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RAMAN SPECTROSCOPIC INVESTIGATIONS OF SARCOPLASMIC RETICULUM MEMBRANES

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SUMMARY

Raman spectra are presented for sarcoplasmic reticulum membranes Interpretation of the 1000–1130 cm^{-1} region of the spectrum indicates that the sarcoplasmic reticulum membrane may be more fluid than erythrocyte membranes that have been examined by the same technique The fluidity of the membrane also manifests itself in the amide I portion of the membrane spectrum with a strong 1658 cm^{-1} band characteristic of C=C stretching in hydrocarbon side chains exhibiting *cis* conformation This band is unaltered in intensity and position in H_2O and in ^2H_2O thus obscuring amide I protein conformation Of particular interest is the appearance of strong, resonantly enhanced bands at 1160 and 1527 cm^{-1} attributable to membrane-associated carotenoids

INTRODUCTION

Polypeptides, proteins, carbohydrates and phospholipids are being subjected to Raman spectroscopy in a number of laboratories An extensive catalog of Raman responses of these biological materials is being accumulated that promises to contribute to the understanding of molecular and macromolecular configurations in aqueous suspension Raman spectroscopy has been extended to membranes [1] but the initial reported work yielded only a poorly resolved spectrum because of a high fluorescence background Very recently, however, the Raman spectra of hemoglobin-free human erythrocytes [2, 3] and thymocyte plasma membranes [4] were reported by several researchers

A particularly simple and interesting membrane for study is the muscle sarcoplasmic reticulum membrane It contains few proteins, of which the major one is a $(Ca^{2+}+Mg^{2+})$-ATPase with a molecular weight of approximately 106 000 [5] This $(Ca^{2+}+Mg^{2+})$-ATPase is thought to comprise up to 70 % of the total protein associated with the membrane Despite its simple composition, this membrane is highly effective in the transport of Ca^{2+} Present evidence indicates that the $(Ca^{2+}-Mg^{2+})$-ATPase protein consists of only a single unit The lipid-protein weight ratio in the vesicles studied was 60 mg lipid per 100 mg protein [6] We report here the initial

results of Raman spectra of sarcoplasmic reticulum membranes obtained on samples suspended in H_2O and in ^2H_2O

EXPERIMENTAL

Vesicles of fragmented sarcoplasmic reticulum were prepared from lobster muscle as described previously [7] Suspensions contained 20 mg of sarcoplasmic reticulum protein/ml and were examined at this concentration The homogeneity of the preparations was checked by electron microscopy [7]

Ca^{2+} transport was measured as previously described [8] Only preparations showing significant rates of Ca^{2+} transport ($> 2\ \mu$mol Ca^{2+}/mg protein per min) were examined spectroscopically Thus the sarcoplasmic reticulum membranes studied were fully functional

Deuteration of the membranes was achieved by equilibrating the membranes in 99 9 % ^2H_2O for 12 h at 4 °C [2] This provided essentially complete deuteration of all exchangeable hydrogens

The samples were sealed in melting-point capillaries and illuminated transversely with a Spectra-Physics Model 165 Argon ion laser operating at 488 nm The capillaries were housed in a heat sink whose temperature was controlled by a circulating water-glycol bath The temperature of the sample was measured by a calibrated platinum resistance thermometer in contact with the capillary heat sink The scattered light was collected at right angles to the capillary and laser plane and investigated with a SPEX Model 1400 0 75-meter double monochromator equipped with 2400 groove/mm Jobin-Yvon holographic gratings The signal was detected by an RCA C31034A-02 photomultiplier tube housed in a thermoelectric cryostat maintained at 70 °C At this temperature the tube exhibited 30 % quantum efficiency and an undiscriminated dark count rate of less than 4 photons/s The unique aspects of the spectrometer are reported elsewhere [9]

RESULTS AND DISCUSSION

A number of Raman spectra of lobster sarcoplasmic reticulum vesicles were obtained at temperatures near lobster biological temperatures ($\sim$ 10 C) Each sample was bleached in the laser beam for 30 min before beginning the spectral run of about 8 h duration These spectra were highly reproducible with representative spectra being shown in Figs 1–3 Tentative phospholipid and protein vibration assignments are given in Table I

