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Influence of solvents on the intramolecular energy transfer in NADH and NADPH

WEBER[1] and VELICK[2] have shown that in aqueous solutions of NADH the excitation energy of the adenine moiety can be transferred to the nicotinamide moiety: the radiation of 260 mμ absorbed mostly by the adenine excites the fluorescence of the nicotinamide with quite the same effectiveness as the 340 mμ radiation absorbed by the nicotinamide itself. Such an intramolecular energy transfer does not take place when NADH is dissolved in propylene glycol or in ethylene glycol monomethyl ether.

These results were interpreted by WEBER as connected with the fact that the NADH molecule can exist in different configurations: the "folded" one in which the rings of adenine and nicotinamide are in close proximity and the "unfolded" form in which these rings are separated. Evidently the first configuration would be more stable in aqueous solutions and the second in the organic solvents.

Exploratory experiments with B. H. J. BIELSKI (Brookhaven National Laboratory, Upton, N.Y., U.S.A.) had indicated that in methanol, NADH showed little energy transfer between its moieties. The present paper is concerned with the dependence of the probability of energy transfer upon the concentration of methanol in water–methanol mixtures.

The preparations of NADH that we used were from "Serva" (Heidelberg) and "Calbiochem" (U.S.A.); the preparation of NADPH was from "Serva". No significant difference in fluorescence between the two preparations of NADH was observed. According to the certificates from the firms, the preparations contained 95 % of the reduced form of the corresponding substances. Methanol was purified on a column with 25–30 theoretical plates; water was twice distilled in quartz; Tris for the buffer was purified by sublimation and the hydrochloric acid by distillation.

The fluorescence was excited by the lines of mercury 334 and 247.5 mμ, isolated from the spectrum of a super high-pressure mercury lamp (1000 W) with the aid of the monochromator SP-2 (Zeiss, Jena). All measurements were made at room temperature in the same cuvette fixed in front of the cathode of a photo-multiplier tube. The stray light was excluded by an appropriate (blue-green) filter which did not transmit the exciting light.

In spite of the careful purification the solvent had a weak fluorescence when excited by 247.5 mμ. To eliminate this effect of background, measurements were made first with only solvent in the cuvette; then the solvent was removed with a syringe and the NADH solution was introduced with a syringe into the same unmoved cuvette and measurement was repeated. Subtraction of the first value from the second gave the net fluorescence of the investigated substance.

The results are presented on Figs. 1 and 2. As one can see from Fig. 1, the fluorescence intensity at 334 mμ increases gradually to nearly twice the value when water was replaced as solvent by methanol. Concurrently the intensity of the fluorescence excited by 247.5 mμ decreases. This is evidence that the probability of the energy transfer from the adenine moiety to the nicotinamide diminishes. The energy transfer virtually disappears in a mixture with a high methanol content.

The ratio of the fluorescence intensities at the two wavelengths of excitation ($I_{247.5}/I_{334}$) can be regarded as a measure of the probability of the energy transfer

or of the average degree of folding of the dinucleotide molecules. As one can see from Fig. 2, this probability drops approx. 4 times in solvents with methanol in high concentration. The residual fluorescence excitation at 247.5 mμ can be due in considerable degree to the weak absorption of the exciting light by the nicotinamide moiety itself.

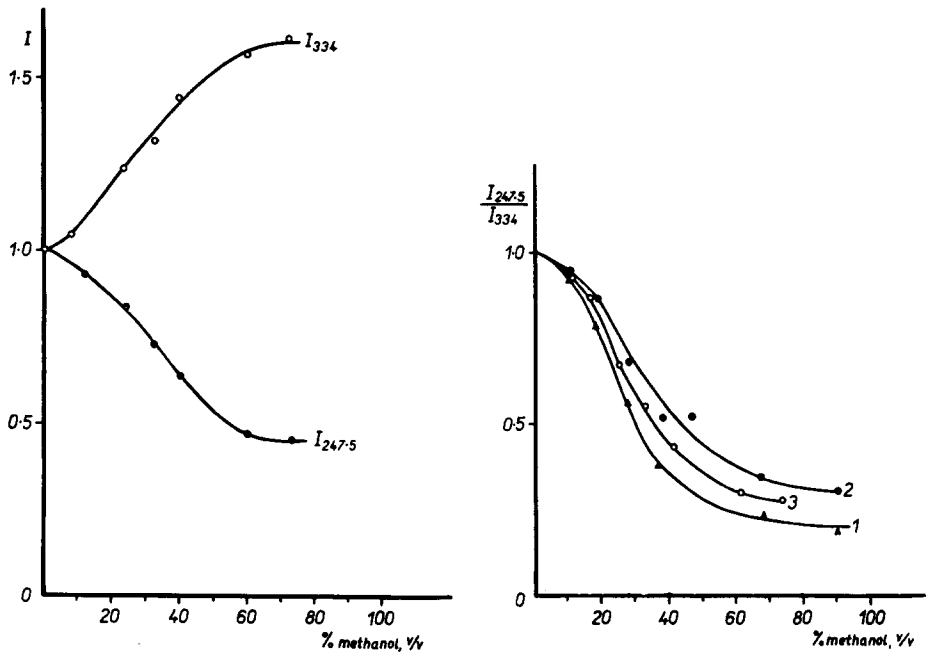

Fig. 1. Relative intensities of fluorescence of NADH and of NADPH dissolved in methanol–water as function of methanol concentration with excitations at 247.5 mμ and at 334 mμ. Temperature, 22°.

Fig. 2. Decrease in probability of energy transfer or of degree of folding within molecules as function of methanol concentration. The relative probabilities are measured by the ratios of fluorescent intensities excited at the two wavelengths 247.5 mμ and 334 mμ. 1 and 2, NADPH and NADH dissolved in 10^{-3} M water–methanol; 3, NADH in mixtures in which Tris–HCl buffer is present in amounts which would produce pH 9 and $I = 0.05$ in a similar volume of water alone.

Little significant difference was found in the results obtained with and without the Tris buffer, pH 9.0, $I = 0.05$. (The quantities of HCl and Tris contained in the water–methanol mixtures were the same as in an equal volume of water possessing pH 9, $I = 0.05$)

With NADPH we obtained similar effects (Fig. 2, Curve 1).

VELICK referred to the possible connection between the magnitude of the dielectric constant of the medium and the degree of folded character of the dinucleotide. The dielectric constant of a mixture of methanol and of water is almost linearly dependent on the volume per cent of either component. No simple quantitative relationship was found between the average dielectric constant of the mixtures and the probability of energy transfer. That factors other than electrostatic ones may play

an important role is brought out by the fact that the charged particles of the buffer ($I = 0.05$) had little effect on the probability of energy transfer.

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