THE REDUCTION OF COBALAMIN*. A PULSE RADIOLYSIS STUDY

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SUMMARY

The reaction of B_{12} with e^-_{aq} and CO_2^- has been investigated by the pulse radiolysis technique. The decay of the e^-_{aq}, B_{12} and the formation of B_{12r} were found to be concomitant bimolecular processes with specific rates ranging from $5x10^{10}M^{-1}sec^{-1}$ at pH 6.1, to $3x10^{10}M^{-1}sec^{-1}$ at pH = 11.1.

The reduction of B_{12} by CO_2^- was also a bimolecular process with a specific rate of $1.2x10^9M^{-1}sec^{-1}$. CO_2^- was found to be more specific toward the metal ion (90% yield). No reaction whatsoever was detected when trying to reduce B_{12} with O_2^-. B_{12r} reacted with molecular oxygen.

INTRODUCTION

Vitamin B_{12} is a Co(III) complex of biological interest with a known structure (1). It has been subjected to many studies providing kinetic and stability data (2,3). The reduced form, B_{12r}, has been suggested as an active mediator in biological reactions (4).

The application of pulse radiolysis to the study of the reduction of molecules of biological interest by hydrated electrons or other reducing agents, and the subsequent electron transfer reactions in these species, was recently shown (5-7). This method enables to produce reducing species (e^-_{aq}, CO_2^- etc.) in a homogeneous aqueous solution within the microsecond time range.

In the present investigation on reduction of vitamin B_{12} by e^-_{aq} and CO_2^- it has been shown that whenever reduction occurs it is mainly on the Co(III) ion.

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* Abbreviations: Cyanocobalamin - Vitamin B_{12},B_{12}; Aquocobalamin - B_{12a}; Hydroxycobalamin - B_{12b}; Reduced cobalamin (Co(II)) - B_{12r}; Hydrated electron - e^-_{aq}.

EXPERIMENTAL

Materials - Cyanocobalamin (K & K crystallized) and hydroxycobalamin hydrochloride (Sigma) were used to prepare the solutions. All other materials were of analytical grade and were used without further purification.

Methods - The linear accelarator of the Hebrew University of Jerusalem was used for the pulse radiolysis. It was operated at 200 mA and 5Mev. The dose was varied by changing the pulse duration between 0.2 to 0.5 μsec to give a dose range of 400 to 800 rads, respectively (6). These pulses were found to produce 1.-2.x10^{-6} equivalents/liter of the reducing species (e^-_{aq}) as determined by ferrocyanide dosimetry (8).

Vitamin B_{12} solutions ($[B_{12}]$ = 1-3x10^{-5}M) were freshly prepared in triply distilled water before each experiment. All solutions contained 10^{-1}M tert-butanol which reacted with all OH radicals and converted them into the relatively unreactive $CH_2C(CH_3)_2OH$ radicals. No buffers were used and the pH was adjusted by the addition of dilute acid ($HClO_4$) or base (NaOH). Air was removed from the solutions by saturation with other gas (highly purified A, CO_2, O_2 or N_2O) which was bubbled for at least 30 min through the solutions. Samples were subjected to one pulse only. Spectra were obtained by reading the optical densities from oscilloscope traces at various wavelengths using glass filters to eliminate straylight. All traces were analysed for first and second order kinetics; rate constants were calculated only when a good decay curve plot was obtained for at least two half lives (linear correlation higher than 99%).

RESULTS AND DISCUSSION

When fast electrons are absorbed in liquid water, the effect may be described by the overall reaction:

$$H_2O \rightarrow e^-_{aq}\ ,\ H,\ OH,\ H_2O_2,\ H_2,\ H_3O^+$$

The ensuing chemical events occuring in a dilute aqueous solution can be accounted for in terms of the reactions of these initial entities (8).

The radiochemical yields (G = number of radicals, atoms or molecules formed per 100 eV) are $G_{e^-_{aq}} = 2.6$, $G_{OH} = 2.6$, $G_{H_2} = 0.45$, $G_{H_2O_2} = 0.75$ and $G_H = 0.55$.

When argon saturated solutions of $1\text{x}10^{-5}$M-$3\text{x}10^{-5}$M vitamin B_{12} containing 10^{-1}M tert-butanol (pH-6.5) were subjected to a single pulse, fast transmittance changes were observed over the whole wavelength range studied (650-290nm).

