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The circular dichroism spectrum of microsomal membranes. Magnesium-induced aggregation

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SUMMARY

The circular dichroism (CD) spectrum of the microsomal membranes from rat liver in the 250–200-nm range is reported. Spectra of both the smooth endoplasmic reticulum and the rough membranes stripped of ribosomes are similar to those of other biological membranes: The spectrum is characteristic of an α-helical protein, but is shifted 1–2 nm to higher wavelengths. The presence of small amounts of Mg^{2+} causes an aggregation of the membranes as evidenced by a sharp increase in turbidity. The spectral changes accompanying this aggregation are a further bathochromic shift of the ellipticity trough and crossover and a decreased intensity of ellipticity.

In recent years circular dichroism (CD) has been used in attempts to elucidate the conformation of membrane proteins. The CD spectra of membranes from a wide variety of sources are strikingly similar[1-3]. The spectra resemble that of an α-helix, but with a characteristic red shift of the ellipticity troughs (near 208 and 222 nm) and crossover (near 202 nm), and a reduced intensity of the CD bands (most pronounced at the 190 nm maximum[4,5]). There is now convincing evidence[4-8] that the anomalies observed in the CD spectra of membrane suspensions are due to optical artifacts arising from the particulate, turbid nature of the samples. We report here the CD spectrum of the rat liver endoplasmic reticulum, both of the smooth-surfaced vesicles and the rough membranes which have been stripped of ribosomes, in the 250–200-nm region. The effect of Mg^{2+} in increasing the turbidity of the suspensions, with the accompanying spectral distortions, is also reported.

The microsomal fraction from the livers of 2-month old male Wistar rats was fractionated into smooth and rough-surfaced vesicles by the discontinuous sucrose gradient method of Williams *et al.*[9]. The ribosomes were stripped from the rough endoplasmic reticulum as follows: The rough membranes (approximately 10 mg membrane protein 5 ml)

were removed from the sucrose gradient and washed by diluting to 40 ml with distilled water and centrifuging at 78 000 x *g* for 30 min. The pellet was resuspended in 20 ml of 1.0 M NaCl containing 5 mM EDTA (pH 7.6) and layered over 1.4 M sucrose. After centrifugation at 78 000 x *g* for 2 h, the membrane band was removed from the interface and washed with distilled water as before. These stripped membranes (stripped rough endoplasmic reticulum) were suspended in 0.01 M Tris–HCl buffer (pH 7.6) for experimentation. Magnesium was introduced by diluting the membrane suspension with 0.01 M Tris (pH 7.6) containing $MgCl_2$ to the final Mg^{2+} concentration desired. The smooth membranes were removed from the sucrose gradient and washed by the procedure used to produce the stripped rough endoplasmic reticulum and suspended in Tris buffer. Pellets of the washed membrane preparations were fixed in 5% Tris-buffered glutaraldehyde, stained with osmium tetroxide and uranyl acetate, sectioned, and post-stained with lead citrate for electron microscopy. The rough endoplasmic reticulum sample examined in the electron microscope was washed twice in 5 mM $MgCl_2$ by centrifugation and resuspension prior to fixing in Tris buffer containing 5 mM $MgCl_2$. Protein was determined according to Lowry *et al.*[10], organic-extractable phosphorus by the method of Chen *et al.*[11], and RNA by the method of Hallinan and Munro[12] assuming $E^{1\%}_{1cm}$ (260 nm) = 300. CD measurements were made at room temperature in a Durrum–Jasco Model J-10 instrument at protein concentrations of about 0.1 mg/ml in a 1-mm path length cell. Molar ellipticities were generated assuming the average weight of an amino acid to be 115. Turbidity was measured in a 1-cm cell at 500 nm in a Cary 11 spectrophotometer.

A micrograph of the smooth vesicles is shown in Fig. 1a. The RNA/protein ratio of these membranes is 0.011 ± 0.004 (w/w) where the error represents (as do the errors for the RNA/protein ratios of the rough and the stripped rough endoplasmic reticulum) the extremes from the mean of preparations from four different animals. The lipid/protein ratio is 0.61 ± 0.02 (w/w) by organic extractable phosphorus assuming the lipid phosphorus content is 4%. The error is the extremes from the mean of duplicate samples, and the ratio determined by phosphorus content agrees within experimental error to that calculated by weighing the lipid recovered by Folch washing[13]. The washed rough endoplasmic reticulum sample (Fig. 1b) is studded with ribosomes and has a correspondingly high RNA/protein ratio of 0.20 ± 0.05. The procedure employed to remove ribosomes from the rough endoplasmic reticulum appears to be effective since no ribosomes are evident in the stripped rough endoplasmic reticulum by electron microscopy (Fig. 1c) and the RNA/protein ratio is 0.019 ± 0.008, within experimental error of the value obtained for the smooth endoplasmic reticulum.

