CIRCULAR DICHROISM OF LIVER CELL MEMBRANE ORGAN SPECIFIC PROTEIN

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Summary

Previous work from this laboratory has shown that liver cell plasma membranes from a variety of mammals contain an organ specific protein (Neville, 1968). This paper reports the purification of this protein under nondenaturing conditions and describes the results of ultraviolet circular dichroism studies performed on the purified protein. The results indicate that virtually all of the residues in the membrane protein are in the α-helix configuration. The data suggest that liver organ specific membrane protein has a rod-like structure.

The initial isolation of liver cell membrane organ specific protein was accomplished by alkaline extraction of membranes and purification by preparative disc electrophoresis in 9 M urea at pH 2.7. Antibody generated by the protein gave a single precipitin line with EDTA extracts of liver homogenates or liver cell plasma membranes. Organ and membrane specificity were demonstrated by the fact that EDTA extracts of liver fractions other than cell surface membranes and EDTA extracts of other organs failed to show the presence of the protein when assayed by immunodiffusion (Neville, 1968).

Previously I have referred to the class of cell surface membrane proteins which are immunologically specific for a specific cell type as eigen proteins. The protein described here is called liver eigen protein or LEP.

Methods

Rat livers were homogenized in 3 mM $MgCl_2$ and the membranes along with other sedimenting particles were spun down at 40,000 X g. The pellet was washed twice in 3 mM $MgCl_2$ and placed in a dialysis bag with an equal volume of water and dialyzed at 4° C for 24 hours against 1 mM Na-EDTA pH 7.

The solubilized protein was freed of particulate matter by centrifugation, concentrated by pressure dialysis, and purified by gel filtration on a Sepharose 4B column. Column eluate was monitored by absorbance at 280 mμ and by Ouchterlony plates utilizing the antiserum generated by the protein previously purified in urea by disc electrophoresis.

Purity of the isolated protein was determined by disc electrophoresis at pH 9.4 and immunoelectrophoresis in 2% agar at pH 7.0.

Lipid content was assayed by refluxing 6 mg of lyophilized protein with 2:1 chloroform methanol for one hour, filtering, and weighing the evaporated residue.

Hexose content was assayed by the phenol sulfuric acid method (Dubois et al., 1956).

The amino acid composition was determined on a Phoenix Amino Acid Analyzer following acid hydrolysis for 24 hours at 110° C.

The C.D. measurements were made with a Cary model 6001 CD attachment to a Cary 60 recording spectropolarimeter. The calibration was checked by the procedure recommended by the manufacturer. All measurements were made in 0.5 mm cells with constant nitrogen purging. The solvent consisted of an aqueous solution of 1 mM EDTA adjusted to pH 7.0 with NaOH. Protein concentration ranged between 0.01 - 0.1% and was determined by Rayleigh optics in a Model E ultracentrifuge using the conservation of mass equations (Van Holde, 1967).

The mean residue molecular ellipticity obtained is given by:

$$\theta = \frac{\theta^{o} M}{100\, \ell c}$$

where M is the mean residue molecular weight here determined as 114 from the amino acid analysis, θ^{o} is the observed ellipticity in degrees, ℓ is the cell pathlength in dm and c is the protein concentration in g/cm^3.

Results

The elution profile after the second pass through Sepharose 4B is shown in Fig. 1. The U.V. absorbing peak is coincident with the peak in antigen activity as determined by immunodiffusion. The peak material runs as a single band on disc electrophoresis at pH 9.4, and a single arc is detected by immunoelectrophoresis at pH 7.0. Hexose and lipid could not be detected.

The CD spectrum shown in Fig. 2 exhibits the three optical transitions associated with α helical residues (Timasheff and Gorbunoff, 1967). The absolute magnitude of these transitions per peptide residue is equivalent to that exhibited by peptides of complete helical content (see Table I). By inference LEP is essentially completely helical in content.

The only known naturally occurring proteins of complete α helical content are the α proteins derived from muscle and epidermoid structures. The similarity of CD spectra of several muscle α proteins and LEP are summarized in Table I. The amino acid composition shown in Table II is similar to that of paramyosin in that the percentage of charged amino acids is high while that of proline is low (Cohen and Holmes, 1963).

Table I

Comparison of Circular Dichroism of Liver Eigen Protein, α Proteins, and α Helical Polypeptides. All measurements done in H_2O with various added salts. Ellipticities have not been corrected for refractive index.

