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Enzymichromism: Determination of the Dielectric Properties of an Enzyme Active Site

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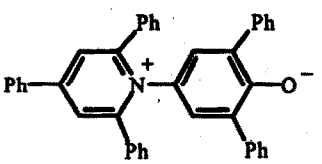
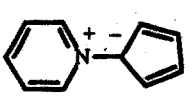
Abstract: The negative solvatochromic and enzymichromic properties of 4-carbamidopyridinium cyclopentadienylide, **3**, are presented. The 78 nm bathochromic shift of the long wavelength absorption band of **3** upon going from water to the horse liver alcohol dehydrogenase (HLADH)-NADH ternary complex is consistent with a low dielectric for the hydrophobic binding pocket of this enzyme.

Measuring the solvational properties of protein interiors is a difficult problem but one that is extremely important in understanding the chemical specificity and rate accelerations of enzyme catalysts. It has been argued on theoretical grounds that enzymic catalysis requires conditions analogous to gas phase reactions.[1] However, there is some theoretical[2,3] and experimental[4,5] evidence against the description of enzyme active sites as cavities of low dielectric. The similar pK_a values of amino acid side chains in proteins compared the intrinsic pK_a of the corresponding amino acid side chains in bulk water provides indirect evidence against a low active site dielectric.[2] Fersht and his coworkers[4] have determined apparent effective dielectrics ranging from $\varepsilon_{eff} = 50\text{-}100$ for subtilisin on the basis of changes in the pK_a of the active site histidine with changes in charged surface residues by site-directed mutagenesis. These values are comparable to the dielectric constant of liquid water $\varepsilon_{H_2O} = 78$ at 25 °C.

Solvatochromic indicators such as Reichardt's $E_T(30)$ betaine, **1**, have seen wide use as empirical measures of solvent polarity of organic solvents.[7] Early work by Kosower and Ramsey[8] suggested that the small, pyridinium cyclopentadienylide, **2**, shows a strong *negative solvatochromic shift* of the long-wavelength intramolecular charge-transfer absorption band.[9]. We reasoned that the amide analog, 4-carbamidopyridinium cyclopentadienylide, **3**, should bind to the enzyme HLADH in analogy to the weak binding of substituted benzamides ($K_i = 0.5$ mM for benzamide).[10]



1



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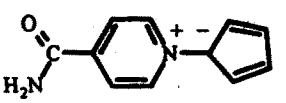


3

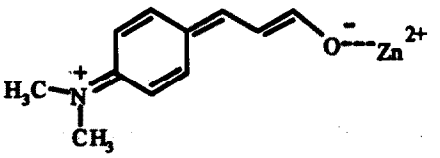
Like **1**, the dye **3** shows a significant, but less pronounced, negative solvatochromism. Thus changing from water (λ_{max} = 519 nm) to benzene (λ_{max} = 562 nm), the long-wavelength UV-vis absorption band undergoes a shift of 42 nm. The dye is insoluble in aliphatic hydrocarbon solvents up to undecane. Table 1 summarizes the solvatochromic and enzymichromic properties of **3** under several conditions.

Table 1 Summary of Solvatochromic and Enzymichromic Properties of 4-Carbamidopyridinium Cyclopentadienylide[a]

Conditions	λ_{max}	dielectric constant
H_2O	519 (518)[b]	78.4
Ethanol	548	24.6
1-Propanol	551	20.3
1-Octanol	556	10.3
Methylsulfoxide	547	46.7
Dimethylformamide	548	37.8
Acetonitrile	543 (545)[b]	43.7
Acetonitrile, 0.5 M LiBr	552	-
Acetonitrile, 10 mM $Zn(Acetate)_2$	542	-
Acetone	555	20.7
Dichloromethane	556	8.9
Tetrahydrofuran	556	7.6
Tetrahydrofuran, 50 mM LiCl	556	-
Benzene	562	2.3
1,4-Dioxane	563	2.2
H_2O, HLADH- NADH ternary complex[c]	597	-

[a]at 25 °C, under argon. [b]Kosower, E. M. and Ramsey, B. G. *J. Am. Chem. Soc.*, **1959**, *81*, 856-860. [c]at pH 9.2 (50 mM PP_i), 1 mM NADH, ≈1 mM **3**, 100 μN enzyme. The sample, in 0.1 cm cuvettes, was referenced against buffer, 1 mM NADH, and the same concentration of dye. The spectrum was recorded immediately after adding the enzyme.

