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Spectroscopic evidence for β structure in rat adipocyte plasma membrane protein

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SUMMARY

Infrared spectra in the Amide I region of fat cell plasma membrane indicates the presence of a significant proportion of β conformation in the membrane proteins. This is noted both in spectra of dried membrane films and membrane suspensions in ^2H_2O. The effect of various solvents is described. This finding illustrates the diversity in protein conformation in plasma membranes obtained from various mammalian cell types.

The nature of the conformation of proteins in cellular membranes has received considerable attention. A prominent early suggestion on the nature of membrane structure was that some of the membrane protein is disposed as thin layers absorbed on the polar surfaces of bimolecular lipid leaflets[1]. In contemporary terms, this would correspond to two dimensionally extended sheets of β conformation. Examination of the plasma membranes of erythrocytes[2-4], Ehrlich ascites tumor cells[5,6] and *Micrococcus lysodeikticus*[7] by infrared spectroscopy and ultraviolet spectropolarimetry has failed to detect β structure under usual conditions, and this has led to the conclusion that the secondary structure of membrane proteins is composed of combinations of α helical and "unordered" conformations. However, partial conversion to β structure can be produced in the membranes by lipid extraction, exposure to low pH and a variety of organic solvents. Moreover, infrared spectra of rat liver mitochondria[8] show absorbtion bands near 1630 cm^{-1} and 1690 cm^{-1}, highly suggestive of the presence of the antiparallel β conformation. This feature is particularly prominent in isolated inner mitochondrial membranes. Furthermore, the proportion of peptide linkages in the β conformation appears to vary with the metabolic state of the mitochondria[9]. Analogous energy dependent conformational changes have been observed in erythrocyte ghosts[10]. Recently, Choules and Bjorklund[11] reported that the mycoplasma membrane contains a considerable portion of its protein in the β conformation.

We report here on the infrared spectra of the plasma membrane of the rat adipose

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cell. These spectra indicate that the proteins of this mammalian plasma membrane, as isolated, contain a significant amount of β structure.

Isolated fat cells were prepared according to Rodbell[12] using 2 mg/ml crude collagenase (Worthington) in the presence of 1 mg/ml D-glucose. The plasma membrane was prepared by the method of McKeel and Jarret[13], modified as follows: The nuclear spin was omitted, and the final pellet was resuspended, layered onto a sucrose gradient (5 mM Tris–HCl, pH 7.4, 1 mM EDTA) ρ 1.1–1.2, and centrifuged at 40 000 rev./min for 45 min in a SW 41 rotor.

Human erythrocyte ghosts were prepared according to Dodge *et al.*[14] as modified by Fairbanks *et al.*[15]. Protein, 5′-nucleotidase (EC 3.1.3.5), NADH oxidase (EC 1.6.99.3) and succinate–cytochrome *c* oxidoreductase (EC 1.3.99.1) were measured by methods previously described[16].

For infrared spectroscopy of lyophilized membrane, the particles were washed twice in 5 mM sodium phosphate (pH 8.0) and resuspended in this buffer (1–2 mg/ml protein). 25 μl was placed on an AgCl disc in a rectangular layer and the disc was immediately immersed in liquid N_2. The frozen films were then lyophilized. Some films were also dried *in vacuo* over anhydrous $CaSO_4$. Dried (non-lyophilized) films were lipid extracted by immersion in the appropriate solvent for 1 h followed by extensive rinsing in the same solvent. All film specimens were stored overnight *in vacuo* over anhydrous $CaSO_4$.

For infrared spectroscopy in ^2H_2O, the isolated membranes were washed twice with 5 mM phosphate in ^2H_2O (p^2H 8.0) and resuspended in that buffer. Spectra of ^2H_2O suspensions were obtained using matched Perkin Elmer absorbance cells with IRTRAN windows, path length 0.1 mm. Spectra were obtained with a Perkin Elmer Model 221 spectrophotometer.

Assay of enzymatic markers indicated that the fat cell plasma membrane preparation was 75 to 80% pure with approximately 20% contamination by smooth endoplasmic reticulum and less than 5% contamination by mitochondria (Table I).

