OBSERVATIONS ON THE STRUCTURE OF COBAMIDE COENZYMES[1]

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The structure of the adeninylcobamide (AC) coenzyme has been shown to be based upon that of pseudovitamin B_{12} (Barker et al. 1960b, Weissbach et al. 1960). Both compounds contain the adeninylcobamide structure. The coenzyme differs from the vitamin by lacking a cyano group and containing an extra adenine nucleoside unit that can be released from the AC coenzyme by exposure to light. Actually the photolysis reaction is complex and two colorless, ultraviolet light absorbing compounds are formed in varying ratios, but the adenine nucleoside, previously referred to as the "peak 1 photolysis product" (Weissbach et al. 1960), is usually the more abundant. Further elucidation of the structure of the cobamide coenzymes requires a knowledge of the structure of the nucleoside and its sugar-like component, the mode of attachment of the nucleoside to the cobamide, and the influence of the nucleoside moiety on the properties of other parts of the coenzyme. We wish to report the results of some experiments on the structure of the nucleoside and its mode of attachment to the cobamide. These experiments have been done mainly with the crystalline 5, 6-dimethylbenzimidazolylcobamide (DBC) coenzyme (Barker et al. 1960a) but the AC and benzimidazolylcobamide (BC) coenzymes have also been used in some experiments.

The DBC coenzyme was previously shown to contain an adenine moiety that

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can be liberated by acid hydrolysis or by treatment with cyanide ion (Weissbach et al. 1959). Like the AC coenzyme, the DBC coenzyme also releases an adenine nucleoside on exposure to light. The identity of this nucleoside with that formed by photolysis of the AC coenzyme is indicated by close similarities of the two compounds with respect to the elution pattern from a Dowex-50 column, movement in paper ionophoresis at acid and alkaline pH, ultraviolet absorption spectrum ($E_{max.}$ at 258 mμ at pH 7, no shift of $E_{max.}$ in 0.1 N NaOH) and color reactions with orcinol and other sugar reagents.

The adenine and sugar components of the nucleoside moiety can be obtained by mild acid hydrolysis (0.1 N HCl, 100°, 90 minutes) of either the nucleoside or the DBC coenzyme. Because of the stability of the benzimidazole-ribose bond, no ribose is released from the coenzyme under these conditions. The products of hydrolysis are separated by differential elution from a Dowex-50 column. Adenine was identified by its spectral, chromatographic and ionophoretic properties (Barker et al. 1960a). The sugar showed the same properties, whether obtained by acid hydrolysis of the nucleoside or of the DBC coenzyme. By paper ionophoresis it was found to behave as a neutral molecule in acid (0.2M acetic acid pH 3.0) or alkaline (0.3M sodium carbonate pH 9.3) solution, whereas in 0.1M sodium tetraborate pH 9.3 it formed a complex that moved as an anion with a mobility 0.7 times that of picric acid. The sugar could not be identified with any common sugar, but it was shown to react with ferricyanide (Park and Johnson 1949), orcinol (Mejbaum 1939), periodate (Dixon and Lipkin 1954) and a number of other sugar reagents. The molar reducing value with ferricyanide and the molar absorbancy at 665 mμ in the orcinol test are approximately 0.85 and 0.23, respectively, of the corresponding values for ribose. The molar concentration of the sugar was determined by the absorbancy at 665 mμ in the orcinol reaction on the assumption that the sugar and the intact DBC coenzyme react equivalently in this test. In the orcinol test, the colored product derived from the sugar or from DBC coenzyme shows a second absorbancy maximum at 432 mμ which is from 1.5 to 3.0 times greater than that at 665 mμ depending on the exact experimental conditions. In the

periodate reaction one mole of sugar consumes approximately 3 moles of periodate and yields 3 moles of formate(Rabinowitz and Pricer 1957) but no formaldehyde (MacFadyen 1945). These results indicate that the sugar is an aldose with three vicinal hydroxyl groups.

The sugar has remarkably high R_F values on paper chromatography in solvents such as butanol-ethanol-water and butanol-acetic-water (4:1:5). In the latter solvent, for example, the R_F of the sugar (0.82) is almost twice that of glyceraldehyde (0.45). The sugar is readily detected with an indole-trichloroacetic acid reagent (Heyrovsky 1956) with which it gives a pink color on heating. The high R_F indicates that the sugar contains two or more non-polar groups. A 2-deoxy structure is excluded by the absence of malondialdehyde (Waravdekar and Saslaw 1959) as a product of periodate oxidation.

Although the nucleoside sugar has not been identified, its position of attachment to adenine has been established by studying the change in spectrum of the nucleoside as a function of pH. Over the pH range from 3.9 to 10.0 the spectrum is similar to that of adenosine, exhibiting an almost constant $E_{max.}$ at 280 mμ. At pH 1.9 both compounds have a slightly lower $E_{max.}$ at 256 mμ. These properties indicate that the nucleoside adenine is substituted in the N-9 position (Friedrich and Bernhauer 1956).

Both the displacement of adenine from the DBC coenzyme by cyanide ion and liberation of the nucleoside by photolysis, suggest that the nucleoside is attached to cobalt in the position occupied by the cyano group of cyano cobalamin. This possibility was further investigated by determining whether the nucleoside moiety interferes with the addition of cyanide to the cobalt. For this purpose the rates of cyanide addition to DBC coenzyme (containing the nucleoside) and to aquocobalamin (lacking the nucleoside) were compared at different concentrations of cyanide and hydrogen ion. The results indicate that under a variety of conditions DBC coenzyme reacts more slowly than aquo-cobalamin by at least two orders of magnitude. For example, at pH 4.0 (0.4 M sodium acetate) aquocobalamin reacts readily with 0.002 M HCN as judged by the absorbancy increase at 361 mμ; the conversion to cyanocobalamin

is 50% complete in approximately 7 minutes at 23° C. By contrast, no reaction of the DBC coenzyme was detected during 30 minutes exposure to 0.04 M cyanide under the same conditions. Thus the kinetic evidence also supports the view that the adenine nucleoside is closely associated with the cobalt atom of the DBC coenzyme.

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