Brönsted Basicity of Vitamin B12s

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Summary The pK_A of hydrido-cobalamin is determined from the E–pH diagram obtained from cyclic voltammetry of vitamin B 12r, adjusting the sweep rate to eliminate the effect of hydrogen evolution and the kinetic influence of the base-on/base-off reaction.

THE nature of the cobalt(I) derivative of vitamin B 12 (B 12s) has been studied recently especially its possible protonation to a hydridic structure.[1–5] It has been established that the cobalt hydride is formed in an acidic medium[5] although it decomposes with evolution of molecular hydrogen according to equation (1).[2–4]

$$Co^{I} + H^{+} \rightleftharpoons Co^{II} + 1/2\ H_2 \qquad (1)$$

This decomposition, which hampers the use of conventional analytical procedures below pH 5[2,3] has so far precluded the measurement of the pK_A of the cobalt hydride.

Any method devised for measuring the pK_A has to ensure that decomposition is negligible down to pHs lower than the pK_A. We now report such a method; the determination of the B 12r–B 12s equilibrium potential as a function of pH using cyclic voltammetry (CV). The equilibrium potential not only reflects the acid-base properties of B 12s but also those of B 12r. In B 12r the protonation affects the nitrogen atom of the 5-6-dimethylbenzimidazole in the nucleotide side-chain (Bzm.) Three forms of B 12r may therefore be present in solution; the base-on form, the base-off protonated form, and the base-off unprotonated form, the relative proportions of each depending upon the pH. We will refer to the pK_A of B 12r as pK_A (II) and that of B 12s as pK_A (I). pK_A (II) has been approximately estimated as 2·5 by e.s.r. spectroscopy.[6] It is expected that the two pK_A's will appear on the equilibrium E-pH diagram. The B 12r base-off species is more reducible than the base-on species, so the electrochemical reduction of B 12r is kinetically dependent upon the interconversion between the three possible species at pH's above pK_A (II).

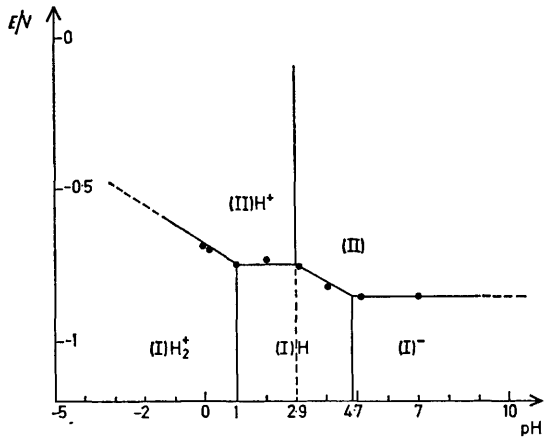

FIGURE 1. Cyclic voltammetry of vitamin B 12r; (A) $HClO_4$ 1·2 M, B 12r 2·28 mM (B) pH = 7·2, B 12r 1·1 mM, tetrabutyl ammonium *p*-toluenesulphonate 0·7 M.

Above pH = 2·9, the CV waves of vitamin B 12r are distorted by adsorption of the reactants but below this pH the reduction process is not significantly perturbed. Adsorption was eliminated by addition of a sufficient amount of tetrabutylammonium *p*-toluenesulphonate (0·7 M) to the solution and it was checked that further additions did not modify the CV curves.

Two types of behaviour are displayed by the reduction curves of vitamin B 12r; (i) below pH = 2·9 one reversible wave is observed between 0·5 and 250 Vs^{-1}. It is characterized by a separation between the cathodic and anodic peaks close to 56 mV. Only at very low sweep rates does a slight irreversibility appear accompanied by a tendency for the cathodic trace to become S-shaped instead of peak-shaped and for the anodic trace to be closer and closer to the cathodic one (Figure 1A). These observations are indicative of a fast charge transfer to B 12r leading to a Co^I species which slowly decomposes according to equation (1) giving rise to a slow catalytic process. Above 0·5 V s^{-1} the interference of this process is practically negligible so that the standard potential can be determined either as the mean value of the cathodic and anodic peak potentials or by adding 28 mV to the cathodic peak potential.

(ii) Above pH = 2·9, the CV pattern shows two cathodic and one anodic waves. The first cathodic wave tends to become more and more S-shaped on raising the sweep rate. As discussed in detail in ref. 7, these data indicate the interference of the base-off/base-on reaction into the reduction process. However, at low sweep rates, one reversible wave is obtained which features the reversible reduction of the three B 12r species at equilibrium (Figure 1B).

Here again there is no difficulty in determining the standard potential by the same methods as above, provided, this time, the sweep rate is low enough, *i.e. ca.* 0·02 V s^{-1}.

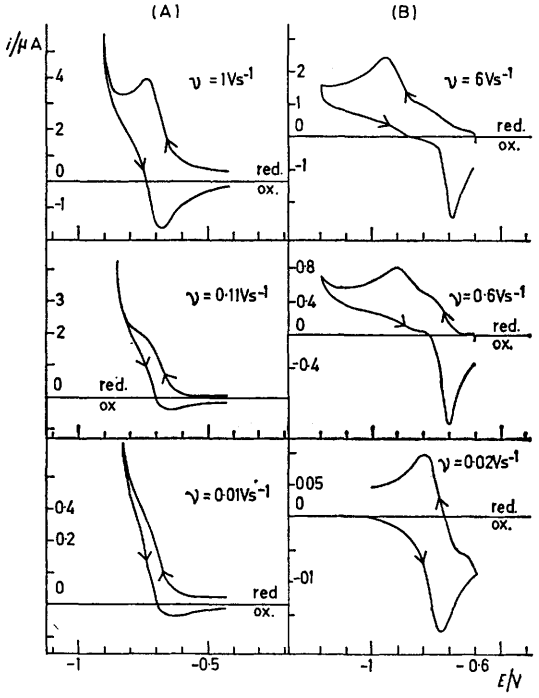

FIGURE 2. B 12r — B 12s standard potential *vs.* pH.

The E-pH diagram (Figure 2) is composed of two horizontal segments and two oblique segments with a slope close to 60 mV/pH unit. The pH values that show the transitions between them are successively 1, 2·9 and 4·7. The second value is close to the approximate result obtained previously for pK_A (II).[6] We have verified by acid-base titration under an argon atmosphere of a solution of B 12r prepared by exhaustive electrolysis of B 12a on platinum that 2·9 is indeed the correct value for pK_A (II). The third value is equal within experimental error to the pK_A of the free Bzm.

It may therefore be concluded with little ambiguity that pK_A (I) = 1. It follows that, in its range of stability, *vs.* $Co^I + H^+$, the hydridocobalamin bears a proton on the cobalt atom and also another one on the nitrogen atom of the Bzm. Furthermore the phosphate group in the nucleotide side-chain is also likely to be protonated in this

pH range.[8] However, this reaction will occur at nearly identical pH's in the Co^{II} and Co^{I} species leading to no detectable influence on the E-pH plot.

The value found for pK_A (I) emphasizes that the Brönsted basicity of vitamin B 12s is so weak that it can react in water as a powerful nucleophile in a large range of pH provided that the corresponding reactions are faster than the hydrogen evolution shown in equation (1).

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