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CONCANAVALIN A STIMULATION MODIFIES THE LIPID AND PROTEIN STRUCTURE OF RABBIT THYMOCYTE PLASMA MEMBRANES

A LASER RAMAN STUDY

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SUMMARY

(1) We have compared the laser Raman spectra of isolated plasma membranes from resting rabbit thymocytes and cells mitogenically stimulated with concanavalin A.

(2) Major alterations in the CH stretching, CH deformation and CC stretching regions indicate a different lipid architecture in the membranes from activated cells.

(3) Spectral changes in the Amide I and II regions, by reference to the spectra of model compounds indicate greater protein amidation in the membranes from stimulated cells.

INTRODUCTION

Mitogenic stimulation of rabbit thymocytes with concanavalin A modifies a diversity of plasma membrane functions [1–5]. It also increases the turnover of a 55 000 dalton (monomer) glycoprotein [6] that is responsible for high-affinity concanavalin A binding [7]. However, comparisons of purified plasma membranes from resting and stimulated thymocytes by dodecyl sulfate-polyacrylamide gel electrophoresis [6] show no significant differences in protein composition or proportion. In contrast, mitogenic stimulation induces the reacylation of membrane lysophosphatides preferentially by polyunsaturated fatty acids [8], producing an increase in net phospholipid unsaturation [9]; the altered plasma membrane function of activated thymocytes might therefore involve a modification of lipid-protein interactions. To test this possibility we have extended earlier laser Raman analyses of plasma membranes isolated from resting thymocytes [10] to the examination of membranes isolated from concanavalin A-stimulated cells.

Abbreviation: HEPES, 4-(hydroxymethyl)-1-piperazinylethane-2-sulfate.

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EXPERIMENTAL

Chemicals

Egg lecithin and sphingomyelin were obtained from Lipid Products (South Nutfield, U.K.) asparagine and glutamine (analytical grade) from Fischer (Freehold, N. J., USA), poly-L-asparagine and melittin from Sigma Biochemicals (St. Louis, Mo.), acrylamide and *N,N'*-methylenebisacrylamide from Bio-Rad, (Richmond, Calif.), concanavalin A from Miles Laboratories (Elkhart, Ind.), and 2-(3-carboxypropyl)-2-tridecyl-4, 4'-dimethyl-3-oxazolidinyl oxyl (5-nitroxide stearate) from Syva (Palo Alto, Calif.).

Preparation of thymocyte membranes

Microsomal membranes were prepared from rabbit thymocytes cultivated with or without concanavalin A as in ref. 6. Washed cells were resuspended at a concentration of $5 \cdot 10^7$ cells/ml in 0.075 M KCl/0.065 M NaCl/0.25 mM $MgCl_2$/0.01 M 4-(hydroxymethyl)-1-piperazinylethane-2-sulfate (HEPES), pH 7.5, and disrupted by nitrogen cavitation. We sedimented nuclei, mitochondria and lysosomes at $6 \cdot 10^5$ $g \cdot$ min (Beckman centrifuge J 21) and pelleted the small particle fraction at 10^7 $g \cdot$ min (Beckman Spinco ultracentrifuge L2 65, Rotor 60Ti). We then washed the microsomes, first with 0.01 M HEPES (pH 7.5) and then with 0.001 M HEPES, pH 7.5 (10^7 $g \cdot$ min) to eliminate trapped cytoplasmic proteins and to dissociate membrane-bound ribosomes. After the last washing, microsomes were resuspended in 0.001 M HEPES/0.001 M $MgCl_2$ (pH 8.2) applied to a dextran gradient (Dextran T-150 Pharmacia, Sweden) (maximum density 1.09) as in ref. 2 and centrifuged for 10^8 $g \cdot$ min (Beckman Spinco L2 65, Rotor SW 56). The two membrane fractions obtained, plasma membranes and endoplasmic reticulum [6] were collected from the gradient and washed twice in 0.005 M phosphate (pH 8.0) by pelleting them at $1.2 \cdot 10^7$ $g \cdot$ min.

Liposomes

Egg lecithin liposomes ($\pm$melittin) were prepared as in ref. 10 by sonication (Sonic Dismembranator, Quigley-Rochester, Rochester, N.Y., power step 3), using 40 mg phospholipid/ml.

