Intramolecular Transfer of Electronic Energy in Dihydro Diphosphopyridine Nucleotide

DIHYDRO diphosphopyridine nucleotide shows blue-white fluorescence ($\lambda_{max.}$ = 468 mμ) in water solution[1] with a quantum yield[2] of 2 per cent on excitation by mercury 366 mμ radiation. Several reduced N-alkyl nicotinamides show fluorescence almost identical in spectrum and quantum yield with that of dihydro diphosphopyridine nucleotide. The excitation spectrum of the fluorescence[3] of these reduced N-alkyl nicotinamides (Fig. 1) shows complete coincidence with the fractional absorption spectrum over the whole range of wave-lengths explored (220–410 mμ). The fluorescence-excitation spectrum of water solutions of dihydro diphosphopyridine nucleotide at *p*H 7–9 shows a further strong band with a maximum at 260 mμ, a region where 80–90 per cent of the absorption is due to the purine part of the molecule. From these observations it appears that 30 per cent of the photons absorbed by the adenine appear as nicotinamide fluorescence. The integrity of the molecule is required for this energy transfer to show itself: after a solution of dihydro diphosphopyridine nucleotide at *p*H 8·3 has been acted upon by nucleotide pyrophosphatase[4] the 260 mμ band of the fluorescence-excitation spectrum disappears, the resulting spectrum becoming similar to that of the reduced N-alkyl nicotinamides. The enzyme hydrolyses the P—O—P linkage and causes the purine and nicotinamide nucleotides to become independent molecules, which in a 9×10^{-6} *M* solution, the concentration of the experiments, are at an average distance of 300 A. of each other. Solutions of dihydro diphosphopyridine nucleotide in propylene glycol show no detectable 260 mμ band in the fluorescence-excitation spectrum, while solutions in 60 per cent sucrose in water show a 260 mμ band the intensity of which is four-fifths of that in pure water.

Observations of the polarization of the fluorescence of sucrose solutions (Table 1) show that in dihydro diphosphopyridine nucleotide, excitation with either mercury 366 or mercury 254 results in fluorescence with positive polarization which increases rapidly with the viscosity of the solvent. From the rate of change of polarization with viscosity, and using a value of 900 cm.³ for the molar volume of dihydro diphosphopyridine nucleotide in solution, a lifetime of the excited state of $0{\cdot}5 \times 10^{-9}$ sec. can be calculated[5], consistent with the low quantum yield. The values of the polarization for excitation by mercury λ 254 agree with the lifetime calculated for excitation with mercury λ 366. Moreover, they are conspicuously higher than the values obtained on excitation by mercury λ 254 of N-methyl nicotinamide in 60 per cent sucrose, or dihydro diphosphopyridine nucleotide in propylene glycol, thus showing that the transfer of the excitation energy absorbed at 254 mμ in aqueous dihydro diphosphopyridine nucleotide results in the emission of polarized fluorescence and does not lengthen appreciably the lifetime of the excited state of the nicotinamide nucleus. This excludes a metastable state of the adenine as a source of the transfer and indicates a coupled oscillator mechanism[6] as the likely one. In agreement with other authors[7] no fluorescence was observed in solutions of adenine or adenylic acid although a quantum yield of 0·1 per cent would have been detected by the method used. Thus the transfer has to compete with a radiationless transition with a probability of 10^{11} or higher (reciprocal of natural lifetime times quantum yield). A probability of transfer of 10^{11} requires short-range interaction of the molecules concerned[8] and points to the existence in dihydro diphosphopyridine nucleotide of an intramolecular complex similar to the one already described in flavin adenine dinucleotide[9] between the adenine and the *iso*alloxazine. Further evidence for the existence of this complex is given by a study of the absorption spectrum: the ratio E_{260}/E_{340} is 2·85 for intact dihydro diphosphopyridine nucleotide in water, increasing to 3·3 after treatment with nucleotide pyrophosphatase, whereas solutions in propylene glycol show $E_{260}/E_{340} = 3{\cdot}4$.

Table 1. FLUORESCENCE POLARIZATIONS *P* FOR EXCITATION WITH NATURAL LIGHT. OBSERVATION AT 90° TO THE DIRECTION OF EXCITATION

Substance	Solvent viscosity (centipoises)	*P* Mercury-366	*P* Mercury-254
Dihydro diphosphopyridine nucleotide in 60 per cent sucrose	55	0·192	0·055
Dihydro diphosphopyridine nucleotide in 40 per cent sucrose	6	0·158	0·04
Dihydro diphosphopyridine nucleotide in 20 per cent sucrose	2	0·091	0·025
Dihydro diphosphopyridine nucleotide in propylene glycol	56	0·159	<0·02
4 Hydro N-methyl nitocinamide in 60 per cent sucrose	55	0·105	<0·02

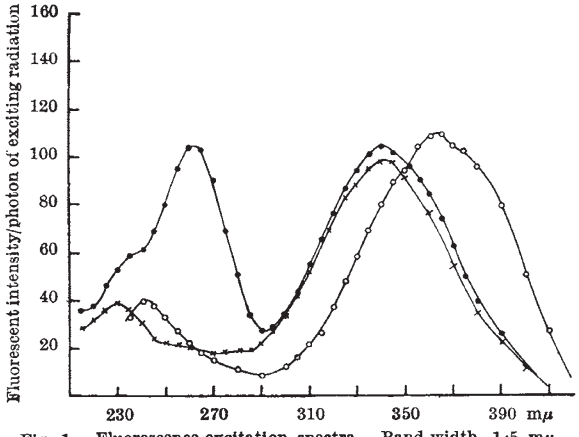

Fig. 1. Fluorescence-excitation spectra. Band-width, 1·5 mμ. Open circles, 4 hydro N-methyl nicotinamide in 0·1 *M* sodium carbonate; dark circles, dihydro diphosphopyridine nucleotide in 0·1 *M tris* buffer *p*H 8·3; crosses, dihydro diphosphopyridine nucleotide in 0·1 *M tris* buffer *p*H 8·3 after incubation for half an hour at 38° with nucleotide pyrophosphatase. The light absorption by the enzyme is negligible throughout

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