nearest-neighbor models, that is, considering the hydrated ions as independent units with octahedral mean coordination geometries. On the contrary, it is interesting to notice that, in the hexagonal crystals of hexahydrate calcium chloride, as well as in other isomorphous hydrates,[4] each cation is surrounded by three H_2O molecules in the positions $(u, 0, 0)$, $(0, u, 0)$, and $(\bar{u}, \bar{u}, 0)$ at the nearest distance (~2.4 Å) and by the other six H_2O molecules at a greater distance (~2.7 Å) in the positions $(v, 0, \frac{1}{2})$, $(0, v, \frac{1}{2})$, $(\bar{v}, \bar{v}, \frac{1}{2})$, $(v, 0, \bar{\frac{1}{2}})$, $(0, v, \bar{\frac{1}{2}})$, and $(\bar{v}, \bar{v}, \bar{\frac{1}{2}})$. These last six water molecules contribute to forming Ca-H_2O-Ca chains along the c axis with Ca-Ca distance of ~4 Å. Chloride ions are interposed between parallel chains and each of them has six water molecules at distances near 3.2 Å and three others at ~3.6 Å. So the main three peaks observed in the correlation function of liquid $CaCl_2 \cdot 6H_2O$ may be explained in terms of an arrangement of nearest neighbor similar to that of the hydrated crystal.

We then constructed a structure model for the liquid using the crystal lattice of $CaCl_2 \cdot 6H_2O$ to describe the distance spectrum around each atom up to a distance which measures the extent of order; in this way the model fulfills the necessary requirements that structurally equivalent atoms have the same distance spectra and that spectra of different atoms are geometrically compatible.

In order to choose the order range around each atom, we considered that deviations from a uniform distribution of distances are evident in the correlation function only up to ~6.5 Å, whereas the shortest Ca-Ca distance in the layers normal to the axis of the Ca-H_2O-Ca chains in the hydrated crystal is ~8 Å. Therefore, only the discrete distances Ca-Ca, Ca-H_2O and H_2O-H_2O shorter than 7 Å among atoms in the same chain were included in the model, thus excluding a positional correlation between atoms of different chains. Two discrete distances Cl-H_2O were also included, while a uniform distribution of distances was assumed for the other interactions.

This model was systematically refined by least squares, fitting a synthetic structure function to values derived from the measurements. Five distances were treated as independent parameters: the shorter Ca-Ca distance (r_{CC}), the two nearest-neighbor Ca-H_2O distances ($r_{C(1)}$, $r_{C(2)}$), and the two Cl-H_2O distances ($r_{A(1)}$, $r_{A(2)}$). The other discrete distances were related to them by the geometry of the model. Mean square deviations of the discrete distances as well as mean distances and mean square deviations characterizing the uniform distributions boundaries were adjusted to obtain the best fit.

The results of the calculation are shown in Figure 1, where observed (circles) and model (solid line) correlation functions are compared: the good agreement proves the compatibility of the model used with the experimental data. The values obtained in the refined model for the independent discrete distances are the following: $r_{CC} = 3.98$; $r_{C(1)} = 2.35$; $r_{C(2)} = 2.69$; $r_{A(1)} = 3.21$; and $r_{A(2)} = 3.62$ Å. Since distances greater than nearest-neighbor ion-water interactions were essential to yield a good fit, the liquid $CaCl_2 \cdot 6H_2O$, unlike less concentrated $CaCl_2$ aqueous solutions, appears to be characterized by a middle range order involving cation-cation correlations and retaining "memory" of the hydrated crystal lattice structure.

It is worth pointing out that this "memory" of lattice structure must be understood, in the sense of the statistical order characteristic of the liquid state, as coming from time average of different instantaneous situations and/or space average of different local structures. In this view discussions as to whether order phenomena observed correspond to "quasi-lattice" structure come down to a secondary question of terminology.

More details about this study will be reported soon.[5]

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(5) The support of this research by the Consiglio Nazionale delle Ricerche is gratefully acknowledged. The calculations were performed at the Centro di Calcolo, University of Cagliari.