Raman spectroscopy

For Raman spectroscopy, samples were transferred to 0.9–1 mm internal diameter Kimex Capillaries. For temperature control, the sample capillaries were placed in a Harney-Miller Cell [11] after sealing. The temperature was regulated by a flow of N_2 monitored by a telethermometer. Temperature control was checked by determining transition temperatures of authentic lipids (dimyristoyl phosphatidylcholine; dipalmitoyl phosphatidylcholine) in the laser beam.

Raman spectra were recorded as in ref. 10, using a Ramalog 4 Raman spectrometer (Spex Industries, Metuchen, N. J., USA) interfaced to an Interdata (Model 70) computer. An Ar^+ laser (Spectra Physics model 164), tuned at 488 nm (300 mW power) was used as an excitation source. The cutoff filter used to eliminate plasma lines appearing above 1200 cm^{-1} was employed for survey spectra and for scans above 1200 cm^{-1}. Because of its background contribution [12] it was not used when inten-

sity ratios in the CC-stretching region were evaluated. The Raman scattering at right angles to the laser beam was detected by a thermoelectrically cooled photomultiplier (RCA 31034) and was recorded in terms of photons/s. The "dark" counts of the photocell were < 100 counts/s. Raman scattering from the samples gave counts in the order of 10^3–10^4/s. Scanning was done through the computer (loaded with the BIE8D Ramancomp Computer Program; Spex Industries). We used the following specifications for scanning. Maximum time and minimum time for each data point were 1 s and 0.5 s, respectively. The photon counts were 10^4–10^5 maximum and 100 minimum. Scanning was in steps of one wave number but was not at a rate linear with respect to time. Rather, scans were incremental between data points and no counts were recorded while the spectrometer was moving between data points. Photon counts were stored in the computer memory during scanning (2–4 scans) and the stored spectra, averaged and smoothed by a least squares procedure (ref. 13; included in the computer program), were ultimately plotted on the Ramalog recorder, using appropriate background suppressions and scale expansions.

To determine the change of Raman scattering with temperature, the samples, in position but with the laser beam occluded, were equilibrated for 20 min at the desired temperature, then equilibrated a further 5 min in the laser beam before scanning.

ESR spectroscopy

This was carried out on a Varian E-9 Spectrometer (Varian Associates, Palo Alto, Calif. USA), using the spin labelling procedures described in ref. 14.

The data reported here are representative of three separate experimental series.

RESULTS AND DISCUSSION

Fig. 1 represents a spectral survey (3000–700 cm^{-1}) of plasma membranes from unstimulated and stimulated thymocytes. Frequencies of relevant scattering peaks for both membrane types and for pertinent model compounds are listed in Table I. Significant differences between the membranes occur in the CH stretching region (3000–2800 cm^{-1}), the Amide I and Amide II regions (1700–1500 cm^{-1}), the in-plane CH deformation region (1500–1400 cm^{-1}), and the CC stretching zone (1200–1000 cm^{-1}).

CH stretching region

In the CH stretching region of membranes from stimulated and control cells, the 2850 cm^{-1} band, due to symmetric CH_2 stretching in methylenes of long acyl chains, is clearly evident. However, the peak at 2890 cm^{-1} in the controls, (cf. also ref. 10) previously assigned to symmetrical CH^3 stretching [15–18], but also present in the spectra of paraffins without methyl groups [18], appears at 2900 cm^{-1} in membranes from stimulated cells. Importantly, the ratios of the intensities at 2900–2890 cm^{-1} to those at 2850 cm^{-1} ($I_{2900-2890}/I_{2850}$) are much greater in the membranes from activated cells (6.6) than in controls (1.3). Extrapolating from the spectra of model lipids under various conditions [15, 16] and from the effect of melittin on the spectrum of phosphatidylethanolamine [10], this difference in $I_{2900-2890}/I_{2850}$

