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Phase Transitions in Combined Rabbit Muscle Sarcoplasmic Reticulum Lipids by Raman Spectroscopy

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Using Raman spectroscopy, we found that the sarcoplasmic reticulum lipids of combined muscles from rabbit leg undergo at least two reversible temperature phase changes, centered at about -15 and 13°C. Below the first transition, the lipid Raman CH st region is characteristic of the hexagonal lamellar gel phase. Above the second transition, the Raman CH stretch region is that of a "melted" lamellar phase, somewhat more rigid than a monophasic lipid system. The composition of the lipids was determined and the possibility of a relation between the major head group types and the phase transitions is discussed. Since SR Ca^{2+}ATPase activiity is enhanced at about 14-19°C, the Raman studies suggest that ATPase activity is enhanced when the 13°C transition is complete.

The temperature-dependent phase behavior of sarcoplasmic reticulum (SR) lipids has not been previously detailed. An abrupt enhancement in the activity of sarcoplasmic reticulum Ca^{2+}-ATPase observed around 15° has been attributed by some to a postulated possible transition of the SR lipid to the liquid crystalline phase[1,2] and by others, to an enzyme conformation transition which requires a fluid bilayer but is not correlated with a lipid phase transition.[3] Recently, we analyzed and compared the phospholipid and cholesterol composition of rabbit SR membrane from various muscle types.[4] Notable differences in composition between fast twitch and slow twitch muscle SR lipids were observed, which led to the proposal of a possible mechanism for regulating ATPase activity and twitch rate by lipid composition. The various studies of SR lipids and ATPase indicate that detailed studies of SR lipid phases are necessary to better understand SR membrane processes. Because Raman spectroscopy is very sensitive to hydrocarbon chain order, and chain packing[5-7], we have studied the Raman CH

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stretch region of lipids extracted from rabbit muscle sarcoplasmic reticulum membrane to examine the temperature dependence of SR lipid phases.

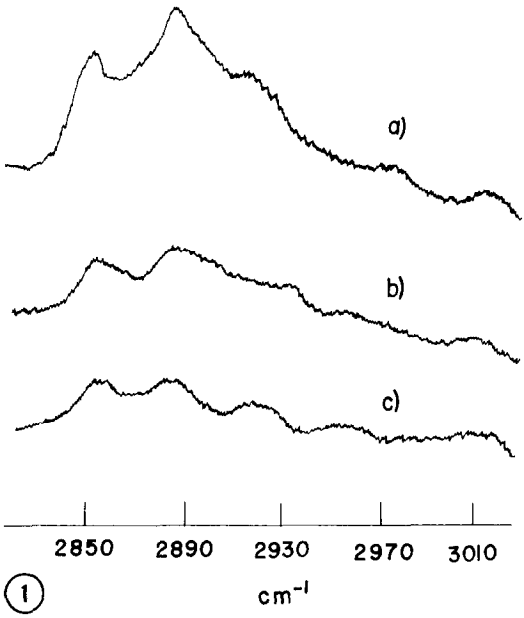
EXPERIMENTAL

Fragmented sarcoplasmic reticulum was prepared from a male white New Zealand rabbit, as previously[4], by the method of MacLennon[8] as modified by Banerjie et. al.[9] Lipids (including cholesterol) were extracted[10] by adding 3 mL $CHCl_3$/MeOH solution (1:2 by volume) to 1 mL R_1 washed sarcoplasmic reticulum suspension (.5 to 15 mg protein/ml) and vortexing for one minute. One mL $CHCl_3$ was added with vortexing. One mL H_2O was added with one minute vortexing. The phases were separated by centrifugatrion. The lower phase was removed for lipid[6] analysis and for Raman sample preparation.

The Raman sample was prepared by evaporating the $CHCl_3$ from an aliquot of the lipid extract under N_2 and dispersing the lipids in triply distilled water. The sample was sealed in a capillary tube and placed in a Harney Miller cell in which sample temperature was controlled within ± 0.5°C by a stream of cooled or warmed N_2 gas and measured by a thermocouple attached to the capillary close to the incident site of the laser beam. Raman-shifted 514.5 nm argon ion laser light scattered from the sample was collected at 90° to the incident beam and analyzed by a Spex 14018 four-slit double monochrometer equipped with a spatial filter to reduce stray light. A cooled RCA-c31034 PM tube was used for photon counting. Spectral bandpass was set at 5 cm^{-1}. Sample integrity was also monitored by measuring acyl vibrational Raman bands (C=O stretch), head group bands (choline symmetric (C_4N^+ stretch), and the side chain C=C stretch at each temperaturue. These bands showed no significant changes which might indicate sample deterioration.