Infrared spectra of lyophilized human erythrocyte ghosts and rat cell plasma membrane are compared in Fig. 1. Both show an Amide I band at 1655 cm^{-1}. The absorbance of the red blood cell membrane declined rapidly below 1645 cm^{-1}, a spectrum indicative of α helical and/or unordered conformations[17–19]. However, the adipocyte plasma membrane shows continued high absorbance down to 1632 cm^{-1} indicating the additional presence of β structure peptide. Fourteen spectra of seven different fat cell

TABLE I

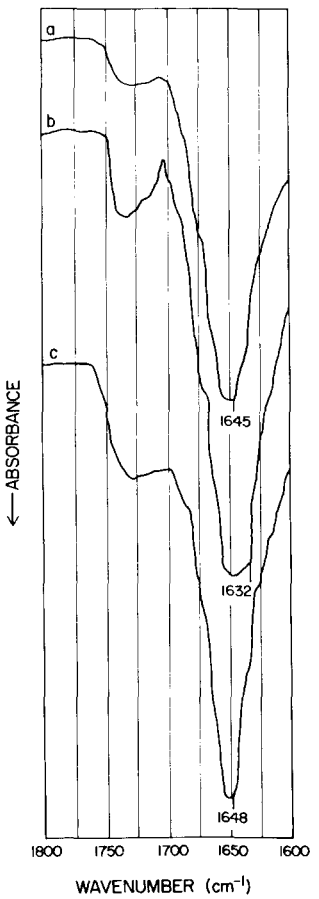
DISTRIBUTION OF MARKER ENZYMES AMONG MEMBRANE FRACTIONS

Specific activity of 5′-nucleotidase (plasma membrane marker enzyme) and NADH oxidase (endoplasmic reticulum marker enzyme) in a representative preparation of membrane fractions. Units are μmoles · mg^{-1} · h^{-1} for 5′-nucleotidase (37°); μmoles·mg^{-1}·min^{-1} for NADH oxidase (25°).

Fraction	*5′-Nucleotidase*	*NADH oxidase*
Plasma membrane fraction	4.06	1.43
Endoplasmic reticulum fraction	1.11	4.58

plasma membrane preparations have yielded these absorbance characteristics between 1640 and 1632 cm^{-1}. When films of plasma membrane are dried *in vacuo* at room temperature, identical spectra are obtained. There is no indication of a band near 1690 cm^{-1}, characteristic of the antiparallel β structure. When adipocyte membranes are dissolved in 90% 2-chloroethanol, a solvent considered helicogenic, and lyophilized films prepared from such solutions, the Amide I band narrows and shifts to 1650 cm^{-1} with shoulders at 1655 cm^{-1} and 1645 cm^{-1} (Fig. 1). This argues strongly that the 1632–1640 cm^{-1} band seen in the native membrane represents a conformational contribution.

2-Chloroethanol is also known to dissociate proteins from lipids in other membrane systems[20]. Thus, the possibility that the protein refolding in 2-chloroethanol, sug-



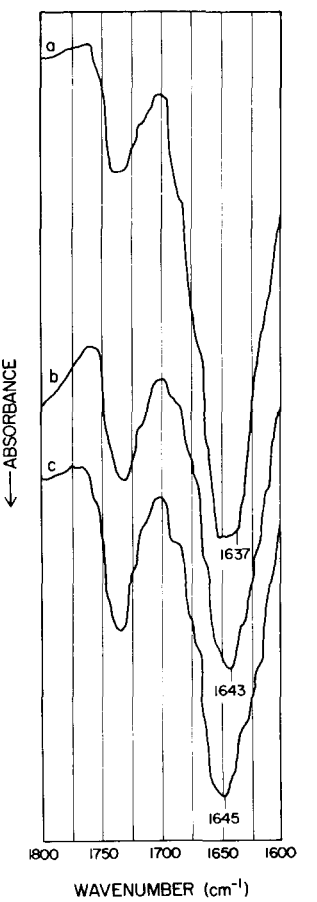


Fig. 1. Infrared spectra freeze-dried of plasma membrane preparations showing the Amide I band. (a) Human erythrocyte ghost, cast from 5 mM sodium phosphate (pH 8); (b) adipocyte plasma membrane, cast from 5 mM sodium phosphate (pH 8.0); (c) adipocyte plasma membrane cast from 2-chloroethanol–water (9:1, v/v).

Fig. 2. Infrared spectra of fat cell plasma membrane cast from various solvents and then lyophilized.(a) Cast from 5 mM sodium phosphate (pH 8.0); (b) cast from chloroform–methanol (2:1, v/v); (c) cast from acetone–water (9:1, v/v).

gested by the change in infrared spectra, may be partially due to disruption of protein lipid interactions must be considered. Indeed, infrared spectra obtained from samples of adipocyte plasma membranes, suspended in other lipid extracting solvents (Fig. 2) and then freeze dried do show proportionally less absorbance at 1632–1640 cm^{-1}. However, when films were cast from aqueous solvents and dried *in vacuo*, subsequent lipid extraction caused no significant alteration in the Amide I band (Fig. 3).