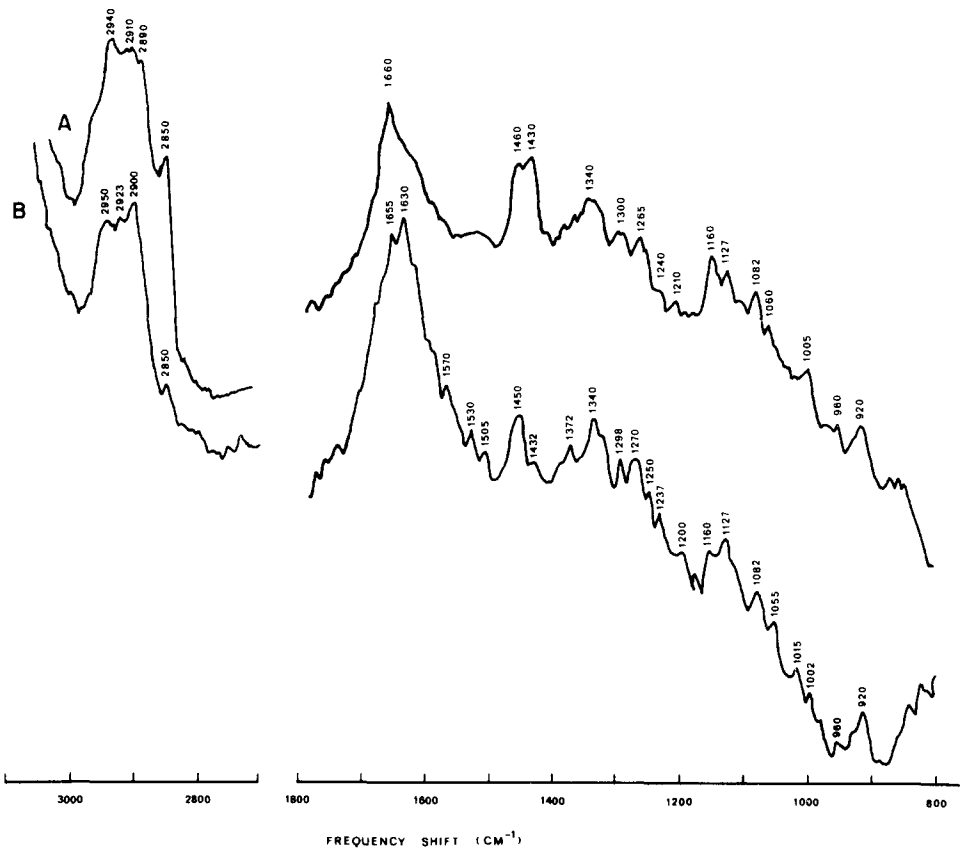

Fig. 1. Survey Raman spectra in the 3000–700 cm^{-1} region of plasma membranes isolated from quiescent (A) and concanavalin A-stimulated (B) thymocytes. Excitation wavelength 488 nm; power 300 mW; temperature 20 ±2 °C. Slits 200 μm (resolution 5 cm^{-1}). Raman intensities are in photons/s, with intensities of maximum CH stretching peaks and Amide I peaks approx. $1.3 \cdot 10^4$ and $8.6 \cdot 10^3$ photons/s, respectively, and intensity at 1000 cm^{-1} approx. $3 \cdot 10^3$ photons/s. For measurement of intensity ratios in CC stretching region, cutoff filter was not used, giving a flat background in this region. The height of the maximum CC stretching band is approx. 10^3 photons/s. Photon-counting precision is ±3 %.

suggests a marked modification of lipid structure in stimulated membranes. In view of recent observations on paraffins lacking CH_3 groups, the ratio change cannot be interpreted as due to exclusively decreased mobility of terminal methyl groups [19].

CH-in-plane deformation region

As noted in ref. 10, at 25 °C the membranes of resting cells exhibit a strong doublet, peaking at 1460 and 1430 cm^{-1}. However, the membranes from stimulated thymocytes show a strong, somewhat broad band at 1450 cm^{-1} with a minor band at 1432 cm^{-1}.

In proteins the prominent CH-banding peak is broad and lies near 1450 cm^{-1}. In dipalmitoyl phosphatidylcholine and dioleoyl phosphatidylcholine above the gel → liquid-crystal transition temperature the CH-in-plane deformation band occurs at

TABLE I

RAMAN SCATTERING FREQUENCIES OF PLASMA MEMBRANES ISOLATED FROM QUIESCENT AND CONCANAVALIN A-STIMULATED RABBIT THYMOCYTES AND MODEL COMPOUNDS

sh, shoulder; ν, stretching; δ, deformation; asym, asymmetric; sym, symmetric.

