THE EFFECT OF SOLVENT ON THE FLUORESCENCE OF SCHIFF BASES OF PYRIDOXAL 5' PHOSPHATE

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SUMMARY.

In non aqueous solvents, the Schiff base form of PLP absorbing at 330 nm fluoresces either at 525 nm or at 430 nm. The former emission, observed in CCl_4 or $CHCl_3$, is due to H displacement in the intramolecular H bond between phenolic group and imine nitrogen; the latter, observed in H accepting solvents, is due to the ionized form in the excited state. The spectral properties of PLP in glycogen phosphorylase and in aspartate aminotransferase are ascribed to the possibility of H-bonding interaction between the coenzyme and its microenvironment.

There is some evidence that PLP*, bound to an ε amino group of a lysine residue in glycogen phosphorylase (1) or in aspartate aminotransferase (2,3) exists in a hydrophobic environment (4,5). At neutral pH the spectral properties of these two Schiff bases are very different : in phosphorylase, the Schiff base absorbs light at 333 nm and fluoresces at 525 nm (4,6); in aminotransferase it absorbs at 430 nm (at pH < 6.3) or at 360 nm (at pH > 6.3) and emits at 510 or 430 nm (5,7). For model systems : n butylamine, valine or n hexylamine -PLP in various solvents the observed properties of Schiff bases are very different : as previously reported in H_2O or in DMF, the form absorbing at 330 nm emits at 430 nm (5), Shaltiel and Cortijo (4) have observed that the same species in $CHCl_3$ emits at 525 nm. Therefore, to elucidate the effect of the microenvironment, we have studied the fluorescence

* Abbreviations : PLP, pyridoxal 5' phosphate
DMF N, N'-dimethylformamide

of Schiff bases of PLP and n butylamine in solvents of various dielectric constant and H accepting power.

MATERIAL AND METHODS.

As previously described, (5), samples used are PLP A grade from Calbiochem and n butylamine RP Prolabo redistillated in the laboratory. The solvents are solvents Merck for spectroscopy and are not purified before use. Absorption studies are performed with a Cary model 14. Fluorescence spectra are recorded with an Aminco Bowman spectrofluorometer, with a bandwidth of about