Saturation of these solutions with N_2O to convert the e^-_{aq} to OH radicals according to:

$$e^-_{aq} + N_2O \xrightarrow{H^+} N_2 + OH + OH^-$$

eliminated the above mentioned transmittance changes, this being a good evidence that tert-butanol was a good OH radical scavenger and that the radical $CH_2\ C(CH_3)_2OH$ did not react with the vitamin. We therefore conclude that in argon saturated solutions only the e^-_{aq} reacted with vitamin B_{12}.

The decay of the e^-_{aq} absorption band measured at $\lambda = 600$ nm was a bimolecular process having a rate constant of $5.2\text{x}10^{10}M^{-1}sec^{-1}$. This value is in good agreement with those found for the reaction of e^-_{aq} with other Co(III) complexes (9). The theoretical limit for a diffusion controlled reaction ($k_{diff.}$) may be calculated using the Smolochovski equation (10). The calculated value thus obtained, $5.0\text{x}10^{10}sec^{-1}$, is in good agreement with that observed experimentally.

At wavelengths lower than 600 nm, transmittance changes were mainly due to changes in vitamin B_{12} absorbance and to its reaction products. From the analysis of the decay curves of the e^-_{aq} and B_{12} and the formation curves of the product it became evident that the three processes were synchronous (Table I).

The amplitudes of the optical density changes in neutral solutions taken 40 μsec after the pulse are shown in figure 1. The spectrum obtained

TABLE I

Specific Rate Constants of the Reaction Between e^-_{aq}, CO_2^- and Vitamin B_{12} at Various Wavelengths

λ(nm)	$k \times 10^{-10}M^{-1}sec^{-1}$ $e^-_{aq} + B_{12}$ (at pH = 6.1)	$k \times 10^{-10}M^{-1}sec^{-1}$ $e^-_{aq} + B_{12}$ (at pH = 11.1)	$k \times 10^{-9}M^{-1}sec^{-1}$ $CO_2^- + B_{12}$
600	5.2±0.1	3.5±0.2	1.2±0.1
520	5.2±0.1	3.5±0.3	0.9±0.1
470	5.3±0.2	3.5±0.1	1.4±0.1
390	5.2±0.3	3.6±0.1	1.0±0.1
350	3.5±0.5	3.0±0.1	1.0±0.1
310	4.6±0.1	3.3±0.1	1.5±0.1

contains the characteristic absorption bands (λ_{max} = 600, 470, 390 and 310 nm) of B_{12r} as obtained by conventional reduction (3). The observed B_{12r} absorption bands were pH independent and no further changes with time could be observed. As expected, the 350 nm band in B_{12} solutions was shifted to 360 nm in alkaline solutions (B_{12h} at pH 11.1) (11). The efficiency of the $e^-_{aq} + B_{12}$ reaction was found to be 65%. It was calculated by comparing the e^-_{aq} concentration (known from dosimetry) to the experimental decrease of the B_{12} concentration calculated for the optical density changes at 310 nm ($\varepsilon_{B_{12}}$, 310 nm = 5850 $M^{-1}cm^{-1}$, $\varepsilon_{B_{12r}}$, 310 nm = 24300 M^{-1} cm^{-1}(3)). From this calculation it seems that 35% of the e^-_{aq} are reacting with another site or sites in B_{12} without further transfer of the electron to the Co(III) ion. This could be the 5,6 - dimethyl benzimidazol (D.M.B.) which is one of the ligands bound to the central Co(III) ion (the sugar ring and the amide bond are known to react slowly with e^-_{aq} (12)). The observed rate constant of $5.1x10^{10}M^{-1}sec^{-1}$, for the reaction of e^-_{aq}+ D.M.B. at pH 6.5, and the formation of a transient,