Resting	Stimulated	Frequency assignment
2940	2950	ν asym (CH_2+CH_3)
2910	2923	
2890	2900	ν sym CH_3, CH_2
2850	2850	ν sym CH_2
1660	1655	Amide I
	1630	
	1570	Amide II
	1530	
	1505	
1460	1450	δ CH_2, CH_3
1430	1432 (sh)	ν sym COO^-
	1372	CH_3
1340	1340	CH and Trp
1300	1298	CH_2 wag
1265	1270	Amide III
1240 (sh)	1250	
	1237	
1210	1200	Tyr, Phe
1160	1160	ν(C-C) and ν(C-N)
1127	1127	
1082	1082	
1060	1055	
1005	1015	Trp, Phe
	1002	
960	960	ν(C-C)
920	920	

1441 and 1445 cm^{-1}, respectively [15, 18]. Below the transition temperature dipalmitoyl phosphatidylcholine and dioleoyl phosphatidylcholine exhibit prominent CH-deformation bands at 1436 and 1445 cm^{-1}, respectively, and shoulders at 1455 and 1475 cm^{-1}, respectively [15, 18]. Yet another pattern is observed with egg lecithin, which is a mixed chain phosphatide, principally 1-palmitoyl-2-oleoyl phosphatidylcholine. Using liposomes equilibrated at +10 °C, we obtain a strong CH-deformation band at 1440 cm^{-1} and a smaller one 1460 cm^{-1} (Table I). At −10 °C, i.e. well below the transition temperature of −5.5 °C, the bands also occur at 1460 and 1440 cm^{-1} and exhibit respective intensities almost equivalent to those observed at +10 °C.

In model systems consisting of one type of phosphatide with identical fatty acid chains at the 1 and 2 positions, a shift from a single band to a split one clearly correlates with the liquid-crystal → gel transition [15, 18]. This is clearly not necessarily the case with mixed chain phospholipids. Therefore, the differences between

the membranes from unstimulated and stimulated thymocytes, both of which contain mixtures of mixed-chain phospholipids, while indicative of a change in lipid organization, cannot be more precisely interpreted.

CC stretching region

The well resolved bands at 1127, 1082 and 1060 cm^{-1} (1055 cm^{-1} in stimulated membranes) can be assigned to CC stretching vibrations, although some contributions from amino acid CN vibrations also appear in this region (Fig. 1). The intensity ratio 1082 cm^{-1}/1127 cm^{-1}, I_{1082}/I_{1127} is consistently 0.54 in the membranes of stimulated cells, compared with 0.86 for control membranes. In model lipids this ratio decreases with the proportion of *trans* vs. *gauche* configurations; i.e. it drops with lowered alkyl chain mobility [17–19].

The 1655 cm^{-1} band

Plasma membranes from stimulated thymocytes exhibit a prominent Raman band at approx. 1655 cm^{-1}, whereas membranes from resting cells show only a small shoulder at this frequency. Deuteration does not displace the band but makes it more prominent in the membranes from control cells. Organic solvent extraction shows the band to be of lipid origin. Moreover, phospholipids with unsaturated paraffin chains, even egg lecithin (primarily 1-palmitoyl-2-oleoyl phosphatidylcholine) exhibit this band [12]; saturated phospholipids do not [15]. We therefore assign this band to the *cis* C=C stretching vibration of unsaturated paraffin chains. The greater prominence of the band in the membranes of stimulated cells presumably reflects their greater fatty acid unsaturation [9].

Amide I and Amide II regions

Membranes from stimulated thymocytes differ markedly from normal in that they exhibit several sharp bands in the Amide I and Amide II areas, i.e. at 1630, 1570 and 1530 cm^{-1} (Fig. 1). These sharp peaks are not observed in lipid extracts, except for a broad band at 1530 cm^{-1}, but suggestions of the bands are apparent also in the spectra of unstimulated membranes. Concanavalin A itself does not exhibit these bands and is not present in the membranes from stimulated cells [6]; therefore these peaks cannot be attributed to this lectin. The "new" bands also cannot be attributed to alterations in secondary structure of membrane proteins, since conformation-sensitive amide scattering does not occur in this frequency range [20]. Moreover, while the Amide III region (1300–1200 cm^{-1}) suggests a greater proportion of β-conformation in "stimulated membranes" than in membranes from resting cells [10], this should not lead to the appearance of bands below 1650 cm^{-1} [20].

