Response to Koynova and Caffrey

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Dear Sir:

I found the comments of Drs. Koynova and Caffrey (1993) very interesting. However, I disagree with their suggestion that the observed differences in transition temperatures arise because different aspects of the phase transition are being measured by different techniques. Phospholipid phase transitions are cooperative processes that occur over a narrow temperature range, and the temperature at which this transition occurs is the same whether measured by differential scanning calorimetry (DSC), X-ray diffraction, ^{31}P NMR, ^{13}C NMR, or ^{2}H NMR or electron microscopy. If a less direct method, such as the use of fluorescence or spin labels, is employed and it monitors a change occurring at a different temperature, I think it most likely that the probe is not monitoring a different aspect of the phase transition but rather that it is monitoring a pre- or posttransition event. This conclusion is strengthened by the fact that so many methods agree on one specific temperature (or small range of temperatures) for the phase transition and the fact that the phase transition is highly cooperative and would not extend beyond this range. I agree that there may be consistent differences between fluorescence probes and DSC and that these differences may not be limited to the L_α to H_{II} phase transition. However, I think it more likely that the fluorescent probes can measure a premelt phenomenon which may be a required precursor of the phase transition and may reflect an aspect of the structural difference between the high temperature and the low temperature phases but is not really part of the cooperative phase transition seen at higher temperatures. This in essence was the point of my letter (Epand, 1993), i.e., that while the lipid is still in a lamellar arrangement it exhibits differences in its properties which can be monitored by fluorescence probes. Apparently from the analysis of LIPIDAT by Koynova and Caffrey (1993) this kind of pretransition phenomenon can also occur for the L_β to L_α transition. One of the problems about analyzing these effects is that they often are not very large and represent shifts of only a few degrees. Thus, there is always an uncertainty in comparing results from different laboratories with different batches of lipid, different temperature calibrations, buffer, history of the sample, etc. However, I agree that there do seem to be some consistent differences between measurements made using DSC and fluorescence. Where we disagree is whether these differences should be termed different aspects of the phase transition or pretransition phenomenon.

REFERENCES

1. Epand, R. M. 1993. Detection of hexagonal phase forming propensity in phospholipid bilayers. *Biophys. J.* 64:290.
2. Koynova, R., and M. Caffrey. 1993. Mesophase transition temperatures as measured by fluorescence and calorimetry. *Biophys. J.* 65:550. (accompanying letter).