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Letters to the Editor

Temperature Effects on the Polarity of Lipid Bilayers and the Localization of Amphiphilic Flavins in Artificial Membrane Vesicles

In a recent article, Schmidt [5] studied the variation of the fluorescence yield of amphiphilic flavins inserted in bilayer vesicles made from different saturated phospholipids. He showed first that the fluorescence quantum yield increases near the vesicles phase transition from the gel to the liquid-crystalline state, and correlated these observations with his own results (*see* Fig. 5 of [5]) and those of Kotaki and Yagi [3] on the increase of the fluorescence yield of the chromophores in media of differing dielectric constant with decreasing polarity of the solvent. From these data and additional arguments based on the band shifts of the amphiflavins to shorter absorption wavelengths in lower dielectric constant media (*cf.* Table 1 and Fig. 6 in [5]), Schmidt concluded that the flavin nuclei are imbedded more deeply into the hydrophobic portion of the bilayer upon gel to liquid-crystalline phase transition. I would like to report the following comments which show that in his discussion the author neglected to take into account, most probably unduly, the well-known variation of the dielectric constant with respect to temperature (*see* e.g., [4]).

In brief, it can be shown that the expression

$$D = D_0 e^{-LT} \tag{1}$$

where D is the dielectric constant of a liquid at the Kelvin temperature T and D_0 and L are constants specific for each liquid, holds with satisfactory accuracy for a wide variety of solvents examined. Equation (1) shows that D decreases exponentially with increasing T. Therefore, while applying dielectric constant measurements to the localization of any molecular species in lipid bilayers we must of necessity be able to separate the temperature-dependent gel to liquid-crystalline phase transition from the temperature effect on D. In this connection, it is mentioned that some preliminary work from our laboratory (*unpublished*) would seem to indicate that temperature brings about variations of the dielectric constant of the interface polar headgroup/hydrocarbon core measured according to a novel chemical reaction probe method [1]. An alternative hypothesis to explain the aforediscussed data would be the temperature-dependent exclusion of water molecules (dehydration) from the phosphorylcholine/hydrogen belt zone [2] interface. This assumption is compatible with the finding that a temperature rise favors the dehydration of glucose and tetrahydropyran-2-carbinol [6].

I conclude thereby that the observed variations of fluorescence quantum efficiences do not prove unquestionably that the amphiflavins headgroups are displaced more deeply into the hydrophobic core of the bilayer upon phase transition. Although this point is quite reasonable, it may happen that it does not rule out Schmidt's conclusion. It is emphasized, nonetheless, that the above remarks point out clearly the need for the quantitative discrimination between the simultaneous effects of temperature on lipid phase transitions and polarity variations inside biomembranes.

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Reply to: Temperature Effects on the Polarity of Lipid Bilayers and the Localization of Amphiphilic Flavins in Artifical Membrane Vesicles

Dr. Fragata brings forward two alternative explanations for the observed fluorescence increase of vesicle-bound amphiflavins near the lipid phase transition (gel-liquid crystalline), based on two well-known facts:

1) temperature-dependent polarity of solvents (e.g., a membrane), and
2) temperature-dependent exclusion of water molecules.

It appears to me that a decrease of polarity of the lipid-water interface with temperature, and a temperature-dependent exclusion of water molecules are only different aspects of the same phenomenon; therefore I don't comment separately on them.

Although I cannot completely rule out these hypotheses, the bulk of the data (only part of this work is already published) clearly favors my interpretation. In Fig. 4 of our previous paper (s.o.) it had been already shown (just to weaken the argument brought forward by Dr. Fragata) that collision quenching clearly overrules the suggested decrease of water-polarity, which in turn would *increase* the fluorescence quantum yield. Figure 1 of this letter shows that this holds true for solvents of various dielectric constants ($D=4$, 30 and 80). Shape and peak positions of the emission spectra don't reflect any polarity change with temperature, suggesting that "L" in the formula $D=D_0 \exp(-LT)$ is negligible small (i.e., $D=D_0$). Absorption spectra of flavins in different solvents show a similar invariability with temperature in this range.

I summarize the main data supporting my interpretation suggesting a phase-induced *delocalization* of the vesicle-bound flavin nucleus upon temperature change:

– Increase of fluorescence of vesicle-bound flavins with temperature; the *opposite* behavior is observed under isotropic conditions.