A possible explanation for the new bands is that the glutamate and aspartate residues of membrane proteins are more highly amidated in the membranes of stimulated cells. The available Raman literature offers support for this possibility. For example, α-casein and myosin exhibit scattering bands at 1617 and 1625 cm^{-1}, respectively [21], tentatively assigned to Tyr, but occurring in the Amide II region of primary alkyl amides; eight of the 15 aspartate residues and 14 of the 39 glutamate residues are amidated in α-casein. In myosin 9 of 20 aspartates and 6 of 33 glutamates are amidated. In lysozyme [22], where 13 of 21 aspartates and 3 of 5 glutamates are amidated, a 1622 cm^{-1} band is assigned to aromatic amino acids on the basis of the

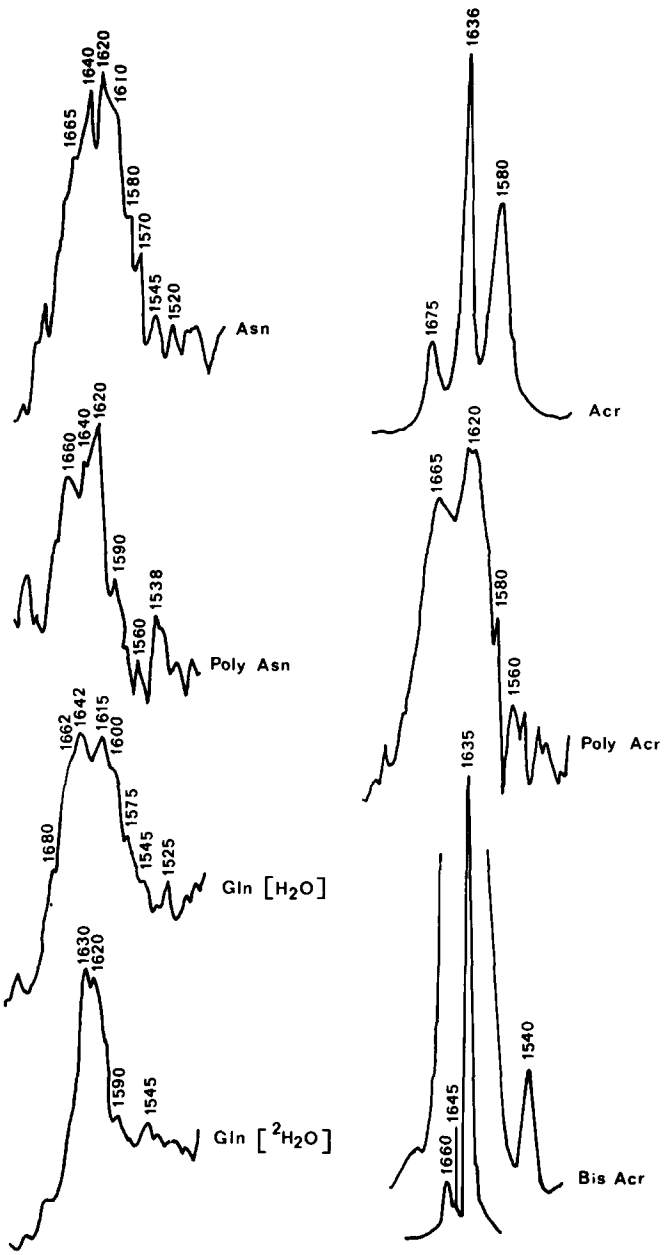

Fig. 2. Raman spectra in Amide I–Amide II regions of several amidated compounds. Excitation at 488 nm; power 300 mW; slits 100 μm (resolution approx. 3 cm^{-1}), temperature 20 °C: Asn = L-asparagine (H_2O); poly (Asn) = poly-L-asparagine (H_2O); Gln = glutamine (H_2O and ^2H_2O); Acr = acrylamide (solid); bis Acr = *N,N'*-methylenebisacrylamide (solid); poly Acr = polyacrylamide. The spectrum of bis Acr is also given at 100 scale expansion to reveal detail.

