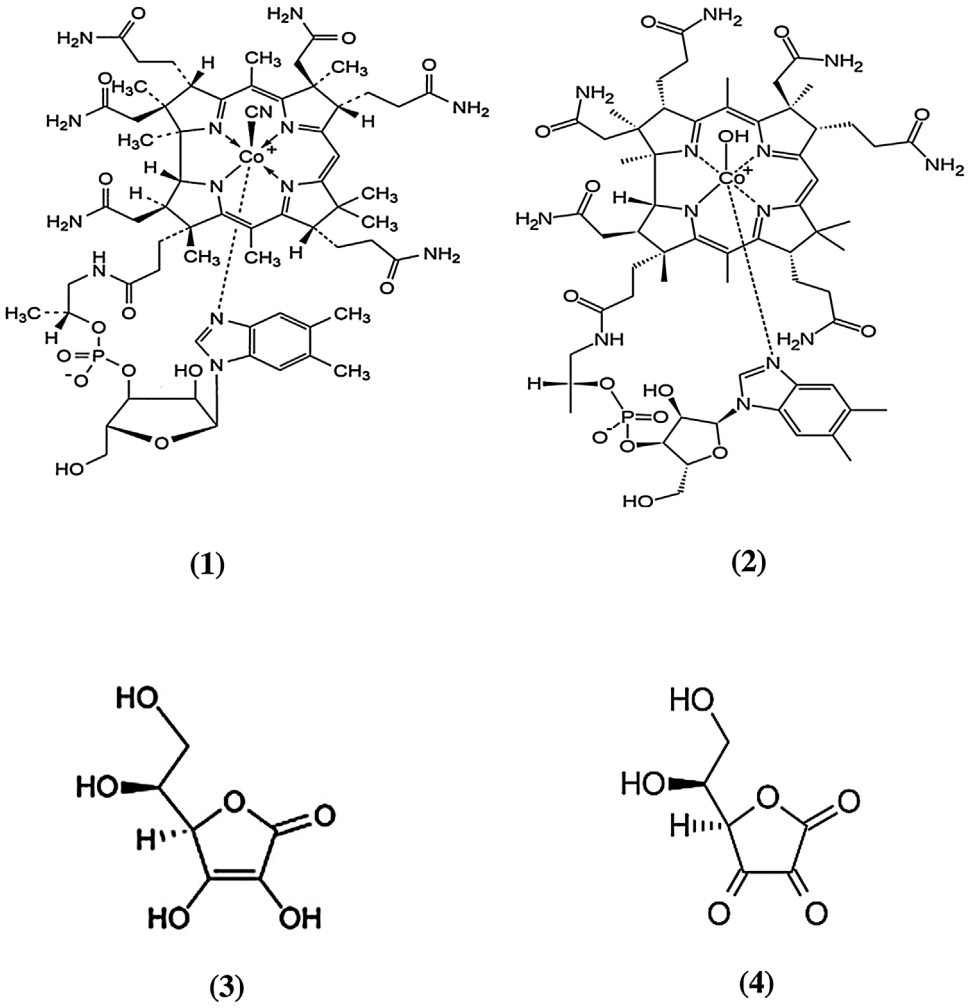

Fig. 1. Chemical structures of cyanocobalamin (1), hydroxocobalamin (2), ascorbic acid (3) and dehydroascorbic acid (4).

Alkyl–cobalamins such as methylcobalamin (MC) also, on photolysis, undergo homolytic Co–X bond cleavage to form B_{12r} and $CH_3^\bullet$ radical [28–30].

$$[Co^{3+}CH_3] \xrightarrow{h\nu} \begin{matrix}[Co^{2+}]\\ B_{12r}\end{matrix} + CH_3^\bullet \quad (3)$$

In the case of B_{12} the Co——CN bond does not photo–dissociate under simple photon excitation [31].

The fate of [Co^{3+} OH] (B_{12b}) on exposure to light of wavelength <350 nm is given by the reaction leading to the formation of [Co^{2+}] and an $OH^\bullet$ radical [32].

$$[Co^{3+}OH] \xrightarrow{h\nu} [Co^{2+}] + OH^\bullet \quad (4)$$

However, this does not apply to the photolysis of B_{12b} on exposure to light at wavelength >350 nm [33].

The photooxygenolysis of B_{12} in methanolic solution (CD_3OD) leads to the formation of two isomeric dioxosecocorrins by a regioselective oxygenolytic cleavage of the corrin macrocyclic ring [34].

Ascorbic acid (vitamin C) (AH_2) (3) is known to enhance the rate of chemical degradation of B_{12} [25,26,31,35–39], probably by promoting the formation of [Co^{2+}], the reduced form of B_{12} in the presence of a reductant [40]. The primary degradation product of AH_2, dehydroascorbic acid (DHA) (4) (Fig. 1), does not exert any effect on the oxidation of [Co^{2+}] [41]. So far the effect of AH_2 on the photolysis of B_{12} has not been reported. The present work is aimed to study the kinetics of photolysis of B_{12} and B_{12a}/B_{12b} in the presence of AH_2 in a wide pH range, to develop rate–pH relationships and determine the range of pH sensitivity of these compounds. The study would throw light on the photostability profile of B_{12} and B_{12a}/B_{12b}, their mode of interaction with AH_2 and subsequent photolysis reactions. It would have an impact on the stability of B_{12} in vitamin preparations.

2. Experimental

2.1. Materials

Cyanocobalamin (B_{12}), hydroxocobalamin (B_{12b}), ascorbic acid (AH_2) and dehydroascorbic acid (DHA) were obtained from Sigma–Aldrich. All reagents and solvents were of the purest form available from Merck. The following buffer systems were used throughout the work.

(a) For photolysis reactions:

KCl–HCl (pH 2.0), citric acid–Na_2HPO_4 (pH 2.5–8.0), H_3BO_3–KCl–NaOH (pH 9.0–10.0) and Na_2HPO_4–NaOH (pH 11.0–12.0).

The ionic strength was 0.002 M in each case.

(b) For assay of B_{12} and B_{12a} (B_{12b} exists as B_{12a} at pH 4.0):

CH_3COOH–CH_3COONa, 0.2 M (pH 4.0)