The CD spectrum of the smooth endoplasmic reticulum is shown in Fig. 2a. The spectrum in Tris buffer (solid line) is similar to those of other membranes from dissimilar sources, resembling an α-helical spectrum which is red-shifted relative to synthetic polypeptide standards[14]. The main trough and shoulder are at 223 and 210 nm, respectively, with the crossover at 204 nm. The stripped rough endoplasmic reticulum spectrum (Fig. 2b, solid line) is essentially identical to that of the smooth endoplasmic reticulum, suggesting no large difference in average membrane protein conformation.

The presence of 2 mM Mg^{2+} affects both membrane preparations similarly (Figs. 2a and 2b, dashed lines). (The effect is not due to Cl^- since NaCl has no effect up to a

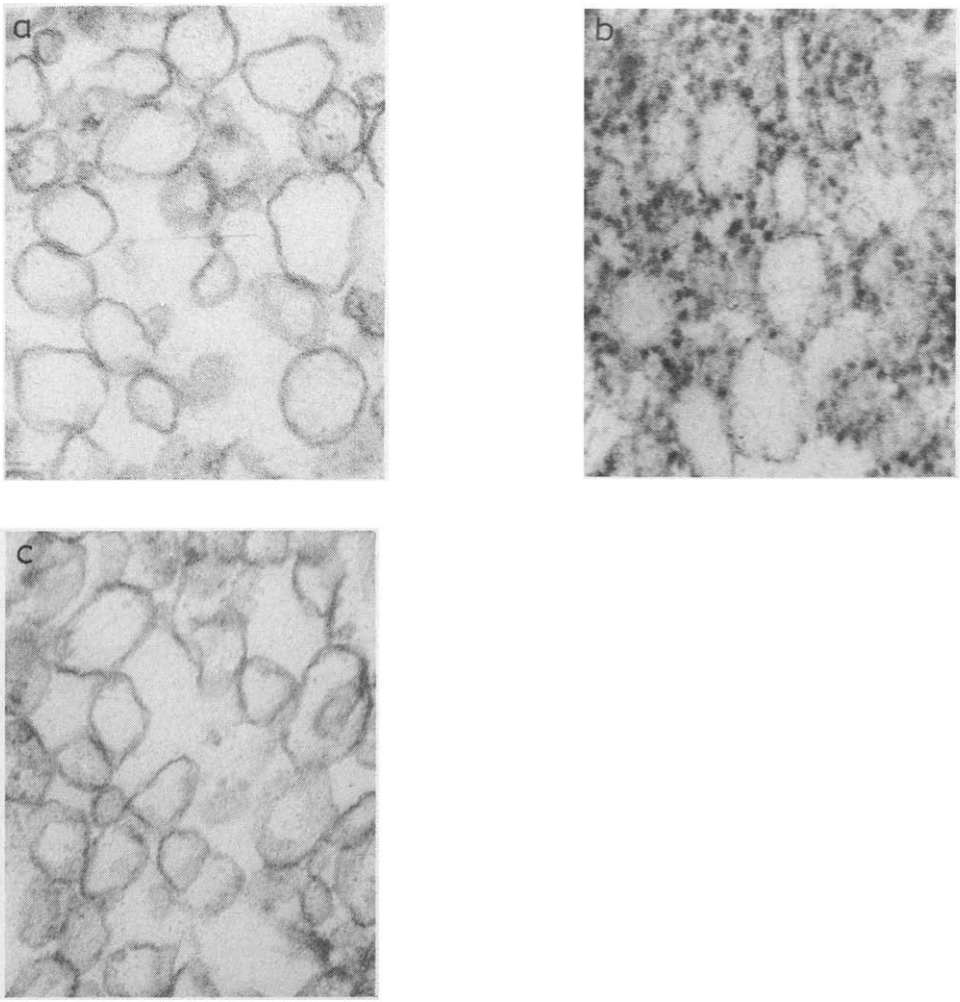

Fig. 1. Electron micrographs of sections of (a) smooth endoplasmic reticulum, (b) rough endoplasmic reticulum, (c) rough endoplasmic reticulum which has been stripped of ribosomes. Sample preparation is described in text. Magnification x 86 600.