spectra of constituent amino acids (although the mixture of aromatic amino acids yields a band at 1575, not 1622 cm^{-1}). We have therefore recorded the Raman spectra in the Amide I and Amide II regions of sphingomyelin, acrylamide, polyacrylamide, Asn, poly(Asn), Gln and melittin (free and incorporated into liposomes). Melittin is amphipathic polypeptide that interacts hydrophobically with phospholipid bilayers [23]. We have studied it because only 4 of its 20 residues should yield distinctive Raman scattering; these are the two glutamines, the amidated carboxyl terminus and a tryptophan. Our results are summarized in Fig. 2 and Tables II–IV.

Sphingomyelin, whose long chain fatty acid is linked to sphingosine through a secondary amide linkage has only two scattering bands between 1700 and 1500 cm^{-1}. A strong peak at 1677 cm^{-1} can be assigned to ν(C=C) of the *trans* double band in the sphingosine residue. The Amide I band occurs at 1645 cm^{-1}, a position typical for secondary amides. No Amide II band is observed, suggesting a *cis* configuration of the amide bond.

In the case of acrylamide (CH_2=CH-$CONH_2$) the major band between 1700 and 1500 cm^{-1} occurs at 1636 cm^{-1} (Fig. 2, Table II) and can be assigned to ν(C=C). The strong Amide I peak occurs at 1675 cm^{-1} and the weaker Amide II band at 1580 cm^{-1}. In the case of *N,N'*-methylenebisacrylamide (CH_2=CH-CONH-CH_2-CONH-CH=CH_2) the major peak in this region, at 1635 cm^{-1} (Fig. 2, Table II) can also be assigned to ν(C=C). The strong Amide I band lies at 1660 cm^{-1}. The presence of the shoulder at 1645 cm^{-1} may indicate non-equivalence of the two -CONH- groups. The weak Amide II band lies at 1540 cm^{-1}, consistent with a *trans* configuration of the amide bond.

TABLE II

RAMAN SCATTERING FREQUENCIES (cm^{-1}) OF ACRYLAMIDE, *N,N'*-METHYLENE-BISACRYLAMIDE AND POLYACRYLAMIDE

Acrylamide	*N,N'*-methylene-bisacrylamide	Polyacrylamide*	Tentative frequency assignment
1675			
		1665	Amide I**
	1660		
	1645 sh		
1636	1635		ν(C=C)***
		1622	Amide II
		1615	
1580		1580	Amide II
	1540	1560	

* Polyacrylamide is a gel in water prepared by copolymerization of acrylamide (75 mg/ml) and *N,N'*-methylenebisacrylamide (1.9 mg/ml). The gels were cast directly in spectrometer capillaries [24].

** Due to $-\overset{O}{\overset{\|}{C}}-NH_2$ or $-\overset{O}{\overset{\|}{C}}-NH-$.

*** Due to vinyl groups of acrylamide or *N,N'*-methylenebisacrylamide [25]. Groups are reduced during polymerization.

TABLE III

RAMAN SCATTERING FREQUENCIES (cm^{-1}) OF CRYSTALLINE L-ASPARAGINE, L-ASPARAGINE IN H_2O AND ^2H_2O SOLUTION AND OF POLY-L-ASPARAGINE IN SOLID FORM AND IN H_2O SOLUTION

Relative intensities of solid forms in parentheses. s, strong; sh, shoulder.

