Structure of Dihydrodiphosphopyridine Nucleotide

WEBER[1] has recently reported on the intramolecular transfer of excitation energy in dihydrodiphosphopyridine nucleotide. In addition to the strong band having its maximum at 340 mμ, the excitation spectrum of the 462 mμ fluorescence also shows a maximum at 260 mμ, a region where the absorption is due to the purine part of the molecule.

Kaplan *et al.*[2] have identified an isomer of diphosphopyridine nucleotide which comprises 10–15 per cent of a highly purified commercial preparation of diphosphopyridine nucleotide. The isomer was characterized by optical rotation studies as the α-glycoside of nicotinamide riboside as distinct from the biologically active β-isomer of diphosphopyridine nucleotide. This assignment of configuration about the C_1 of ribose was substantiated by the synthetic preparation of nicotinamide mononucleotide[3] to give a mixture of the β- and α-anomers in a 4 : 1 ratio.

The α-isomer of diphosphopyridine nucleotide was reduced chemically with sodium dithionite[4] and the product was examined spectrofluorometrically. Unlike the β-isomer, the excitation spectrum of the 462 mμ fluorescence of α-dihydrodiphosphopyridine nucleotide shows a strong band only when activated at 340 mμ and not when irradiated at 260 mμ, indicating that excitation energy transfer is not as efficient with the α-isomer as was observed with the β-isomer. The 462 mμ fluorescence resulting from 260 mμ and 340 mμ excitation of α- and β-dihydrodiphosphopyridine nucleotide is shown in Fig. 1.

Further evidence for the differences in interaction in the α- and β-isomers of dihydrodiphosphopyridine nucleotide may be found in their spectrophotometric behaviour in water and propylene glycol. Weber[1] reported that the ratio E_{260}/E_{340} is 2·85 for the intact β-isomer of dihydrophosphopyridine nucleotide, whereas this ratio becomes 3·4 in propylene glycol. A similar examination of the reduced α-isomer reveals that the E_{260}/E_{340} ratio is the same in water as in the propylene glycol.

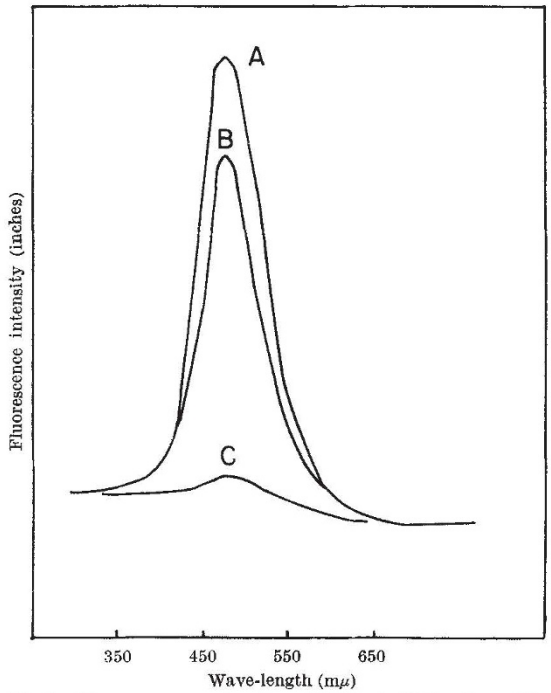

Fig. 1. Fluorescence spectra of α- and β-dihydrodiphosphopyridine nucleotide. Curve *A*, α- and β-forms excited at 340 mμ; curve *B*, β-form excited at 260 mμ; curve *C*, α-form excited at 260 mμ. These values are relative since the intensities of the exciting light have not been determined

Studies similar to those described for the α-isomer of dihydrodiphosphopyridine nucleotide have been carried out with deamino-dihydrodiphosphopyridine nucleotide (the hypoxanthine analogue[5]) and with the 3-acetylpyridine analogue[6]. The latter analogue clearly showed the transfer of excitation energy since excitation at 260 mμ gives a 478-mμ fluorescence[7] which is also elicited by excitation at 365 mμ. The absorption spectrum of this analogue behaved much like β-dihydrodiphosphopyridine nucleotide in water and propylene glycol. The deamino-dihydrodiphosphopyridine nucleotide, however, did not show properties of energy transfer, since excitation of the purine ring did not result in fluorescence of the dihydropyridine nucleus.

In accordance with the postulate by Weber[1] that the adenine and the dihydropyridine rings form a complex in β-dihydrodiphosphopyridine nucleotide, it appears that such complex formation cannot occur in the α-isomer.

Further studies on the structural conformation of diphosphopyridine nucleotide and dihydrodiphosphopyridine nucleotide are being examined in this laboratory through the use of pyridine coenzyme analogues.

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A Possible Negative Feedback Phenomenon controlling Formation of Alkaline Phosphomonoesterase in *Escherichia coli*

WE have already reported[1] that in a phosphate-deficient medium the amount of ribonucleic acid in *E. coli* decreased remarkably, while there was an appreciable increase of deoxyribonucleic acid, protein and viable cell numbers. At the same time we also studied activities of some enzymes presumed to be related to the degradation of ribonucleic acid, for example, ribonuclease phosphodiesterase and phosphomonoesterase, and found a marked increase in that of phosphomonoesterase. This communication is concerned with this formation of phosphomonoesterase in *E. coli*.

The strain used was *E. coli B* and the medium contained per litre: 3·0 gm. NaCl, 0·25 gm.