CHARGE TRANSFER AND EXCITATION ENERGY TRANSFER BETWEEN REDUCED AND OXIDIZED PYRIDINE NUCLEOTIDES

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The purpose of this communication is to describe two types of interaction between reduced and oxidized pyridine nucleotides.

1. Charge Transfer Interaction

Frozen mixtures of reduced and oxidized pyridine nucleotides in 0.1 M Tris-HCl, pH 8.3, show an intense, bright yellow color. The color disappears as the mixture is warmed to room temperature and is not produced in frozen solutions of either the oxidized or the reduced form of pyridine nucleotides. The above phenomenon is clearly noticeable in pyridine nucleotide mixtures containing as little as 10 mM DPN plus 2 mM DPNH. Spectra of DPN-DPNH mixtures at 77° K are shown in Fig. 1. It is seen that 2 mM DPNH alone has very little absorption between 390 mμ and 500 mμ**. However, addition of increasing amounts of DPN, which by itself has negligible absorption in this range, results in a considerable increase in the absorbancy of the mixture. Double reciprocal Benesi-Hildebrand plots of DPN concentration vs. absorbance between 390 and 420 mμ yield straight lines as shown in Fig. 2. The abscissa intercept corresponds to a complex formation constant of $K = 175\ M^{-1}$ and the

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** The interruption of spectra below 390 mμ is due to limitations (lack of quartz optics) of the instrument used for low temperature spectroscopy.

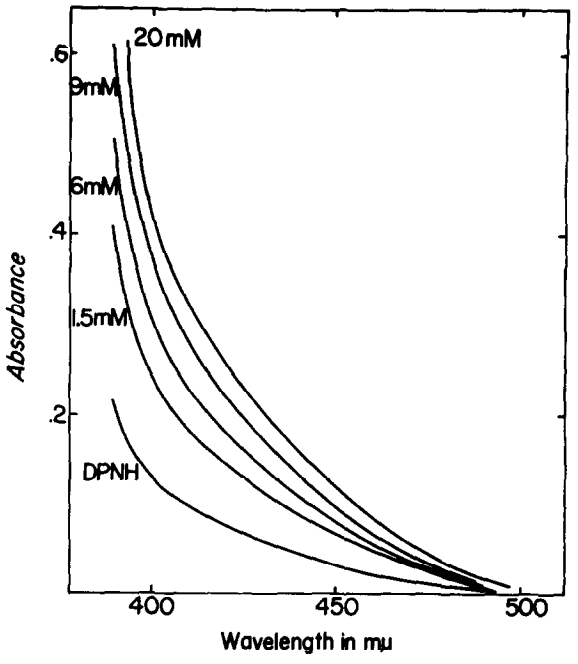

Fig. 1. Spectra of DPNH and DPN-DPNH mixture at 77° K in 100 mM Tris-HCl, pH 8.3. DPNH, 2 mM and DPN as indicated. Light path, 2 mm. The spectra are corrected for DPN contribution.

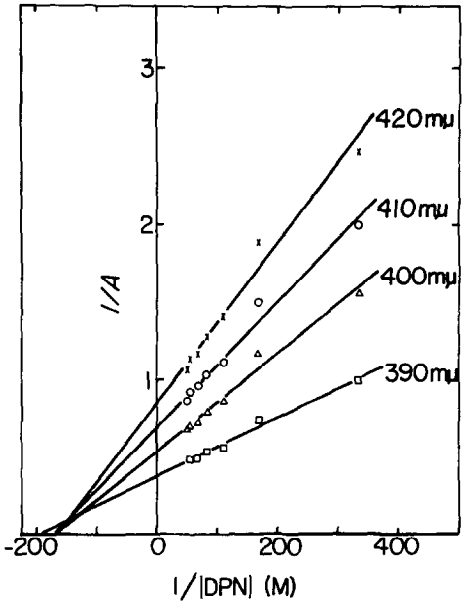

Fig. 2. Benesi-Hildebrand plots of absorbance versus DPN concentration in DPN-DPNH mixtures at various wavelengths. Conditions were the same as in Fig. 1, except that DPNH contribution has been subtracted and absorbance (A) has been corrected for a light path of 1 cm.

