Letters to the Editor

Mesophase Transition Temperatures as Measured by Fluorescence and Calorimetry

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Dear sir:

In the January, 1993, issue of *Biophysical Journal*, Dr. R. Epand comments on an interesting discrepancy in the lamellar-hexagonal phase transition temperature (T_h) recorded by differential scanning calorimetry (DSC) and by fluorescence anisotropy (Epand, 1993). He notes that in several instances T_h determined by fluorescence anisotropy is lower than that observed by calorimetry. Furthermore, thermotropic changes in the fluorescence characteristics of the bilayer phase while approaching T_h were suggested to reflect a biologically relevant change in the properties of the bilayer.

While these observed differences are worthy of note and of further systematic study, a word of caution is in order. It is entirely possible that the differences in T_h observed by DSC and by fluorescence originate from a difference in the inherent sensitivities of the two techniques. In the case of DSC, it is the system heat capacity change, reflecting a change in molecular motional freedom, that is sensed. In contrast, the motional freedom of a fluorescent probe is responded to in a fluorescence anisotropy measurement. Accordingly, it is possible that in passage from one phase to another the two techniques might not respond in perfect register.

The above discussion focuses on the lamellar-hexagonal phase transition which involves a dramatic structural reorganization of the lipid and water phase compartments. We thought it might be instructive therefore, to determine if, by comparison, the lamellar gel-lamellar liquid crystal phase transition temperature (T_m) is method-dependent. In this case, the phase change involves a chain order/disorder transition while the bulk of the system remains lamellar. For purposes of the comparison, we conducted a search of the lipid thermodynamic data in LIPIDAT (1993) listed under dielaidoylphosphatidylethanolamine (DEPE). The latter was chosen since, under conditions of full hydration, it exhibits both a lamellar gel-lamellar liquid crystal and a lamellar liquid crystal-hexagonal phase transition, and because it is one of the lipids cited in Dr. Epand's letter. The search produced the following results. By using calorimetry and fluorescence techniques the T_m values are 37.8 ± 1.5°C (n = 42) and 34.2 ± 1.6°C (n = 5), respectively. The latter are averages of data collected under a variety of conditions. However, we did restrict the search to fully hydrated DEPE with ≤0.15 M salt and 5 ≤ pH ≤8 and to T_m values recorded in the heating and cooling directions that differed by ≤2°C. While limited in scope, the analysis shows that a discrepancy in T_m determined by calorimetry and by fluorescence does exist and is in the same direction as noted above for T_h. A similar albeit less pronounced difference in T_m determined by fluorescence and calorimetry was observed from a search of LIPIDAT for several other phospholipids. This includes the phosphatidylcholines which are not particularly well disposed to forming nonbilayer phases.

In sum, a systematic study of the methods used to monitor lipid phase transitions is needed to establish precisely the parameters responded to by a given technique on either side of and during a transition. This is particularly pertinent to methods where potentially perturbing exogenous probes are used. Until such information is in hand, a discussion of the significance of a difference in lamellar-nonlamellar transition temperature (T_h) determined by disparate techniques must remain speculative.

REFERENCES

1. Epand, R. M. 1993. Detection of hexagonal phase forming propensity in phospholipid bilayers. *Biophys. J.* 64:290.
2. Caffrey, M. 1993. Lipid Thermotropic Phase Transition Database (LIPIDAT). NIST Standard Reference Database 34 (NIST, SRDP, Gaithersburg, MD 20899).