BIOCHEMISTRY

Partial Synthesis of Vitamin B₁₂ Coenzyme and Analogues

THE structure of the vitamin B₁₂ coenzyme¹ has been determined recently by X-ray crystallography². It seemed possible (cf. ref. 3) that a synthesis of this compound, which contains a 5'-deoxyadenosyl group attached direct to the cobalt atom by a cobalt-carbon bond, could be achieved from reduced hydroxocobalamin and a suitably 5'-substituted adenosine derivative. 5'-Tosyladenosine is known⁴ to cyclize easily to the tosylate of *cyclo*adenosine but the 5'-tosyl-2',3'-isopropylidene derivative of adenosine can be prepared without difficulty⁴. Hydroxocobalamin was reduced in aqueous or ethanolic solution with sodium borohydride and reacted with this adenosine derivative. A product which had the properties expected of an isopropylidene derivative of the coenzyme was isolated from the reaction mixture in poor to moderate yield. Following a suggestion from Dr. V. M. Clark, whom we thank for his interest, we then attempted to reduce the cobalamin not just to the brown vitamin B₁₂, (ref. 5), believed to be a cobalt II derivative, but to the grey-green compound containing cobalt in a lower oxidation state⁶. This compound can be prepared under anaerobic conditions from hydroxocobalamin with sodium borohydride, chromous acetate in ethylenediamine tetracetic acid buffer at pH 9.5, or with zinc dust and dilute acetic acid. Under acid or alkaline conditions it reacts rapidly with a range of alkylating agents to give good yields of coenzyme analogues. In contrast, vitamin B₁₂, the exclusive product obtained from hydroxocobalamin and chromous acetate at pH 5, is quite unreactive.

The reaction product from fully reduced hydroxocobalamin and 5'-tosyl-2',3'-isopropylideneadenosine was purified via phenol extraction and passage of an aqueous solution through columns of DEAE-cellulose and CM-cellulose in series. The product passed rapidly through the former and more slowly through the latter as an orange zone which emerged as a red effluent. This was concentrated and crystallized from aqueous acetone. Mild acid hydrolysis (0.1 N hydrochloric acid for 16 hr. at room temperature or 15 min. at 90°) gave the vitamin B₁₂ coenzyme as the main product. It was fully active in the enzyme assay of Abeles and Lee⁷; the absorption spectra in neutral and acid solution and the *R_F* value on paper chromatography with wet *sec.*-butanol were the same as those of the natural B₁₂ coenzyme. On treatment with cyanide it yielded adenine and dicyanocobalamin.

Analogous experiments using 5'-tosylinosine or 5'-tosyluridine yielded crystalline products with absorption spectra and other properties similar to those of the coenzyme. In the enzyme assay, however, these analogues behaved as powerful competitive inhibitors of the coenzyme. Furthermore, alkyl halides and sulphates, for example, ethyl iodide and dimethyl sulphate, react with fully reduced cobalamin in aqueous or alcoholic solutions, the colour changing almost instantly from greenish-black to red or orange. Crystalline products have been isolated which presumably contain the alkyl group linked to cobalt. ¹⁴CH₃I yielded the methyl analogue of the coenzyme, which had the same specific activity as the methyl iodide. Similar products have been obtained from reactions with ethylene bromhydrin, chloroacetic acid, acetyl chloride and benzyl chloride. Some of

these products also inhibit the natural coenzyme in the enzyme system.

Work is in progress to characterize these and related compounds and their degradation products, to measure their inhibition indices and to study their effects in biological systems.

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¹ Barker, H. A., Smyth, R. D., Weissbach, H., Toohy, J., Ladd, J. N., and Volcani, B. E., *J. Biol. Chem.*, **235**, 480 (1960).

² Lenhert, P. G., and Hodgkin, D. C., *Nature*, **192**, 937 (1961).

³ Brady, R. O., and Barker, H. A., *Biochem. Biophys. Res. Comm.*, **4**, 373 (1961).

⁴ Clark, V. M., Todd, A. R., and Zussman, J., *J. Chem. Soc.*, 2952 (1951).

⁵ Diehl, H., and Murie, R., *Iowa State J. Sci.*, **24**, 555 (1952). Jaselskis, B., and Diehl, H., *J. Amer. Chem. Soc.*, **76**, 4345 (1954).

⁶ Beaven, G. H., and Johnson, E. A., *Nature*, **176**, 1264 (1955).

⁷ Abeles, R. H., and Lee, H. A., *J. Biol. Chem.*, **236**, 2347 (1961).

Acetylcholinesterase Activity in *Nitella*

It is generally accepted that the process of excitation starts with a localized depolarization of the conducting cell membrane. The adjacent region is then depolarized by the resultant current flow. This process repeats itself at successive points and thereby the impulse is propagated. These electrical phenomena have been widely investigated, but the underlying mechanism is still under discussion. Nachmansohn¹ has suggested a theory according to which the acetylcholine system plays an essential part in bioelectrogenesis. Much evidence has been accumulated in favour of this theory during recent years. The evidence is based on experiments with animal cells, mostly nerve and muscle fibres of vertebrates and invertebrates. However, there are plants which resemble nerve and muscle in being able to propagate impulses by electric currents. There is an excitable membrane separating the cell from its environment, in which a sharp decrease in electrical impedance occurs during the passage of an impulse.

Nitella flexilis is a freshwater alga with large single cells. When an internode is stimulated, an action current is generated and conducts the impulse². It appeared of interest to investigate whether the acetylcholine system is present in *Nitella*. There is a considerable number of difficulties to be overcome for testing this problem. But it appeared possible to test whether acetylcholinesterase is present. If the presence of this enzyme were established, this would be an indication that the substrate may also be present and may be hydrolysed there. It has been found that *Nitella* indeed is capable of hydrolysing acetylcholine. This communication is a preliminary report on the properties and concentration of the cholinesterase in *Nitella*.

The branches were dissected in small particles, dried on filter paper and pre-soaked in the incubation fluid for 1 hr. In previous experiments with other tissues it had been observed that in the first hour the rate of hydrolysis seemed to be much higher than in the following hours. Apparently some substrate is taken up by the tissue. After the pre-incubation the tissue was dried, weighed, homogenized, and then placed into the incubation fluid. Following such