molar extinction coefficient of the DPN-DPNH complex at 400 mμ is 740. The straight line relationship between DPN concentrations and absorbance changes in the Benesi-Hildebrand plots indicates that the DPN-DPNH complex involves only one molecule of DPN. The absorbance decrease (data not shown) resulting from dilution of a DPN-DPNH mixture further suggests that the complex also involves one molecule of DPNH. DPNH plus TPN or DPNH plus NMN yield similar spectra as DPNH-DPN mixtures, and at 10 mM TPN or NMN the spectra duplicate the effects with 10 mM DPN. These results suggest that color formation in frozen mixtures of reduced and oxidized pyridine nucleotides is only due to interaction of the nicotinamide moieties and that adenosine and the additional phosphate of TPN play no important role. The absence of an EPR signal near g = 2 in DPN-DPNH mixtures at 98° K excludes the possibility that color formation in these systems is due to free radicals.

The above data strongly suggest that oxidized and reduced pyridine nucleotides interact to form a charge-transfer complex. This conclusion is supported by the fact that (a) the complex formation is reversible, (b) the characteristic absorption of the complex vanishes as the frozen mixture is brought to room temperature, and (c) the color formation is not due to the presence of free radicals. That pyridine nucleotides and related pyridine derivatives are capable of charge-transfer interaction is known (1-6).

2. Interaction by Transfer of Excitation Energy

The conformation of DPNH (7-10) in water allows an intramolecular transfer of electronic energy from the adenine moiety to the dihydronicotinamide portion of the molecule. Thus, Weber has shown that excitation of the adenine moiety of β-DPNH by irradiation at 260 mμ causes fluorescence emission by the dihydronicotinamide system (7). The intensity of this emission is of the same order of magnitude as would result from excitation of the dihydronicotinamide moiety itself at 340 mμ. This type of intramolecular energy transfer has also been shown with α- and β-TPNH as well as with cyanide adducts of α- and β-TPN (11).

Our studies show that in addition to such intramolecular interactions, energy transfer also occurs intermolecularly from oxidized to reduced pyridine nucleotides. Thus, the excitation spectra of aqueous solutions of α-DPNH, β-DPNH and β-TPNH exhibit a considerably increased intensity of the band at 260-262 mμ after addition of α-DPN, β-DPN or β-TPN to any of the above reduced nucleotides (Fig. 3)*. By contrast, the excitation maximum at 343-348 mμ is virtually unaffected. As shown in Table 1 there are no significant quantitative differences between the four combinations of reduced and oxidized β-DPN and β-TPN. Similarly, within limits of the accuracy of measurements and purity of the samples, the increase of the 260 mμ excitation band of α-DPNH is the same when induced by either α-DPN or β-DPN. Adenosine, but none of its phosphate derivatives, produces an effect on the DPNH excitation spectrum

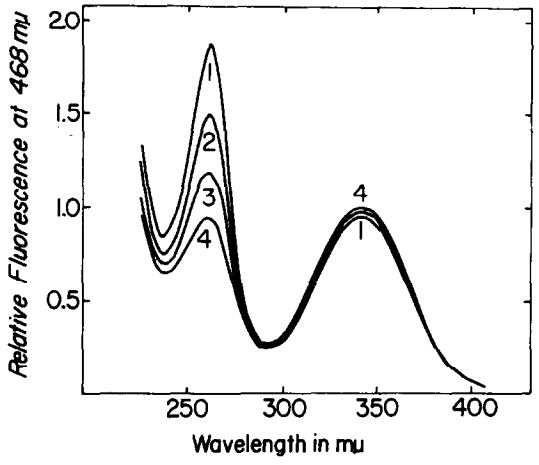

Fig. 3. Excitation spectra of DPNH and DPN-DPNH mixtures. Concentrations and conditions are the same as in Table 1. Traces 1 to 3, respectively 50, 33.3 and 16.7 μM β-DPN in 3.3 μM β-DPNH; trace 4, 3.3 μM β-DPNH alone. The fluorescence intensities are expressed in relation to the 343 mμ maximum of β-DPNH alone, which is assigned an arbitrary value of 1.

