SPECTROPHOTOMETRIC STUDIES OF DIPHOSPHOPYRIDINE NUCLEOTIDE TRYPTOPHAN INTERACTION

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Recently two different types of experimental evidence have appeared which may shed some light on the nature of the interaction between pyridine nucleotide coenzymes and the various apoenzymes. A characteristic fluorescence spectrum is observed when the DPNH*-apoenzyme complexes are excited with light at 340 mu, the DPNH absorption maximum. The same fluorescence spectrum is obtained when the complex is activated at 280-290 mu by several enzymes including lactic dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase (Velick 1959), and glutamic dehydrogenase (Frieden 1960). This implies a possible energy transfer process from the aromatic amino acids in the protein structure to the dihydro-nicotinamide moiety of the coenzyme-apoenzyme complex.

Within the last few months two reports have appeared describing an interaction of indole derivatives (including tryptophan) with nicotinamide derivatives (including DPN, TPN, N-benzyl nicotinamide, and N-methyl nicotinamide) to form a yellow color complex in alkaline aqueous solutions (Cilento and Giusti 1959, also Alivisatos et al 1960). Moreover, Cilento and Giusti report

*Abbreviations: DPNH dihydro-diphosphopyridine nucleotide, DPN diphosphopyridine nucleotide, and TPN triphosphopyridine nucleotide

that tryptophan is the only amino acid capable of this interaction with nicotinamide derivatives. The possible significance of the last two reports in explaining the coenzyme-apoenzyme interaction prompted an investigation of the reaction in this laboratory.

Method:

Reagents used were DPN (Sigma beta DPN 98% pure), L-tryptophan (California Corporation for Biochemical Research, grade A (CfP), and N-benzyl nicotinamide (Bios).

Attempts to measure the difference spectrum were made in two different ways using the Cary model 11 recording spectrophotometer. By one method individual absorption spectra were obtained for tryptophan and DPN at 8×10^{-5} M concentration against water in the reference cell. The summation of the individually determined spectra for DPN and tryptophan was compared with an independently measured spectrum of combined DPN and tryptophan, each at 8×10^{-5} M.

In an attempt to detect a possible difference spectrum having a very low extinction coefficient the following "tandem cell" technique was employed (Laskowski et al 1960, and Foss 1960). Two cuvettes were placed in tandem in each beam of the spectrophotometer. Unmixed solutions of tryptophan and DPN were placed in separate cells in the reference beam of the instrument. Mixed solutions of tryptophan and DPN were placed in both cuvettes of the test beam of the instrument. Concentrations of the unmixed solutes were adjusted to twice that of the mixed solutions since the light path was half as long as through the former solutions. Using this technique it is possible to directly record difference spectra at higher solute concentrations than is possible by the method described in the previous paragraph.

Spectrophotometric studies were carried out at acid and

alkaline pH values (approximately 6 and 11) and at concentrations up to 4 X 10^{-3} M in each solute.

N-benzyl nicotinamide was also examined for interaction with L-tryptophan by the "tandem cell" technique.

Results and Discussion:

Spectrophotometric studies by the methods described above failed to reveal evidence of interaction between L-tryptophan and DPN (or N-benzyl nicotinamide) in the visible or in the ultraviolet range of the spectrum.

Though Alivisatos et al used buffered solutions Cilento and Giusti did not. Therefore the lack of a difference spectrum in our experiments can not be attributed to this difference in technique.

DPN alone is yellow in alkaline solutions; however, the DPN tryptophan mixed solution spectrum is precisely the same as that obtained by adding the individually recorded DPN and tryptophan spectra. Alivisatos et al reported difference spectra resulting from the interaction of these two solutes in the ultraviolet region of the spectrum. A correction added to reprints distributed after publication attributed this to light scattering. We also have observed artifacts in the ultraviolet range of the spectrum under conditions of very high absorbance.

It is possible to experimentally produce similar absorption peaks, where none exist, by recording difference spectra at very high optical densities (Alan H. Mehler also Fridovitch et al). Anomalous absorption spectra may be accounted for by stray light or fluorescence in the system as previously reported (Mehler et al, also Buell and Hansen).

Alivisatos et al employed solutions with very high optical densities (60 to 90 optical density). The spectrum of tryptophan with an optical density of 6 determined against a tryptophan

solution of 5 optical density in the reference beam of the spectrophotometer has false peaks at 255 and 295 in our instrument. These peaks are both in the spectral region where the optical density of the solution is changing rapidly with wavelength. The exact position and amplitude of these peaks vary with the concentration of the tryptophan and probably also with the particular instrument used. These false peaks we attribute to increased intensity of stray light as the slit width is increased to a maximum in the high optical density region of the true absorption curve. Examination of the data of Alivisatos et al reveals that all four spectra given fall in regions of rapid optical density change with wavelength regardless of the region of the spectral region where this rapid change takes place.

The limited description of Cilento and Giusti's work does not allow scrutiny for a similar explanation of their results.

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