Crystal	Asn		poly (Asn)		
	H_2O	^2H_2O	Solid	H_2O	Tentative assignments
1665 (0.2)	1665 sh	1645	1660 (0.93)	1660 s	Amide I (residue and skeletal)
1637 (1.0)	1640 s		1640 (1.0)	1640 s	
1622 (0.53)	1620 s		1615 (0.66)	1620 s	Amide II (residue); dipolar antisymmetric CO_2^- stretching
	1610 sh	1612			
1582 (0.63)				1590	Amide II (skeletal); degenerate NH_3^+ deformation
	1580		1580 sh	1560	
	1570			1538	
	1545				
1530					
	1520				

During polymerization of acrylamide and *N,N'*-methylenebisacrylamide, the C=C bonds of these molecules are reduced. The Raman spectrum of polyacrylamide thus exhibits no ν(C=C) bands (Fig. 2, Table II) and the 1700–1500 cm^{-1} region is dominated by amide bands. The strong peak at 1665 cm^{-1} most probably represents the Amide I band, while the peaks at 1622–1615 cm^{-1} and 1580 cm^{-1} and below can be reasonably identified with the Amide II bands of the primary and secondary amide linkages, respectively, in the polymer.

Crystalline asparagine exhibits well resolved scattering bands at 1665, 1637, 1622 and 1582 cm^{-1} (Table III). The spectrum of Asn in H_2O (Fig. 2) is complicated by the broad H-O-H deformation band of water (1640–1630 cm^{-1}). The strong peaks at 1637 cm^{-1} (crystal), 1640 cm^{-1} (H_2O) and 1645 cm^{-1} (^2H_2O) must represent Amide I bands; we cannot now explain why we find no decrease in frequency in ^2H_2O. The strong band near 1620 cm^{-1} (crystal; H_2O) presumably constitutes the Amide II band (primary amide), since it disappears upon deuteration. The band near 1610 cm^{-1} is attributed to antisymmetric CO_2^- stretching. It is not observed in crystalline Asn. The peaks below 1600 cm^{-1} presumably represent degenerate NH_3^+ deformation; their intensity is much reduced in ^2H_2O.

The Raman spectrum of poly-L-asparagine (molecular weight 10 000–20 000) fits that of Asn (Table III, Fig. 2) and the strong peaks at 1640 cm^{-1} are taken to be residue Amide I bands. The skeletal Amide I band lies at 1660 cm^{-1}. The peaks at 1615 cm^{-1} (solid) and 1620 cm^{-1} (H_2O solution) are taken to constitute sidechain Amide II bands.

Crystalline glutamine (anhydrous) exhibits strong bands at 1685, 1642, 1620, 1600 and 1585 cm^{-1}. (Table IV, Fig. 2). The spectrum of Gln in H_2O is complicated

TABLE IV

RAMAN SCATTERING FREQUENCIES (cm^{-1}) OF CRYSTALLINE L-GLUTAMINE, L-GLUTAMINE IN H_2O AND ^2H_2O FORM, AND INCORPORATED INTO LIPOSOMES

Relative intensities for solid form in parentheses. s, strong; sh, shoulder.

Gln			Melittin		Tentative assignments
Crystal	H_2O	^2H_2O	Anhydrous	Liposomes	
1685 (0.46)					
	1680 sh		1680 (0.38)		
	1662 sh		1665 (0.23)		Amide I (residue and skeletal); ν(C=C) in liposomes; H_2O deformation
1642 (0.53)	1642 s			1658	
		1630 s	1635 sh	1635 sh	
				1620 sh	
1620 (0.75)		1620 sh			
	1615 s		1610 (1.00)		Amide II (residue) dipolar antisymmetric CO_2^- stretch Trp in melittin
1600 (1.00)	1600 sh			1600	
1585 (0.48)			1585 (0.86)	1585 sh	Amide II (skeletal) degenerate NH_3^+ deformation; Trp in melittin
	1575		1560 (0.55)		
1545 (0.28)	1545			1545	
	1525		1528 (0.33)		
				1510	

by the H-O-H deformation band of water. The 1642 cm^{-1} peak of crystalline Gln is most probably the Amide I band. This shifts to 1630 cm^{-1} in ^2H_2O. The strong peaks at 1620 and 1615 cm^{-1} in the case of the solid and H_2O solution, respectively, presumably constitute the Amide II bands, since they disappear upon deuteration. The strong scattering at 1600 cm^{-1} is most reasonably attributed to antisymmetric CO_2^- stretching. The bands between 1600 and 1500 cm^{-1} are assigned to degenerate NH_3^+deformations.

