## BBA Report

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# Raman spectroscopic study of human erythrocyte membranes

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SUMMARY

The Raman spectrum of human erythrocyte membranes in the 1000–1500 $cm^{-1}$ region is reported. The results indicate the presence of considerable fluidity in the hydrocarbon chains of the phospholipids.

Infrared[1,2] and Raman[2,3] spectroscopy have recently been shown to yield detailed information about fluidity of hydrocarbon chains in phospholipid–water mixtures. In this note we report the first results of the use of Raman spectroscopy to study biomembranes.

Fig. 1 shows the Raman spectrum of a suspension of erythrocyte membranes in the 1000–1500 $cm^{-1}$ region. Four Raman lines are seen, at 1110, 1340, 1420 and 1445 $cm^{-1}$. All these bands are attributable to the hydrocarbon chains of the fatty acids, with a possible small contribution from the $CH_2$ groups of cholesterol. The proteins present do not have any strong Raman bands in this region of the spectrum. The Raman bands appear on a broad, intense, fluorescent background.

The spectrum was obtained using instrumentation which has been described previously[2,4]. Because of the intense fluorescence, the spectrum was recorded digitally at 1 $cm^{-1}$ intervals, with an integration time of 100 s at each data point. Total time for obtaining the spectrum was thus about 14 h. Laser power (He–Ne 632.8 nm) at the sample was 40 mW. During this period the sample showed no apparent signs of decomposition This was confirmed by successfully reproducing the spectrum in Fig. 1 with the same sample as well as with a fresh sample. The spectrum in the 1100 $cm^{-1}$ region was also reproduced in a shorter time using a Krypton ion laser (647.1 nm) giving approx. 200 mW power at the sample.

The sample was prepared according to the method of Dodge *et al.*[5] and was hemoglobin free as determined by the electronic spectrum (Cary 14). Prior to obtaining the

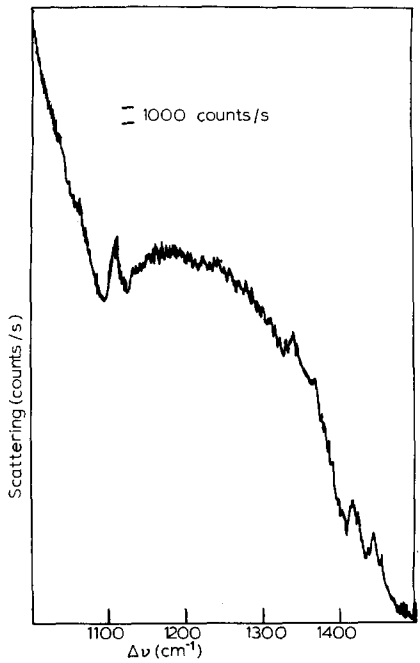


Fig. 1. Raman spectrum of human erythrocyte membranes. The sample was suspended in buffer. The spectrum shown is unsmoothed, digitally collected, as a result of using photon counting amplification. The spectrum has been off-set to put the lowest point at zero on the scale.

Raman spectrum, the sample was centrifuged to concentrate the ghosts with respect to low molecular weight impurities. While the fluorescence is strong, this represents just a small amount of impurity, as fluorescence is approximately $10^6$ times as strong as Raman emission.

Previous studies[2,3] have shown that the 1100 $cm^{-1}$ region of the Raman spectrum is a good diagnostic for fluidity of fatty acid chains in phospholipids. For crystalline chains, two relatively intense bands at 1130 and 1060 $cm^{-1}$ are found, with a weaker band at 1090 $cm^{-1}$. At higher temperatures, with increasing fluidity, these collapse to a broad central maximum centered near 1080 $cm^{-1}$.

Fig. 1 shows this broad band as well. Its maximum is at 1110 $cm^{-1}$. The presence of the single broad band in this region indicates that the fatty acid chains are fluid in erythrocyte membranes.

These findings appear to be somewhat different from those of spin label experiments[6] which indicated that the hydrophobic region in erythrocyte membranes was less fluid than that in either phospholipid vesicles or neural membranes. It is important, however, to make a number of points relevant to a comparison between the two experiments: First, fluidity varied somewhat in ref. 5 with different spin labels. Second, Raman measurements look only at the fluidity of the hydrocarbon chains, whereas in a diffused spin label (and to a certain extent in a covalently bound spin label) one examines the fluidity of the

entire hydrophobic region. Third, measurements by different spectroscopic techniques always involve averaging over different time scales, and consequently can yield apparently conflicting results which are, in fact, in agreement.

The observation of a maximum at higher frequency from that seen in phospholipid–water bilayers[2,3] is also of interest. Several effects, not yet investigated, may be responsible. These include the presence of considerable amounts of unsaturated fatty acids and the interaction of the phospholipids with cholesterol.

These results point up the power of Raman spectroscopy as a non-destructive, small sample (routinely less than 1 $\mu$l) technique for studying biomembranes.

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REFERENCES

1 B.J. Bulkin and N. Krishnamachari, *Biochim. Biophys. Acta*, 211 (1970) 592.
2 B.J. Bulkin and N. Krishnamachari, *J. Am. Chem. Soc.*, 94 (1972) 1109.
3 W.L. Peticolas and J.L. Lippert, *Proc. Natl. Acad. Sci. U.S.*, 68 (1971) 1572.
4 B.J. Bulkin and A. Noguerola, *Proc. Conf. on Computers in Chemical Education and Research*, 1971, 1.47.
5 J.T. Dodge, E. Mitchell and D.J. Hanahan, *Arch. Biochem. Biophys.*, 100 (1963) 119.
6 W.L. Hubbell and H.M. McConnell, *Proc. Natl. Acad. Sci. U.S.*, 64 (1969) 20.