The largest spectral shift relative to water is seen in the presence of the HLADH-NADH ternary complex where λ_{max} = 597. A control experiment with 1 mM **2** showed no change in the UV-visible spectrum upon addition of enzyme. This shows that **2** does not bind to the active site of the enzyme at this concentration. It provides indirect evidence that the spectral changes associated with binding of **3** to the enzyme-ternary complex are due to binding at the active site, and are not due to non-specific association of the dye with the protein. A similar 66 nm bathochromic shift in the absorption maximum of *N, N*-dimethylamino-cinnaminaldehyde upon binding to the enzyme-NADH ternary complex is consistent with significant stabilization of the ground state zwitterionic structure **4**, upon binding to the active site zinc.[11]



4

In this case, model studies employing metal ion complexes suggest that most of the enzymichromism of this substrate is due to stabilization of the structure 4 and not to the protein dielectric since this substrate displays *positive solvatochromism*, in the absence of metal ions.[12] In the case of 3, the small 9 nm shift in λ_{max} upon going from acetonitrile to 0.5 M LiBr in acetonitrile suggests that only a small stabilization of the ground-state structure by the active site metal ion is expected. We conclude that the extremely large bathochromic shift in the long wavelength charge transfer band of 3 upon binding to the enzyme active site is due to an extremely low dielectric environment in the binding pocket. These results are consistent with several reported crystal structures of HLADH ternary complexes that show a deep 20 Å long hydrophobic barrel for binding the substrate as shown in Figure 1. Further theoretical and experimental work is in progress to characterize the enzymichromic properties of 3.

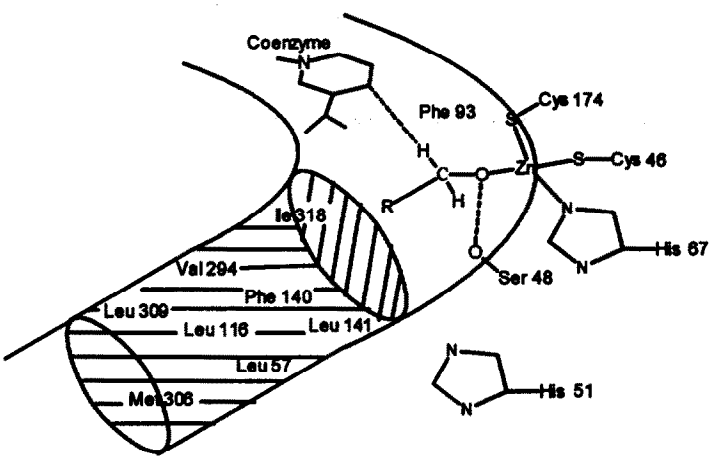


Figure 1. Schematic representation of the active site region in liver alcohol dehydrogenase, showing catalytic zinc with its ligands, the nicotinamide-binding site, and the hydrophobic substrate-binding barrel. (after Pettersson, G. *CRC Crit. Rev. Biochem*, 1987, *21*, 349-389).

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7. For a recent review see, Reichardt, C. *Chemia*, **1991**, *45*, 322-324.
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9. The term *negative solvatochromism* refers to a hypsochromic (blue) shift in the absorption maximum with *increasing* solvent polarity. In analogy to the thermochromism, piezochromism, and halochromism of **1**, we suggest that the color change of **3** upon binding to a protein interior be termed "enzymichromism".
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