The spectra of melittin (Table IV) are not easily analyzed, since we have not been able to obtain satisfactory spectra in solution (owing to fluorescence). Moreover, we observe a strong band at 1610 cm^{-1} which we attribute to the Trp residue (we find that pure Trp exhibits a ring vibration near 1612 cm^{-1}). We suggest that the peak at 1665 cm^{-1} represents the skeletal Amide I band. In liposomes this is obscured by the very strong ν(C=C) band 1658 cm^{-1} owing to unsaturated fatty acids. The shoulder at 1635 cm^{-1} in the solid and in liposomes is assigned to the residue Amide I band. Residue Amide II bands are obscured by the contributions of Trp, which presumably also add to the 1585 cm^{-1} band.

ESR

Our ESR experiments with 5-nitroxide stearate show maximum hyperfine splitting of 54.5 G for membranes derived from resting cells and 56.0 G for membranes from concanavalin A-activated thymocytes, indicating lesser probe mobility in the latter.

GENERAL DISCUSSION

If one extrapolates the behavior of simple lipid systems [15–18] to the differences between the membranes of resting and stimulated cells, the greater value of $I_{2900-2890}/I_{2850}$ in the latter case could be taken to indicate a constrained lipid mobility in the membranes of stimulated cells. The lower value of I_{1082}/I_{1127} leads to a similar conclusion, particularly since stimulation with concanavalin A does not induce significant changes in membrane protein composition or proportion [6] and since I_{1082}/I_{1127} is not sensitive to changes in protein architecture. Our ESR data, indicating a lesser lipid probe mobility in "stimulated" membranes, is also consistent with the differences of Raman scattering in the CH and CC stretching regions, but our analysis of the CH in-plane deformation zone allows no conclusions other than that "stimulated membranes" differ in lipid organization from "resting membranes". Indeed we cannot exclude the possibility that the changes observed in the CH and CC stretching regions do not reflect altered acyl chain mobility, but that they, as well as the modifications in the CH deformation zone, derive from the increased lipid unsaturation in the membranes of stimulated cells [9] signalled also by their prominent ν(C=C) band at 1658 cm^{-1}.

Our experiments with diverse amidated compounds support our suggestion that the plasma membranes from stimulated cells are more highly amidated than the membranes of control cells. However, our data also demonstrate how sensitive the amide vibrations of residue amides are to local environment and configuration. "Stimulated membranes" might thus also differ in the environment of their Asn and Gln residues. Certainly the residue Amide I and Amide II bands of glutamine and asparagine lie in the frequency range of the "new" bands observed in the membranes from stimulated cells. Moreover, the spectra of these membranes also show a depletion of structure near 1400 cm^{-1}, the symmetric carboxylate COO^- stretching region; this would be expected with conversion of COO^- to $CONH_2$. (CO_2^- asymmetric stretching and degenerate NH_3^+ deformation, prominent in Asn and Gln, do not yield sharp bands in proteins [20–22].) Finally there is sufficient asparatate+glutamate in membrane proteins generally (> 20 residues % [26]) to account for the large changes observed, while there is not enough acetylated amino sugar (which does exhibit amide bands in the region under discussion [27]).

A possible change in side chain amidation is of general interest, since it is established that high levels of glutamate and aspartate amidation produce unusual visco-elastic properties in certain proteins [28]. It is relevant also to an important, more specific topic, the action of L-asparaginase. L-Asparaginase acts as an immunosuppressant, inhibiting the mitogenic response of lymphocytes to phytohemagglutinin concanavalin A. Fidler and Montgomery [29] have shown that asparaginase treatment of lymph node cells or peripheral blood lymphocytes produces cell surface modifications that inhibit binding of concanavalin A and blastogenic stimulation by this lectin; depletion of cellular asparagine is not a factor. The recent studies of Waithe et al. [30] also indicate that L-asparaginase inhibits lymphocyte blastogenesis by its action on the plasma membrane.

Our Raman spectra fit the concept that concanavalin A-induced blastogenic stimulations increases the amidation of membrane aspartates (± glutamates) but we require other evidence to prove this hypothesis. We are therefore extending our

Raman analyses to the glycoprotein whose turnover is stimulated by concanavalin A [7]. We are also testing for possible changes in amidation by monitoring the effects of L-asparaginase and by comparing the proteins of resting and stimulated cells, using isoelectric focusing.

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