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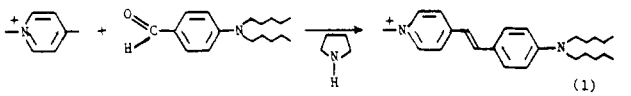
Received February 24, 1979

An Unexpected Blue Shift Caused by Differential Solvation of a Chromophore Oriented in a Lipid Bilayer

Sir:

As the first experimental realization of a qualitative theoretical scheme[1] for the design of electrochromic[2] molecular probes of membrane potential, we have prepared 4-(*p*-dipentylaminostyryl)-1-methylpyridinium iodide (di-5-ASP)[3] (eq 1). The calculations indicate that most of the positive



(1)

charge in the ground state is concentrated in the pyridinium ring and that it shifts to the aniline ring upon excitation.[1] This charge migration is a component of our interpretation of an unusual blue shift in the visible absorption spectrum when di-5-ASP binds to lipid bilayer membranes.

The "unexpected" nature of this blue shift is apparent from inspection of the data in Table I. Spectra of di-5-ASP obtained in less polar solvents than H_2O are red shifted and sharpened; since the ground state can be solvated more effectively than the vertically excited state of a polar chromophore, this behavior is quite common. Another common experimental observation is that a lipid bilayer or a micelle shifts the spectrum of a bound chromophore in the same direction as a nonpolar solvent. This is indeed true for di-5-ASP bound to sodium dodecylsulfate micelles, but lecithin vesicles induce a significant blue shift (an accompanying increase in extinction suggests that the probes are more uniformly solvated than in water).

A possible explanation for this behavior might involve aggregation[4] due to the high effective concentration of the probes in the membrane.[5] On the other hand, an aggregate is expected to have lowered extinction and quenched fluorescence; vesicle bound di-5-ASP has a higher extinction (Table I) and a fluorescence which is approximately a factor of 100 stronger than aqueous di-5-ASP. If the proportion of lipid to probe is varied, it is only at ratios lower than 100:1 that the fluorescence intensity and extinction begin to drop and the absorption maximum begins a further blue shift (to 450 nm at 1:1 egg lecithin-di-5-ASP); above ratios of 100:1 the spectral parameters are essentially constant. The fact that we observe typical evidence for aggregation only under forcing conditions assures us that di-5-ASP obeys the usual criteria for this behavior and that we must find an alternate explanation for the (concentration independent) blue shift.

Our explanation is based on the most reasonable orientation of the bound probe in the lipid bilayer, as depicted in Figure

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Table I. Absorption Spectra of Di-5-ASP[a]

solvent	λ_{max}, nm	ϵ_{max}, A/M·cm $\times 10^{-4}$
water	475	2.8
ethanol	494	4.5
butanol	498	3.9
10^{-2} M aqueous sodium dodecyl sulfate	485	3.7
2 mg/mL sonicated egg lecithin	464	3.7
2 mg/mL sonicated dipalmitoyl lecithin	460	4.1

[a] Data obtained on a Cary 118 or a Beckman 25 spectrophotometer over a range of probe concentrations.

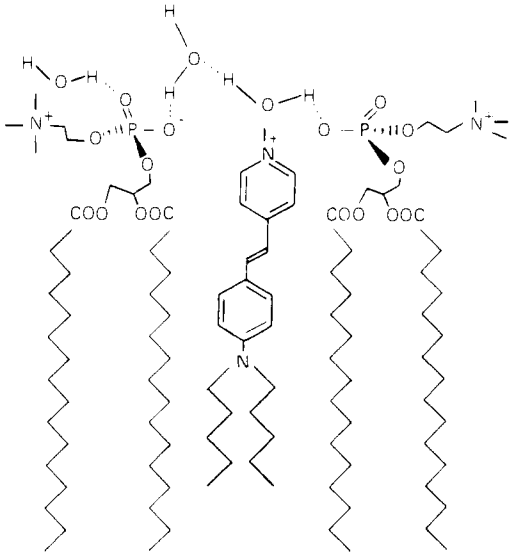

Figure 1. Orientation of di-5-ASP which maximizes stabilizing polar and hydrophobic interactions with adjacent lipid molecules and the aqueous interface in a membrane.