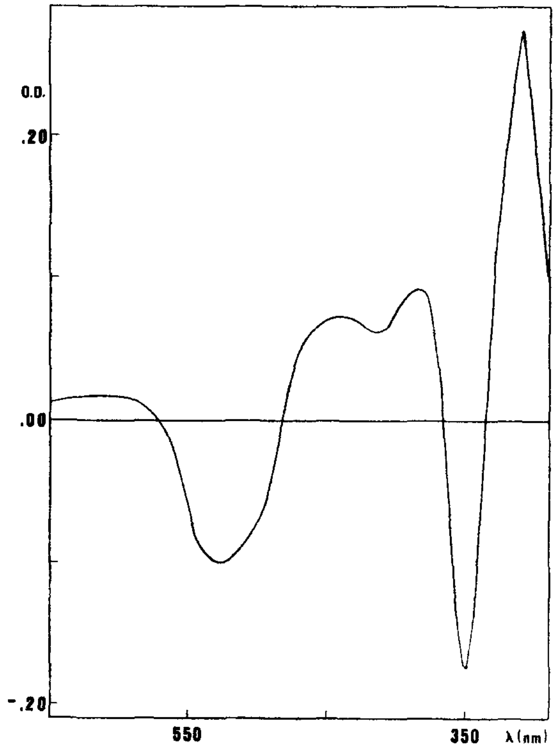


Fig. 1 Spectrum obtained in the reaction of e^-_{aq} and 10^{-5}M argon saturated solution of B_{12} containing 10^{-1}M tert-butanol (pH-6.1), taken 40 μsec after the pulse.

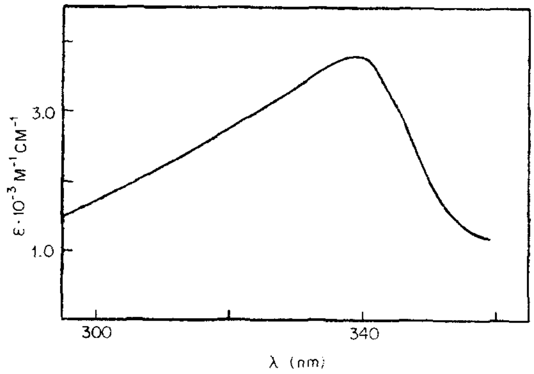
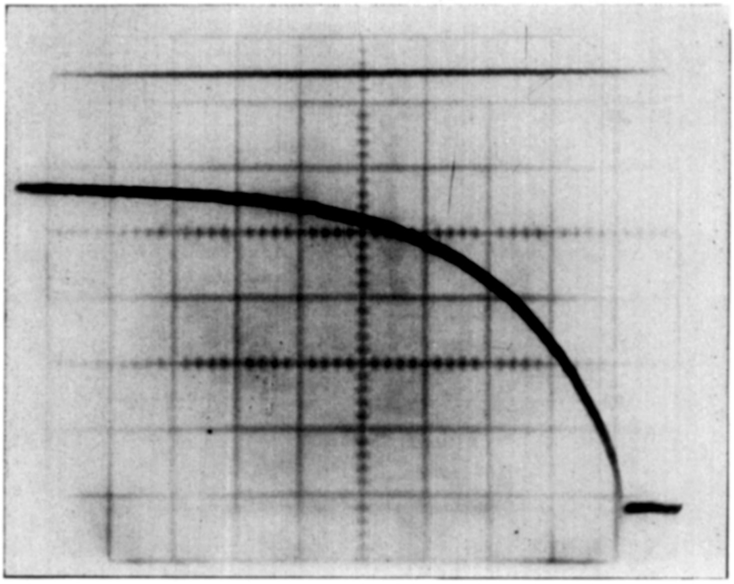
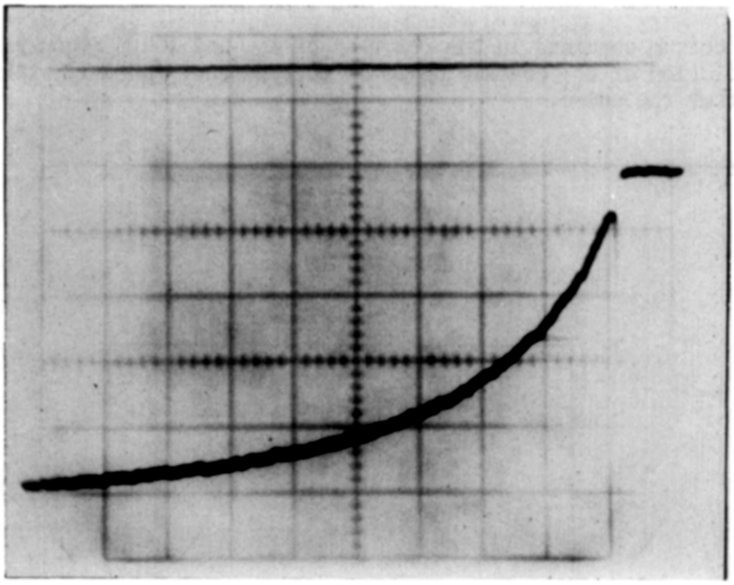


Fig. 2 Spectrum of transient obtained from the reaction e^-_{aq} + 5,6 - dimethylbenzimidazole in argon saturated solution.