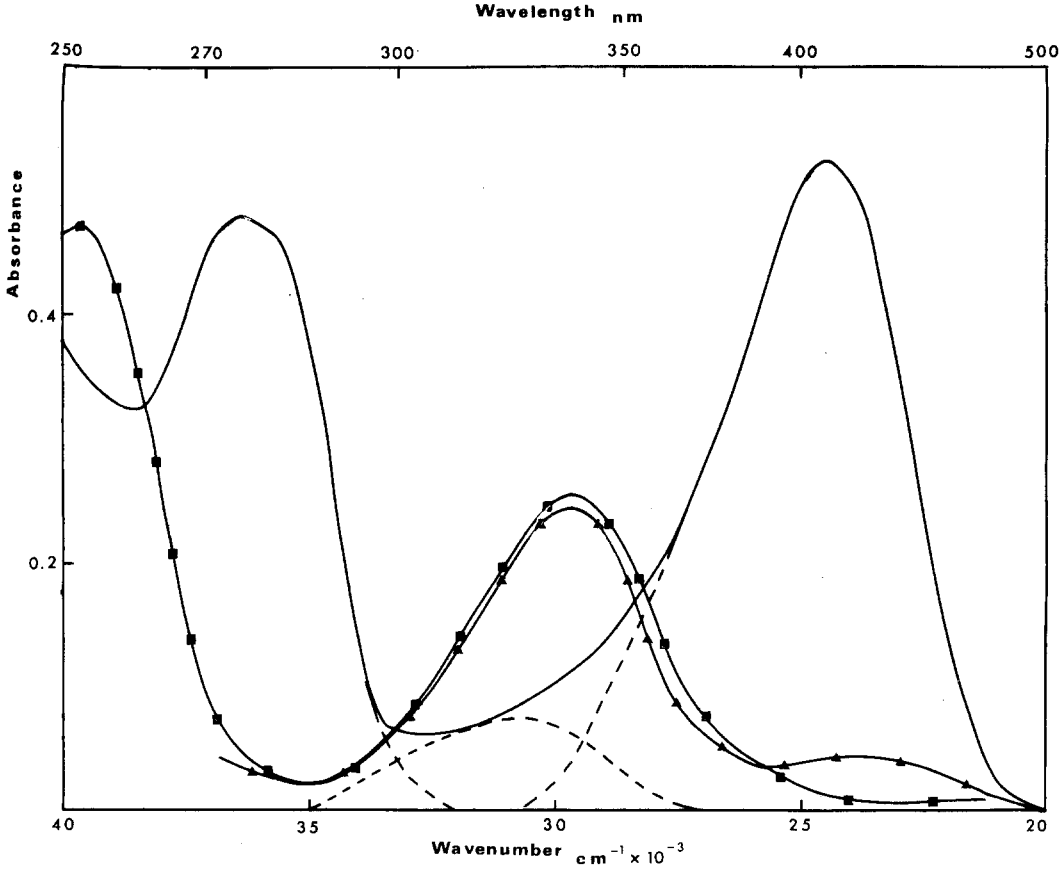

Fig. 1 : Absorption spectra of Schiff base (PLP 7 x 10^{-5}M, n butylamine 2.5 x 10^{-4}M) in various solvents : ■—■ dioxane; ▲—▲ DMF; —— H_2O, the dotted line represents decomposition of this spectrum.

10 nm. Emission and excitation spectra are not corrected ; according to Chen (8) for the emission spectra recorded with a monochromator blazed at 500 nm and a photomultiplier 1 P 28, corrections need not be applied between 350 and 430 nm and are less than 15 % at 530 nm. In this study we do not determine absolute quantum yields, but only relative yields.

RESULTS.

Absorption spectra. Figure 1 gives the spectra of Schiff bases of PLP and n butylamine in various solvents, including H_2O at a pH where the pyridine nitrogen is deprotonated in the Schiff base (4). The spectra are indicated in

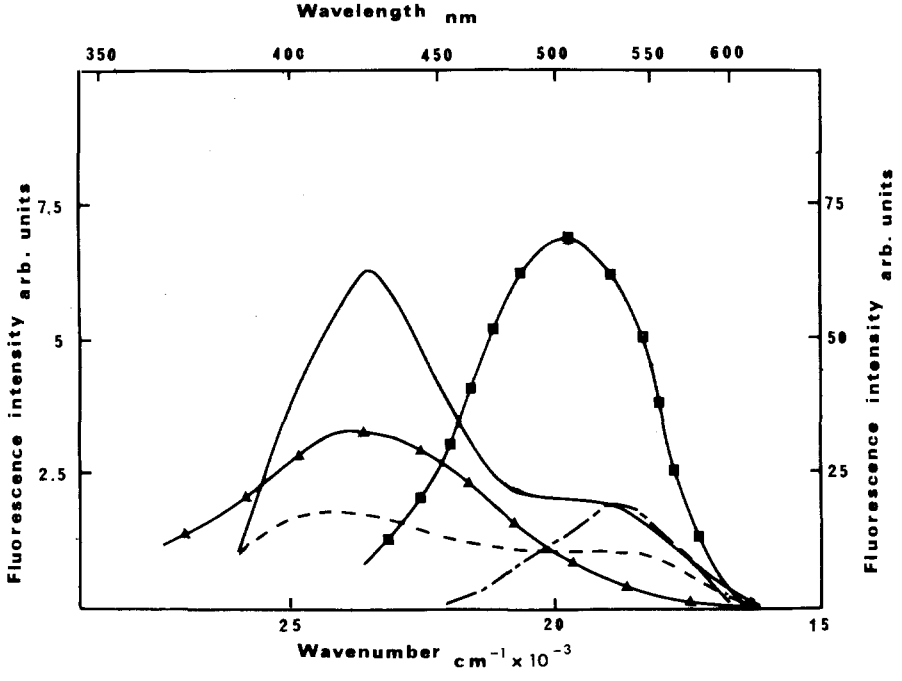

Fig. 2 : Fluorescence spectrum of Schiff base of PLP and n butylamine in various solvents. The spectra correspond to an absorbance of 0.1 at the wavelength of excitation.