Polymer	Author	θ 222 mμ	θ 209 mμ	θ 191 mμ
Liver eigen protein		-38 000	-36 500	+89 000
Poly-L-glutamic acid pH 4.3	Adler et al., 1968	-40 000	-36 000	+90 000
Poly-L-lysine pH 11.2	Townend et al., 1966	-39 800	-38 900	+71 500
Light meromyosin Fx 1	Oikawa et al., 1968	-44 450	-41 290	+98 470
Paramyosin	Oikawa et al., 1968	-38 940	-36 300	+87 000

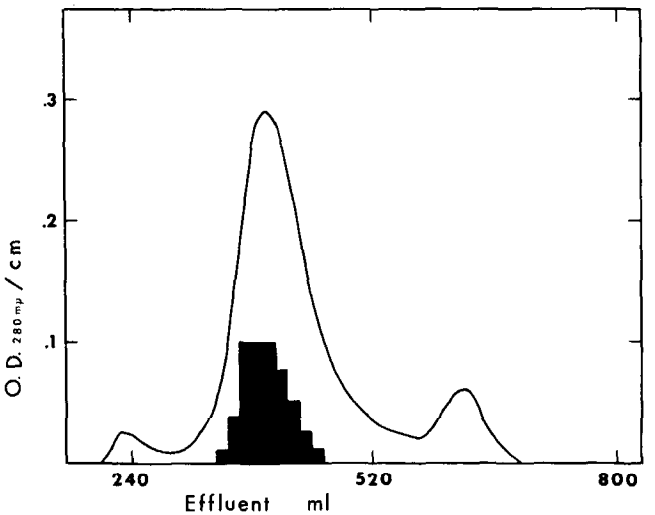

Fig. 1. Purification of LEP by gel filtration on a 5x32 cm Sepharose 4B column. The solid line indicates the elution profile of 280 mμ absorbing material. The blackened area represents the relative concentration of LEP as determined by immunodiffusion.

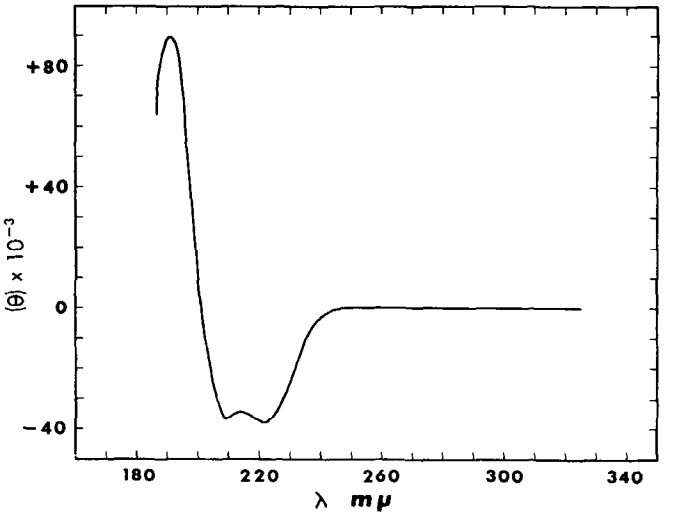

Fig. 2. Ultraviolet circular dichroism of liver eigen protein in 1 mM EDTA pH 7.0. The units of θ are in deg cm^2/decimole of peptide.

Table II
Amino Acid Composition of Liver Eigen Protein.

AA	% Moles	AA	% Moles	AA	% Moles
Glu	20	Val	6	Phe	2
Asp	11	Leu	12	Meth	2
Lys	8	Isoleu	5	His	1
Arg	7	Thr	5	Pro	1
Ala	8	Ser	5	Cyst	0
Gly	5	Tyr	2		100

Lipid < 1.6% Hexose < 1.2%

Discussion

The CD data indicates that LEP is essentially completely α helical in structure. Steric considerations require an α helical structure to behave as a rigid rod and hydrodynamic data indicate that this is the case for the helical polypeptides and the soluble α proteins (Lowey et al., 1963).

The α proteins have on the basis of x-ray diffraction data a coiled-coil structure of 2 or more chains (Crick, 1952; Cohen and Holmes, 1963). Diffraction data are not yet available on LEP, however, it would not be surprising to find that LEP also has a multichain structure twisted into a super helix. Preliminary hydrodynamic data suggest that LEP is either a two or three chain structure of length between 700-1100 Å.

The significance of a helical rod-shaped protein in the cell membrane is unclear. Certainly a rod-like molecule will impart rigidity to the membrane and its primary function may be structural.

The relationship between the α proteins in muscle and the contractile process is at present unclear. It is possible however that membranes have contractile elements and LEP may play a role in cell locomotion, pseudopod formation, and pinocytosis.

I have postulated that different cell types may contain specific cell surface proteins or eigen proteins which function in the processes of cell recognition. If this is the case eigen proteins, although immunologically distinct, may share common physical properties. Liver eigen protein has two unique properties. It is solubilized from membranes by chelating agents, and it has a rod-like structure. Work is now in progress to see whether membranes from other cell types contain organ specific proteins sharing these physical properties.

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