Carotenoids

Intense bands were observed at 1160 and 1525 cm^{-1} in our sarcoplasmic reticulum vesicles

Lippert [3] previously noted in his Raman spectra of erythrocyte ghosts the appearance of intense bands of unknown origin at 1160 and 1527 cm^{-1} Wallach [2] reported similar spectra and after detailed parametric studies [10] was able to make a high-confidence assignment of these lines to resonance Raman scattering from membrane carotenoids [11]

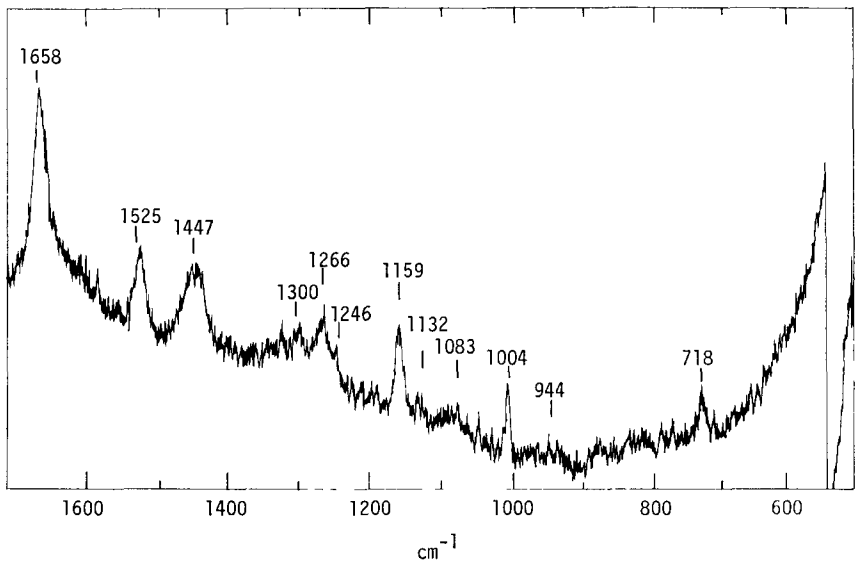

Fig 1 Raman spectrum of sarcoplasmic reticulum membranes in H_2O Maximum signal, 3 10^3 photons/s, wavelength, 488 0 nm, power, 200 mW, resolution, 4 cm^{-1}, time constant, 30 s, temperature, 10 °C

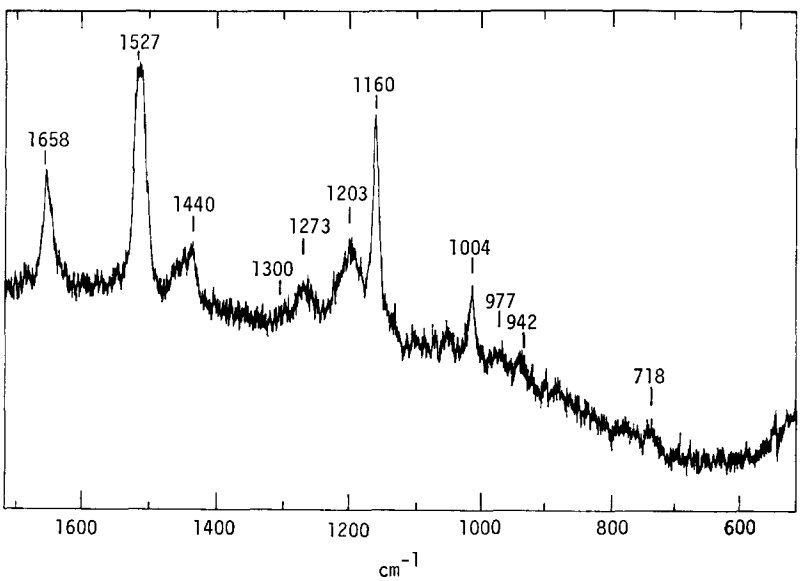

Fig 2 Raman spectrum of sarcoplasmic reticulum membranes in ^2H_2O Maximum signal, 3 10^3 photons/s, wavelength, 488 0 nm, power, 200 mW, resolution, 4 cm^{-1}, time constant, 30 s, temperature, 10 °C