—·—·—·— in CCl_4, excitation at 339 nm

– – – – – – in dioxane, excitation at 337 nm

———— in DMF, excitation at 335 nm

—▲—▲— in H_2O, excitation at 325 nm (scale on the right)

—●—●— in H_2O, excitation at 410 nm.

wavenumbers to facilitate the correlation to the transition energy.

Emission spectra. Figure 2 shows the fluorescence spectra of the Schiff base form absorbing at about 330 nm ; they correspond to an absorbance of 0,1 at the maximum of the absorption band. In aqueous solvent, the emission corresponding to the excitation at 410 nm is also indicated.

The results are summarized in the table with the physical characteristics of the used solvents.

DISCUSSION.

From the results indicated in the table, it appears that three phenomena are observed : a blue shift of the absorption at 330 nm by increasing the polarity of the milieu, the appearance of a form absorbing at 410 nm when the dielectric constant is high and two emission bands (430 and 525 nm) varying with the composition of the milieu.

TABLE

Solvent	dielectric constant	refractive index	λ absorption nm	λ emission nm	$\Delta\nu_{abs}$ / abs in CCl_4 (330 nm band)
CCl_4	2,2	1,457	339	533	0
Dioxane	2,2	1,420	337	413 530	200 cm^{-1}
CH_3CN	37,5	1,342	335 ∿425	425 520 520	400 cm^{-1}
DMF	38	1,427	335 425	425 520 ∿520	400 cm^{-1}
H_2O	80	1,333	325 410	428 505	1250 cm^{-1}

Absorption and emission spectra of a Schiff base PLP-n butylamine in various solvents.

In aqueous solvent, the two absorption bands have been attributed to the tautomeric forms (enol-imine and keto-enamine) of the Schiff bases (5,9). In non aqueous solvents, the same tautomers are observed, but the population is inverted (4,9). The absorbance of the Schiff base at 330 nm (enol-imine form) is too high to be due to an $n \rightarrow \pi^*$ transition ($\varepsilon = 3500$) so the blue shift obtained by increasing the polarity of the solvent is very surprising : the best explanation is the formation of a hydrogen bond between the molecule and the solvent, which is slightly stronger in the ground state than in the excited state (10).

In aqueous solvent for the Schiff base PLP-n butylamine or in DMF for the Schiff base PLP-valine, we observed that the emission at 510 nm occurs from the keto-enamine absorbing at 410 nm and the emission at 430 nm occurs from the enol-imine tautomere absorbing at 325 or 333 nm. In nonpolar solvents such as