1. The positive charge in the ground state, centered in the pyridinium ring, is in a polar environment; thus, the ground-state stabilization should be comparable with that in H_2O. If the charge migrates to the aniline ring upon excitation, the orientation depicted in Figure 1 places the vertical excited state at a higher energy than it might find itself when simply dissolved in water. The blue shift of di-5-ASP, oriented in the ordered bilayer structure of the membrane, can thus be attributed to a differential solvation which is absent in micelles or homogeneous solution.

Since our explanation rests heavily on the orientation of the chromophore, a polarized fluorescence procedure was devised to verify the presumptions used to formulate Figure 1. The vertically polarized 442-nm line of an He-Cd laser (~5 mW, Liconix 4110H) was focused on either the bottom (B) or the middle (M) of a hemispherical oxidized cholesterol bilayer[6] suspended from a 3-mm polyethylene tube in a 3 μM solution of di-5-ASP in 100 mM aqueous KCl. Membrane fluorescence was collected at 90 °C to the exciting beam through a 570-nm interference filter and a polarizing film which could be oriented either horizontally (H) or vertically (V). A cooled EMI 9558QA photomultiplier tube and a Victoreen 1001 electrometer were used to determine the relative intensities of the four polarized fluorescences: BH, BV, MH, MV. According to an analysis developed by Yguerabide and Stryer in their elegant study of fluorescence from suspended spherical bilayers,[7] BV (δ in ref 7) derives intensity from chromophores with their transition moments perpendicular to the membrane and MV (α in ref 7) derives intensity from chromophores oriented parallel; BH and MH are equivalent for any orientation (these are designated γ in ref 7) and can thus be used to normalize BV and MV for variations in the extent of illumination and the efficiency of light collection at the two positions.

The ratio of the normalized values of BV:MV is 6.1 ± 1.2, indicating a substantial preference for a probe orientation perpendicular to the bilayer surface.[8]

Fluorescence, because of its inherently greater sensitivity, has been a much more popular technique for the study of membrane preparations than absorption spectroscopy. Indeed, the fluorescence properties of di-5-ASP are quite remarkable, showing a 100-fold increase in intensity and a 50-nm blue shift upon binding to egg lecithin vesicles. While these features are generally consistent with our picture of the unique interaction of di-5-ASP with the membrane, an unambiguous interpretation is not possible without additional knowledge of the structure and lifetime of the fully relaxed excited state. Interpretation of the absorption (or, equivalently, excitation) spectra, as exemplified in this work, is simplified by the assurance that only the ground-state structure and environment of the chromophore can contribute to the energies of both the ground and excited states.

Acknowledgment. This work was supported by Grant No. GM-25190, awarded by the National Institute of General Medical Sciences.

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Received March 30, 1979

Electrophile-Induced Disproportionation of the Neutral Formyl (η-C_5H_5)Re(PPh_3)(NO)(CHO). Generation of Cationic Rhenium Carbenes of the Formula [(η-C_5H_5)Re(PPh_3)(NO)(CHX)]$^+$ (X = H, OCH_3, OH)

Sir:

We recently reported the isolation[1] and X-ray crystal structure[2] of the neutral formyl complex (η-C_5H_5)Re(PPh_3)(NO)(CHO) (**1**). In this communication, we describe low temperature reactions of **1** with electrophiles, under which conditions a stoichiometric fraction of the formyl ligands are transformed, *without the addition of a reductant*, into methyl ligands. Furthermore, evidence is presented for the intermediacy of a variety of novel cationic rhenium–carbene complexes in these reactions, several of which have been independently generated.

The treatment of **1** (~0.1 M in toluene) with 1 equiv of CH_3SO_3F at −78 °C, followed by warming to room temperature, resulted in the formation of [(η-C_5H_5)Re(PPh_3)(NO)(CO)]$^+SO_3F^-$ (**2**)[1,3] and (η-C_5H_5)Re(PPh_3)(NO)-

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