* α-DPNH has an excitation spectrum very similar to the β-isomer. The excitation maxima for β-DPNH are at 262 mμ and 343 mμ, and the ratio of the intensity of these two bands is 0.9 under the conditions used. By comparison, the respective values for α-DPNH are 260 mμ, 348 mμ and 1.0. The data on α-DPNH agree with the qualitative observations of Suzuki et al. (11), but not with the results of Shifrin and Kaplan (12).

TABLE I

Effect of Oxidized Pyridine Adenine Dinucleotides on the Excitation Spectra of Reduced Pyridine Nucleotides

Additions (μM)		β-DPNH 3.3 μM	β-TPNH 3.3 μM	α-DPNH 2.5 μM
		262:343		260:348
β-DPN	0.0	0.89	0.89	1.02
	16.7	1.12	1.15	1.23
	33.3	1.42	1.45	1.61
	50.0	1.84	1.86	2.08
β-TPN	0.0	0.91	0.93	-
	16.7	1.17	1.12	-
	33.3	1.47	1.46	-
	50.0	1.93	1.83	-
α-DPN	0.0	-	-	1.01
	16.5	-	-	1.25
	33.0	-	-	1.62
	49.5	-	-	2.10

262:343 (260:348), ratio of the excitation band intensities at 262 mμ (260 mμ) and 343 mμ (348 mμ). Emission, 468 mμ; excitation band width, 10 mμ; emission band width, 25 mμ; buffer, 50 mM Tris-HCl, pH 8.3.

similar in magnitude to the DPN effect, while guanosine is much less effective. Thus, in the presence of 6.67 μM DPNH, 33.3 μM adenosine increases the ratio of the 262 mμ : 343 mμ excitation band intensities by 50%, while a similar concentration of guanosine increases this ratio only by 8%. As might be expected, NMN and NMNH do not replace any of the oxidized or reduced pyridine adenine dinucleotides**.

** Guanidines in decimolar concentrations and above decrease the observed intermolecular, as well as the intramolecular, energy transfer effect. Methylguanidinium sulfate also decreases the intensity of the charge-transfer absorbancy of the DPN-DPNH system. These results will be described in a subsequent publication.

Thus, it may be concluded that under certain conditions an intermolecular energy transfer occurs from adenosine and oxidized pyridine adenine dinucleotides to the reduced dinucleotides. The adenine moiety in reduced nicotinamide adenine dinucleotides is essential for this effect. The interaction is affected neither by the stereoisomerism of the riboside-nicotinamide bond nor by the presence of an additional phosphate group in the 2'-position of the molecule*.

In summary, two types of interaction, one indicative of charge transfer and another of excitation energy transfer between oxidized and reduced pyridine nucleotides have been described. These interactions might have significant implications in biological systems where oxidized and reduced pyridine nucleotides coexist in cellular compartments at relatively high concentrations.

EXPERIMENTAL

Low-temperature spectra were recorded with a ratio recording spectrophotometer equipped with a stray light filter, and fluorescence spectra were recorded with Turner fluorescence spectrophotometer Model 210 operated as an absolute spectrofluorometer. Nucleotides were obtained from P-L Biochemicals and Sigma Chemical Co.

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* Because of limitations of the available fluorescence spectrophotometers, the above conclusions are only based on the limited concentration range ($<10^{-4}$ M DPN) used, and complex formation constants for this type of interaction could not be determined by this technique.