concentration of 10 mM.) The spectra are further red-shifted, the main trough to near 225 nm and the crossover to about 208–209 nm. The 210-nm shoulder is less well-defined, and the ellipticity of the main trough is decreased by about 25%. At higher concentrations of Mg^{2+} (up to 5 mM), the trough and crossover are further red-shifted, and the intensity of the negative ellipticity band is correspondingly diminished. These spectral changes induced by Mg^{2+} likely result from the increased scattering of light associated with the aggregation of the membranes[6,8] (see below), and do not likely result from a significant change in secondary structure of the membrane proteins. Production of random coil or β structure at the expense of α-helix, which would decrease the intensity of the CD minimum near 220 nm, would not cause a red shift of both the trough and crossover[14].

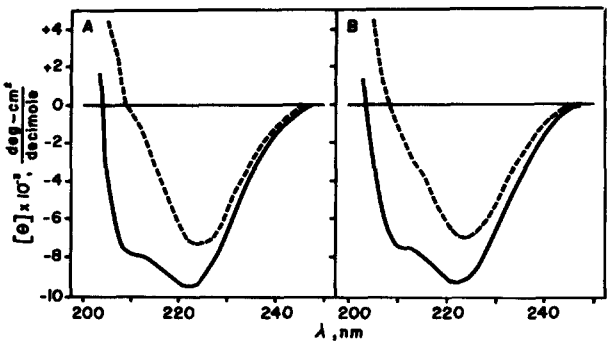

Fig. 2. CD spectra of smooth and stripped rough membranes in 0.01 M Tris (pH 7.6). A. Smooth endoplasmic reticulum; solid line, no Mg^{2+}; dashed line, 2 mM Mg^{2+}. B. Stripped rough endoplasmic reticulum: solid line, no Mg^{2+}; dashed line, 2 mM Mg^{2+}. CD conditions in text.

Measurements of the turbidity of the membrane suspensions show that Mg^{2+} does cause a sharp increase in light scattering. The turbidity (at 500 nm) of both the smooth and the stripped rough endoplasmic reticulum suspensions increases linearly with the concentration of Mg^{2+} up to 5 mM Mg^{2+}, being increased 20% at 1 mM and doubled at 5 mM. The increase in turbidity is independent of membrane concentration in the 0.05 to 0.15 mg/ml protein range. This Mg^{2+}-induced increase in light-scattering is consistent with aggregation of the membranes, but could also result from a change in physical properties of the individual vesicles. Electron micrographs of negatively-stained suspensions of the smooth membranes in the presence and absence of 5 mM $MgCl_2$, however, indicate no apparent different in size or shape of the vesicles. Experiments are in progress to further investigate the origin of this aggregation and its possible significance in regards to the structure of the endoplasmic reticulum membranes.

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REFERENCES

1 S.J. Singer in L.I. Rothfield, *Membrane Structure and Function*, Academic Press, New York, 1971, in press.
2 A.S. Gordon, D.F.H. Wallach and J.H. Strauss, *Biochim. Biophys. Acta*, 183 (1969) 405.
3 D.W. Urry, M. Mednieks and Bejnarowicz, *Proc. Natl. Acad. Sci. U.S.*, 66 (1970) 909.
4 M. Glaser and S.J. Singer, *Biochemistry*, 10 (1971) 1780.
5 D.W. Urry and J. Krivacic, *Proc. Natl. Acad. Sci. U.S.*, 65 (1970) 845.
6 D.W. Urry and T.H. Ji, *Arch. Biochem. Biophys.*, 128 (1968) 802.
7 D.J. Gordon and G. Holzwarth, *Arch. Biochem. Biophys.*, 142 (1971) 481.
8 A.S. Schneider, M.C. Schneider and K. Rosenheck, *Proc. Natl. Acad. Sci. U.S.*, 66 (1970) 793.
9 D.J. Williams, D. Gurari and B.R. Rabin, *FEBS Lett.*, 2 (1968) 133.
10 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
11 P.S. Chen, T.Y. Toribara and H. Warner, *Anal. Chem.*, 28 (1956) 1756.
12 T. Hallinan and H.N. Munro, *Biochim. Biophys. Acta*, 108 (1965) 285.
13 J. Folch, M.L. Lees and G.H. Sloan-Stanley, *J. Biol. Chem.*, 226 (1957) 497.
14 N. Greenfield and G.D. Fasman, *Biochemistry*, 10 (1969) 4108.