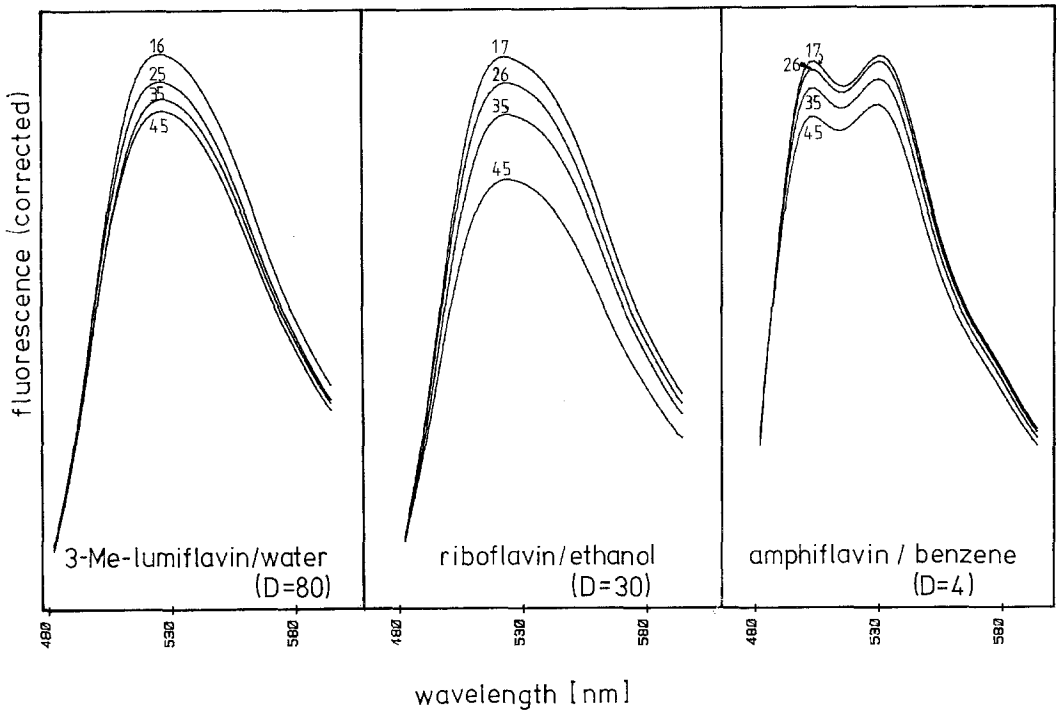

Fig. 1. Fluorescence emission spectra of flavins in solvents of various dielectric constants. Excitation was at 440 nm, and the peak height of the spectra taken at *lowest* temperature was normalized for the purpose of comparison. Different flavins had to be chosen according to their solubility in the various solvents, leaving the chromophore largely undisturbed. Clearly, the shape of the spectra is not significantly changed by temperature, but the fluorescence quantum efficiency *decreases* with temperature, overruling the assumed decrease of polarity, which in turn would *increase* the fluorescence intensity. The fluorescence decrease is mainly due to (temperature-dependent) collision quenching. Temperatures are given in °C

– Peak-shift of the $SO \rightarrow S2$ and $S1 \rightsquigarrow SO$ transitions with temperature, which is not observed under isotropic conditions (the latter transition is documented in Fig. 1).
– Flavin fluorescence quenchers and exogeneous photoreduction substrates have easier access to the flavin in the gel than in the liquid crystalline state of the membrane (*unpublished*).
– Probably the strongest evidence for my hypothesis is the availability of an internal, membrane-intrinsic electron donor for the flavin triplet in the gel-, but *not* in the liquid crystalline state of the membrane (*unpublished*).
– The fluidity of the microenvironment of the flavin increases by a factor of five near phase transition temperature, a value not observable under isotropic conditions in the given temperature range.

Summarizing, the physico-chemical properties (including those not yet published) of membrane-bound flavins investigated so far can be explained in an unconstrained manner on the basis of our hypothesis. So far I don't see the necessity that we have to account for a pure (i.e., isotropic) polarity change within membranes with temperature (following a well-known physico-chemical law).

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