RESULTS

The Raman CH stretch bands for the phospholipid hydrocarbon chains[5-7] are very sensitive to intrachain conformataion (gauche-trans isomerization) and are also sensitive to lateral interactions of hydrocarbon chains. The ratio of peak heights for the Fermi-enhanced Raman-active antisymmetric CH_2 stretch at ~2882 cm^{-1} and the symmetric CH_2 stretch at ~2850 cm^{-1} is about 1.38. The low temperature Raman CH st spectra of the SR lipids are typical of hexagonal phase lipids in lamellar gel (or solid) state. (See Fig. 1a). The spectra above 14°C are typical of melted lamellar dispersions (Fig. 1c). As the hydrocarbon chains become more randomized, the Fermi-enhanced 2882 cm^{-1} Raman band broadens and decreases in peak height. The peak height ratio I_{2882}/I_{2850} is used in the present study to monitor the packing and "fluidity" of the bilayer interior of the SR lipids as a function of temperature.



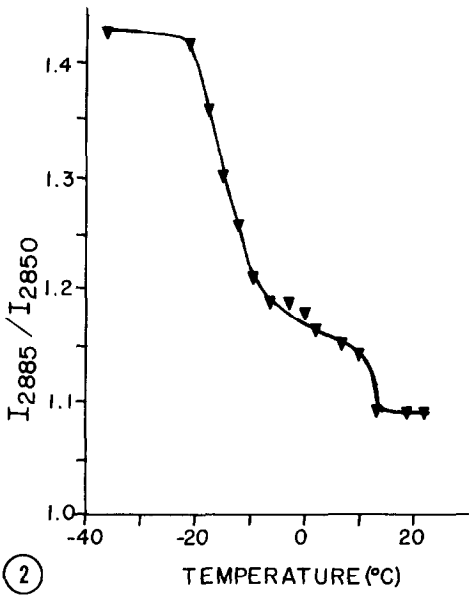


FIGURE 1. Raman CH stretch region for SR lipids at (a) -17 (b) -6 and (c) 20°C.

FIGURE 2. The peak height ratio of the 2882 and 2850 cm^{-1} Raman bands plotted against temperature for rabbit sarcoplasmic reticulum lipids.

Two distinct phase transitions are evident from the peak height ratio I_{2882}/I_{2850} plotted against temperature in Figure 2. The Raman temperature measurements were made three times with different aliquots and gave virtually identical results for the three studies. Phase transitions were centered at about -15° and 13°C.

The difference ΔR between the peak height ratios I_{2882}/I_{2850} immediately after and before each transition is a measure of the average change in fluidity of the total sample during the transition. When compared to ΔR_{max}, the difference between the highest and lowest values of I_{2882}/I_{2850} observed for the sample, a very rough estimate of the fractional change in average bilayer fluidity for each transition is obtained. The lower temperature transition accounts for about 70% of the bilayer melting, while the higher temperature transition accounts for about 30% of the bilayer melting.

The composition of the SR lipid extraction used was analyzed and is presented in Table I. As expected, the phosphatidylcholine/phosphatidyl-

TABLE I
Composition of the Sarcoplasmic Reticulum Sample

Lipid Type	Mole % of Total Phospholipid Content
Phosphatidylcholine	68%
Phosphataidylethanolamine	16%
Sphingomyelin	5%
Phosphatidylinositol	4%
Phosphatidylserine	7%
Cholesterol	9% of total lipid and cholesterol

ethanolamine ratio is high. About 70% of the SR phospholipids are phosphatidylcholine; 16% are phosphatidylethanolamine; and phosphatidylserine, phosphatidylinositol, and sphingomyelin make up the remaining 14%. The cholesterol content measured is less than 10%.

DISCUSSION

The phase changes may be due to morphological changes in which the bilayer undergoes long-range structural reorganization. Or the individual phase changes may be due to "melting" to the liquid crystalline state of a fraction of the lipid components of the bilayer. However, we can determine from the final value of the CH stretch intensity ratio above 14° that the bilayer is in the liquid crystalline lamellar phase, since the ratio value R of about 1.09 is greater than that observed for liquid crystalline lamellar phases of pure synthetic phospholipids such as DPPC.[5] Both the 2890 and 2850 peak height ratio and the 2890 and 2935 cm^{-1} peak height ratio are close to those of lammellar melted phases, and not like those of non-bilayer phases.