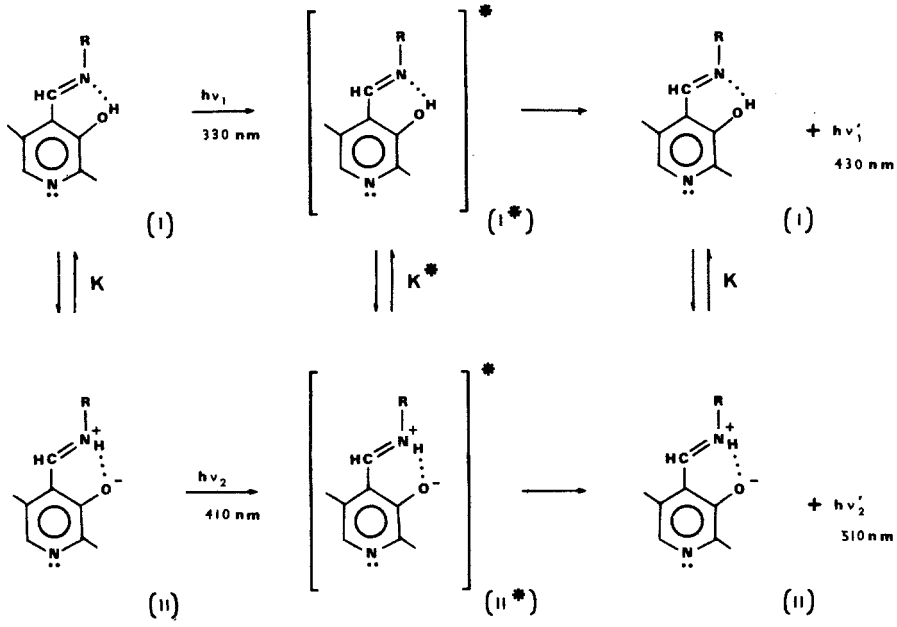

Fig. 3

$HCCl_3$ or CCl_4, from the enol imine tautomere the emission of the keto-enamine is observed. In H accepting solvents as dioxane or DMF the two emissions are observed (for the Schiff base PLP-n butylamine). This phenomenon is very similar to that observed in analoguous compounds: anils (11), and can be summarized as in figure 3. For non polar solvent the equilibrium in the excited state is displaced far toward II^*; in solvent able to accept H (ionisation or H bond) as H_2O, DMF, acetonitrile, dioxane, the equilibrium is displaced toward I^* ... solvent or $I^{*-} + H^+$.

Application to PLP enzymes. From the absorption and emission spectra of the coenzyme in aspartate aminotransferase and in glycogen phosphorylase, it has been shown that the microenvironment of PLP is hydrophobic (4,5). In aspartate aminotransferase the PLP can exists as a deprotonated Schiff base, as a Schiff base protonated on the imine nitrogen and maintained by electrostatic interaction with a group YH^+ or as an unknown form

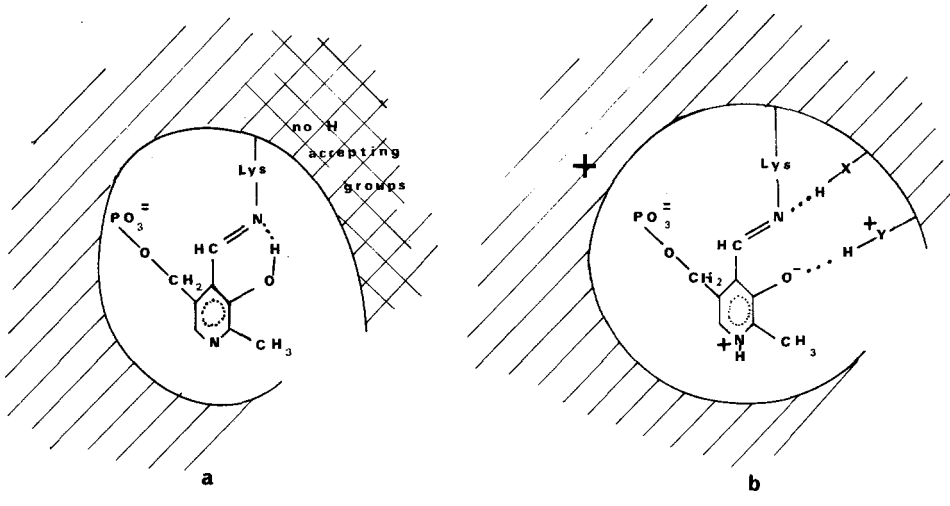

Fig. 4 : Schematic representation of PLP :
a in phosphorylase
b in aspartate aminotransferase (active form).

absorbing at 340 nm (12). According to Martinez-Carrion et al. (13) this form may have a tetrahedral carbon attached to a group X of the protein; the results presented here show that it may be a Schiff base protonated on the phenol group. The most surprising result about glycogen phosphorylase is the emission at 535 nm (14) : it means that PLP in this enzyme is in a hydrophobic region (4) and, from the results of this work, it means also that there is not any H accepting group in the proximity of the PLP and no competition between the protein and the intramolecular H bond of the Schiff base (Fig. 4).

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