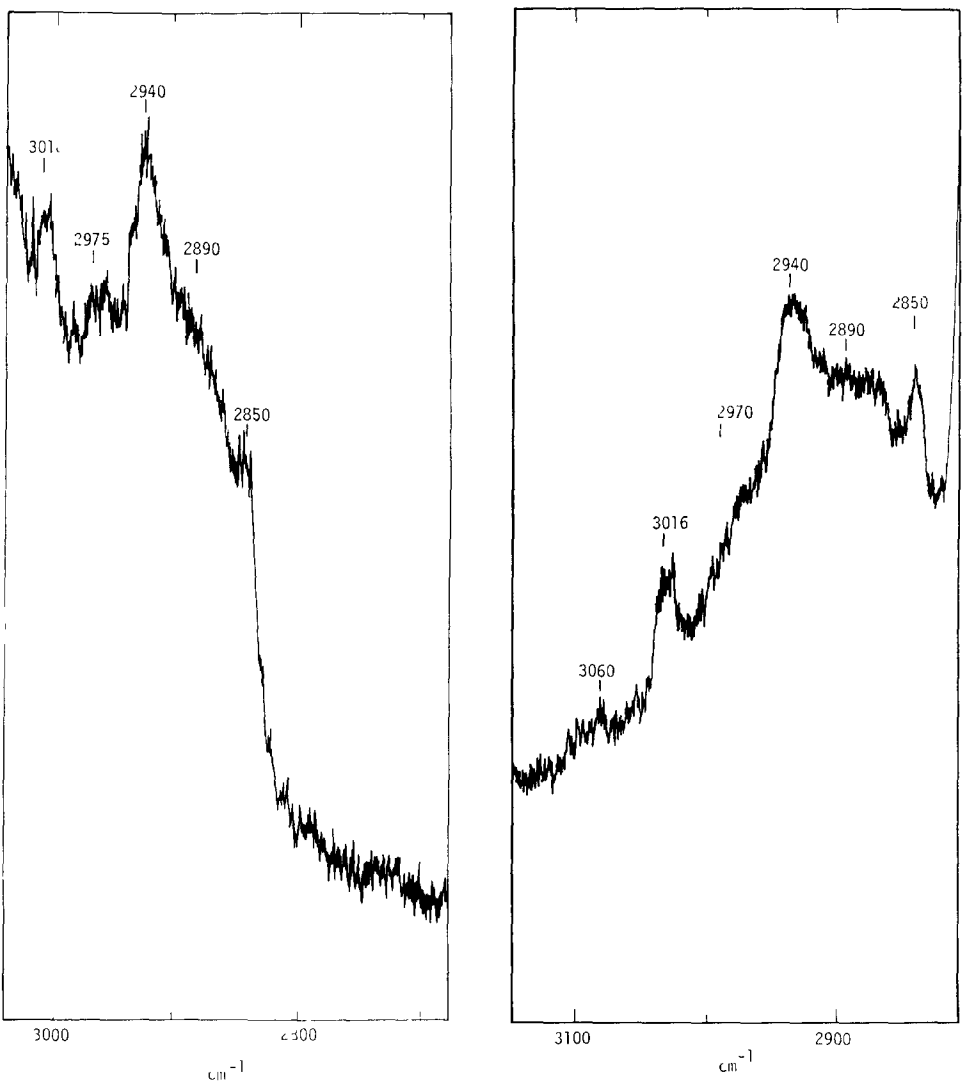

Fig 3 2800–3100 cm^{-1} region of the Raman spectrum of sarcoplasmic reticulum membranes in (a) H_2O and (b) ^2H_2O Maximum signal, 10^4 photons/s wavelength, 488 0 nm power, 200 mW, resolution, 4 cm^{-1}, time constant, 10 s, temperature, 10 °C

Region of 2800–3100 cm^{-1}

In this region, six vibrations are present in ^2H_2O and five in H_2O Considerable research on this region [12–14] indicates that most of the bands can be assigned to hydrocarbon stretching modes The sharp bands at 2850 and 2940 cm^{-1} are assigned to symmetric and antisymmetric CH_2 stretching The 2850 cm^{-1} band is attributable to membrane lipids From comparison of the spectra of fat-free albumin and liposomes Wallach suggests that the 2940 cm^{-1} band can be attributed predominantly to membrane protein [2] However, because of the increased degree of unsaturation