1. λ = 390 nm, 4.17% /div. 50μsec/div.



2. λ = 530 nm, 4.45% /div. 50μsec/div.

Fig. 3 Oscilloscope traces of the decay at B_{12} and formation of B_{12r} obtained in the reaction of CO_2 and B_{12}, $[B_{12}]$ = 1.x10^{-5}M. Vertical displacement corresponds to changes in transmittance; horizontal to time.

λ_{max} = 340 nm; ε_{max} = 4000±200 $M^{-1}cm^{-1}$ (Fig.2), indicate the possibility that the e^-_{aq} will react with the D.M.B. ligand. However, the fact that in aqueous solutions the transient product of the D.M.B. reaction with e^-_{aq} decays at a rate of $4x10^7$ $M^{-1}sec^{-1}$ and no such behaviour could be observed in the experiments with B_{12} ,seems to eliminate this possibility. Thus, if the e^-_{aq} reacts with the D.M.B. ligand, it is transferred to the Co(III) ion in a very fast first order reaction ($\tau_{1/2}$ > 10^{-6}sec).

The e^-_{aq} being a most powerful and unspecific reducing agent, it was desirable to observe the reduction of B_{12} by a less powerful and thus more specific one. The CO_2^- ion radical is one of these species and could be obtained from the reaction: $CO_2 + e^-_{aq} \rightarrow CO_2^-$ ($k=7.7x10^9M^{-1}sec^{-1}$) (12). Solutions of $10^{-5}MB_{12}$ saturated with CO_2 in the presence of 10^{-1}M tert-butanol at pH 6.0 were pulse radiolysed. Under these conditions practically all the e^-_{aq} were converted to CO_2^- ($k_{e^-_{aq} + CO_2}[CO_2] >> k_{e^-_{aq} + B_{12}}[B_{12}]$). It was expected that CO_2^- will reduce B_{12} according to:

$$CO^-_{2aq} + B_{12} \rightarrow B_{12r} + CO_{2aq}$$

The rate constant of this reaction was obtained by observing the rate of appearance and of disappearance of absorption at various wavelengths(Fig.3). From these curves we obtained a rate constant of about $1.1x10^9M^{-1}sec^{-1}$(Table I).

The spectrum taken 4 m sec after the pulse, was similar to that shown in Fig.1. The efficiency of the CO_2^- to reduce the B_{12} was of the order of 90% which shows a very good specifity for the reduction of Co(III) in B_{12}.

Similarily to the reduction by CO_2^-, reduction of vitamin B_{12} by O_2^- , another less powerful reductant (E°=-0.45 V), was attempted. No reaction whatsoever was observed. However, a reaction of B_{12r} with O_2 was observed; its rate constant is of the order of 5 x 10^7 $M^{-1}sec^{-1}$.

REFERENCES

1. R. BONNETT, Chem. Rev. 63, 573, (1963).
2. R.J.P. WILLIAMS et al., J. Chem Soc. (A), (1969), 381, and references therein.

3. D. DOLPHIN, in "Methods in Enzimology" vol XVIII part C., D.B. McCornick and L.D. Wright Eds., Academic Press, New York, p. 35 (1969).

4. R. YAMADA et al., Arch. Biochim. Biophys. 117, 675, (1966).

5. E.J. LAND and A.J. SWALLOW, Biochim. Biophys. Acta. 162, 327, (1968); Biochemistry 8, 2117 (1969); J. Biol. Chem. 245, 1890 (1970); Arch. Biochem. Biophys., in press.

6. I. PECHT and M. FARAGGI, Proc. Nat. Acad. Sci U.S.A., 69, 902, (1972).

7. N.N. LICHTIN, J. OGDAN and G. STEIN, Israel J. Chem. 9, 579, (1971).

8. M.S. MATHESON and L.M. DORFMAN, "Pulse Radiolysis", M.I.T. Press,1969.

9. M. ANBAR and E.J. HART, in "Radiation Chemistry", R.F. Gould, Ed., Adv. Chem. Series No. 81 (Am. Chem. Soc.) 1968.

10. "COMPREHENSIVE CHEMICAL KINETICS", vol. II, C.H. Bamford and C.F.G. Tipper, Ed., Elsevier (1969).

11. J.A. HILL, J.M. PRATT and R.J.P. WILLIAMS, J. Theor. Biol., 3, 423, (1962).

12. M. ANBAR, P. NETA, Int. J. Appl. Rad. Isotopes, 18, 493, (1967).