Lateral phase separation and monotectic phase behavior have been observed in a number of model binary lipid systems when the phase transition temperatures T_c of the pure components differ by 10° or more (See references 11-14 for examples). Significant differences in head group, differences in side chain saturation, and differences in side chain length

of two or more methylene groups are sufficient to prevent "co-crystallization" of different lipids in a binary system. There has been much speculation that lateral phase separation of fluid and non-fluid components in natural membrane lipids may also occur.[15,16] And, it has been suggested that proteins requiring a fluid lipid environment for maximum activity may attain this enhanced activity at temperatures below that required for melting of the entire bulk lipid by preferentially associating with regions of fluid lipid in a phase-separated mixed phase system.[17,18]

Phase transitions for various lipid types in natural systems have been measured by separating the lipids by head group type, and have been observed to have widely differing melting temperatures (T_m). For example, T_m for egg phosphatidylcholine (PC) is reported to be -15 to -17°C.[19] This temperature range is similar to that for the first phase change observed in the SR lipid sample. That transition accounts for about 70% of the entire hydrocarbon chain disordering observed over the entire melting study and PC constitutes about 68% of the phospholipid population in the sample studied. Thus the observations are not in contradiction with the possibility that the lowest observed SR lipid transition may involve PC-rich lipids. T_m reported[20] for egg phosphatidylethanolamine is 5°C, which is very close to the value of the second transition centered at 13°C. Sphingomyelin melting points are generally higher, 38°C for bovine brain SM. PE and SM together make up 21% of the lipids in the SR sample and may be involved in the second transition.

The fact that the higher temperature phase transition is complete at about 14°C, which corresponds to the temperature of enhanced Ca^{2+}ATPase activity, suggests that either the entire bulk lipid must be liquid crystalline for enhanced ATPase activity, or that the highest temperature transition involves lipids enriched with specific types which interact with Ca^{2+}ATPase.

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REFERENCES

1. Davis, D. G., Inesi, G., and Gulik-Krzywicki, T. (1976) Biochemistry 15, 1271.

2. Inesi, G., Milliman, M., and Eletr, S. (1973) J. Mol. Biol. 81, 483.

3. Lippert, J. L., Tyminski, D., and Desmeules, P. J. (1976) J. Am. Chem. Soc. 98, 7075.

4. Borchman, D., Simon, R., and Bicknell-Brown, E.B. (1982) J.Biol. Chem. 257, 14136.

5. Brown, K. G., Peticolas, W. L., and Brown, E. B. (1973) Biochem. Biophys. Res. Comm. 54, 358.

6. Gaber, B. P., Yager, P., and Peticolas, W. L. (1978) Biophys. J. 21, 161.

7. Hill, I. R., and Levin, I. (1979) J. Chem. Phys. 70, 842-851.

8. MacLennan, D. H. (1970) J. Biochem. 245, 4508.

9. Banerjie, R., Epstein, M., Kandrach, M., Zemniak, P., and Racker, E. (1979) Membrane Biochemistry 2, 283.

10. Spector, T. (1978) Anal. Biochem. 86, 142.

11. Galla, J. H. and Scakman, E. (1975) Biochim. Biophys. Acta 401, 509.

12. Wu, S. H., and McConnell, H. M. (1975) Biochemistry 14, 847.

13. Pagano, R. E., Cherry, R. J., and Chapman, D. (1973) Science 181, 557.

14. Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochem. Biophys. Acta 311, 330.

15. Wunderlich, F., Ronai, A., Speth, V., Seeling, J., and Blume, A. (1975) Biochemistry 14, 3730.

16. Oldfield, E. and Chapman, D. (1972) FEBS Fed. Eur. Biochem. Soc. Lett. 23, 285.

17. Raison, J. K. Lyons, J. M., Melhorn, R. J., and Keith, A. O. (1971) J. Biol. Chem. 246, 4036.

18. Lee, M. P., and Gear, A. R. L. (1974) J. Biol. Chem. 249, 7541.

19. Papahadjopoulos, D., Vail, W. J., Jacobson, K., and Poste, G. (1975) Biochim. Biophys. Acta 394, 483.

20. Jacobson, K., Papahadjopoulos, D. (1975) Biochemistry 14, 152.