TABLE I

FREQUENCIES, INTENSITIES AND ASSIGNMENTS OF OBSERVED RAMAN SPECTRA OF SARCOPLASMIC RETICULUM MEMBRANES

Numbers in parentheses are intensities relative to the 1004 cm^{-1} band, numbers in brackets are references helpful in making tentative assignments

Frequency (cm^{-1})		Tentative assignment		
H_2O	^2H_2O	Lipid	Protein	Other
718 (6)	718 (2 5)	$RN^+(CH_3)_3$ [14]		
944 (1)	942 (2)		C-C stretch [22]	
	977 (2)		Amide III [2]	
1004 (10)	1006 (10)		Phe [16]	ν_4(=C-C=) [11]
1040 (3)	1043 (2)		Phe [16]	
1083 (3)		C-C stretch [18]		
1132 (3)		C-C stretch [18]		
1159 (12)	1163 (25)			ν_1(=C-C=) [11]
	1203 (12)			^2H_2O
1246 (2 5)		CH_2 bend	Amide III [2]	
1266 (7)	1273 (5)		CH_2 bend	
1300 (4)	1300 (2)	CH_2 twist [14]		
1323 (4)				
1343 (2)			CH_2 twist [14]	
	1440 (8)		CH_2 bend [14]	
1447 (13)	1450 (7)	CH_2 bend	CH_2 bend [14]	
1525 (12)	1527 (32)			ν_2(=C-C=) [11]
1583 (3)				
1658 (20)	1659 (15)	C=C stretch (*cis*) [19]	Amide I [2]	H_2O
2851	2852	CH_2 *Sym* stretch [12]		
2890	2890	CH_3+CH_2 *Sym* stretch		
2934	2936	CH_2 *Anti-sym* stretch	CH_2 *Anti-sym* stretch	
2974	2970	CH_3*Asym* stretch	CH_3*Asym* stretch	
3018	3016	CH Olefinic stretch [15]		
	3060		Phe [16]	$2\nu_2$(=C-C=) [11]

in sarcoplasmic reticulum vesicles over erythrocytes, significant contributions from short $(CH_2)_n$ chains separated by C=C cannot be ruled out The broad band at 2890 cm^{-1} represents overlapping symmetric stretching of CH_3 and CH_2 of membrane lipids

Of particular interest is the sharp band at 3016 cm^{-1} It can be tentatively assigned to olefinic CH stretch in lipids exhibiting *cis* conformation [15] This interpretation complements the analyses of the 1658 cm^{-1} band in the next section

The very weak band at 3060 cm^{-1} in ^2H_2O is quite possibly $2\nu_2$ of the resonant carotenoids [11] although a strong band due to phenyl CH is expected around 3075 cm^{-1} [16] In sarcoplasmic reticulum vesicle samples in which the carotenoid signal has been bleached out, the 3060 cm^{-1} band also vanishes This supports the $2\nu_2$ carotenoid assignment although the signal-to-noise ratio is not high enough to make this unambiguous

Region of 500–1700 cm^{-1}

This region is characterized by vibrations from both phospholipid and protein

(vibrations attributed to protein backbone structure are referred to as amides A, B and I–VII) In addition, aromatic amino acids exhibit strong Raman signals (attributable to ring vibrations) in this region In the sarcoplasmic reticulum membranes a strong line present at 1004 cm^{-1} is attributable to phenylalanine Following the precedent of other researchers [2, 3], it is used as the intensity standard throughout

This is not a totally valid procedure as a weak resonantly enhanced carotenoid band is found at 1006 cm^{-1} [11] Analysis of the relative carotenoid intensities and membrane intensities in both H_2O and ^2H_2O indicates that approx 13 % of the 1004 cm^{-1} line in H_2O is due to carotenoid, while approx 33 % of the 1006 cm^{-1} line in ^2H_2O is due to carotenoid As only relative intensities are of importance, a better internal standard would be the 1160 and 1527 cm^{-1} carotenoid lines, when they are present, or the 1004 cm^{-1} line when carotenoids are absent