An infrared spectrum of adipocyte plasma membrane particles suspended in buffered ^2H_2O is given in Fig. 4. Here the β signal is predominant, supporting the spectra obtained from dried membranes and indicates that the spectral characteristics of those specimens is not an artifact due to film preparation. Also, it has been shown that careful rehydration of lyophilized mitochondria and erythrocyte ghosts can restore P:O ratios[9] and ATPase★ activity, respectively, to close to pretreatment values. To what extent the apparent increase in β conformation in ^2H_2O as opposed to dried film specimens rep-

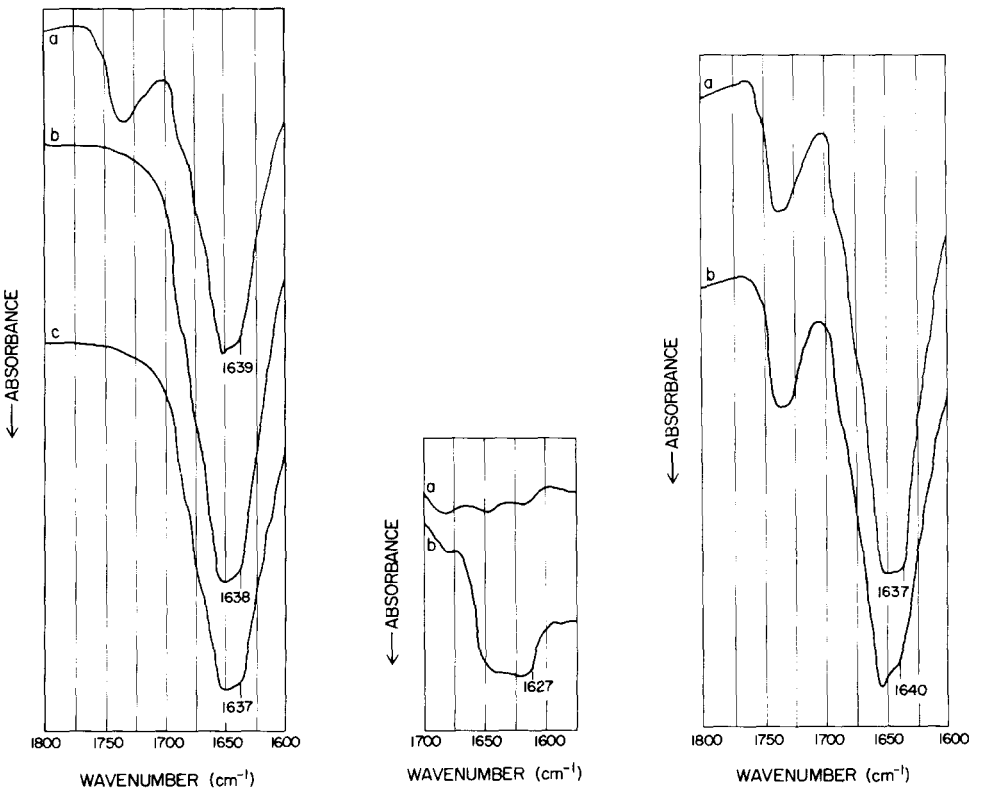


Fig. 3. Infrared spectra of adipocyte plasma membrane cast from 5 mM sodium phosphate (pH 8.0), dried *in vacuo* and then subjected to lipid extraction. (a) No treatment; (b) lipid extracted from dried film with chloroform–methanol (2:1, v/v); (c) lipid extracted from dried film with acetone–water (9:1, v/v).

Fig. 4. Infrared spectra of adipocyte plasma membrane suspended in 5 mM sodium phosphate (pH 8.0) in ^2H_2O. (a) Buffer baseline; (b) membrane spectrum.

Fig. 5. Infrared spectra of freeze-dried preparations of (a) adipocyte plasma membrane and (b) adipocyte endoplasmic reticulum in the Amide I region.

★J.M. Graham, personal communication.

resents the effect of differing solvation, N-deuteration or a specific conformational effect on protein structure by ^2H_2O is not clear.

The spectra of freeze dried adipocyte plasma membrane and endoplasmic reticulum prepared simultaneously, are compared in Fig. 5. The plasma membrane and endoplasmic reticulum fractions are approximately 20–25% cross-contaminated. While the spectra are similar, the absorbance of the endoplasmic reticulum falls off somewhat more rapidly below 1645 cm^{-1}, indicating that the β structure in the plasma membrane spectra is not due to contamination by endoplasmic reticulum. The small mitochondrial contamination is also inadequate to account for this signal.

The above findings document the presence of significant β structure in the plasma membrane of the rat adipocyte. The role of β conformation in membrane structure remains to be defined, especially in relation to its significance in lipid protein interactions. The present finding contrasts with the failure to detect this conformation in unperturbed plasma membrane preparations from red blood cells and Ehrlich ascites cells under similar conditions. The heterogeneity of membrane protein conformation among diverse mammalian (and also bacterial) cells is not surprising and suggests restraint in generalizing models of plasma membrane organization.

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