The spectrum from sarcoplasmic reticulum membrane (unlike that from erythrocyte ghosts [3] which indicated the presence of considerable all-*trans* configuration of lipid hydrocarbon side chain) is characteristic of unsaturated lipids exhibiting some *cis* configuration Of prime importance is the appearance of a sharp band at 1658 cm^{-1} in both H_2O and ^2H_2O Comparison with spectra of previous researchers [17, 18] of lecithin sonicates as well as model saturated and unsaturated fatty acids indicates that the 1658 cm^{-1} line in sarcoplasmic reticulum membranes can be assigned to C=C stretching vibration of the hydrocarbon side-chain of unsaturated lipid in *cis* configuration [19] This assignment is also supported by the appearance of a sharp band at 3016 cm^{-1} The significance of this is that it conceals the weaker amide I vibration which is important in determining protein secondary structure The absence of a sharp band at 1065 cm^{-1}, the appearance of only a weak band at 1132 cm^{-1} (indicative of rigid all-*trans*) and the appearance of a band at 1083 cm^{-1} (indicative of random, fluid side-chain) all indicate that the sarcoplasmic reticulum membrane is less predominantly *trans* than that of erythrocytes (55–70 % reported) [3] As all published spectra to date have been taken at 10–20 C, the increased rigidity of erythrocytes over lobster sarcoplasmic reticulum may be due solely to the difference in biological temperatures (37 °C as compared to approx 10 °C)

The Amide I vibration of proteins occurs in the 1620–1680 cm^{-1} region of the spectrum [20–23] H_2O also exhibits a moderate Raman signal in this region, hence, it has become common practice to also obtain a spectrum of the sample in question suspended in ^2H_2O This removes the competing H_2O signal from the amide I region and allows for easier identification of the amide I vibrations Unfortunately, in the sarcoplasmic reticulum membrane spectra, the dominant feature in the amide I region is the 1658 cm^{-1} band from the membrane lipid The intensity and position of this band is unaffected by deuteration, consequently, it obscures the amide I region

The amide III vibrations occur in the 1200–1300 cm^{-1} region in H_2O Proteins with α-helical structure have amide III frequencies above 1250 cm^{-1}, while those with β and random coil structure have amide III below 1250 cm^{-1} [15–18] In ^2H_2O the amide III is shifted 300 cm^{-1} to the 900–1000 cm^{-1} region The sarcoplasmic reticulum membranes in H_2O (Fig 1) exhibit a shoulder at 1246 cm^{-1} possibly indicative of β or random coil structure although it may be a lipid vibration In ^2H_2O an amide III is possibly present at 977 cm^{-1}, but the background in this region is quite obscuring The 1266–1273 cm^{-1} band does not shift upon deuteration It cannot, therefore, be assigned to α-helical amide III It is probably CH bend in amino

acid residues, and its intensity obscures the presence of α-helical structure It is apparent that without supporting amide I vibrations, the amide III region in sarcoplasmic reticulum membrane spectra is inadequate to describe completely membrane protein conformation

GENERAL COMMENTS

Raman spectra are readily obtainable from actively transporting sarcoplasmic reticulum membranes prepared by standard laboratory techniques Two significant points are immediately apparent from our results The first is the appearance of the two resonantly enhanced bands attributable to carotenoids The same bands were reported previously by Wallach [2] and Lippert [3], although Lippert reported their appearance only in some of his spectra We observe different intensities of these bands in different samples (compare H_2O and ^2H_2O spectra) and we have not observed the resonant carotenoid bands in work at this laboratory on human and animal erythrocytes [24] Furthermore, the resonant bands are absent from our recently obtained spectra of rabbit sarcoplasmic reticulum membranes (Milanovich, F P , et al , unpublished) It is not yet certain whether the carotenoids are membrane constituents, as Wallach suggests, or impurities from the surrounding plasma that were not completely removed in the washing process If they are indeed membrane constituents, their presence and strength might become an excellent indicator of membrane integrity after isolation and washing

Secondly, the 1658 cm^{-1} C=C stretch significantly obscures the amide I vibration in sarcoplasmic reticulum membranes and, quite possibly, in erythrocytes This so complicates the identification of protein amide I that Raman analysis of sarcoplasmic reticulum membranes cannot as yet be considered a reliable indicator of the secondary conformation of membrane protein

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