Gerke, V., & Weber, K. (1984) *EMBO J. 3*, 227-233.
Glenney, J. R., Jr., & Tack, B. F. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7884-7888.
Gould, K. L., Woodgett, J. R., Isacke, C. M., & Hunter, T. (1986) *Mol. Cell. Biol. 6*, 2738-2744.
Hirata, F. (1981) *J. Biol. Chem. 256*, 7730-7733.
Hirata, F., Matsuda, K., Notsu, Y., Hattori, T., & DelCarmine, R. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4717-4721.
Hu, N., & Messing, J. (1982) *Gene 17*, 271-277.
Huang, K.-S., Wallner, B. P., Mattaliano, R. J., Tizard, R., Burne, C., Frey, A., Hession, C., McGray, P., Sinclair, L. K., Chow, E. P., Browning, J. L., Ramachandran, K. L., Tang, J., Smart, J. E., & Pepinsky, R. B. (1986) *Cell (Cambridge, Mass.) 46*, 191-199.
Huang, K.-S., McGray, P., Mattaliano, R. J., Burne, C., Chow, E. P., Sinclair, L. K., & Pepinsky, R. B. (1987) *J. Biol. Chem. 262*, 7639-7645.
Kondo, S., Noguchi, M., Funakoshi, T., Fujikawa, K., & Kisiel, W. (1987) *Thromb. Res.* (in press).
Kreil, G., & Kreil-Kiss, G. (1967) *Biochem. Biophys. Res. Commun. 27*, 275-280.
Kretsinger, R. H., & Creutz, C. E. (1986) *Nature (London) 320*, 573.
Kristensen, T., Saris, C. J., Hunter, T., Hicks, L. J., Noonan, D. J., Glenney, J. R., & Tack, B. F. (1986) *Biochemistry 25*, 4497-4503.
Maki, M., Murata, M., & Shidara, Y. (1984) *Eur. J. Obstet., Gynecol. Reprod. Biol. 17*, 149-154.
Mancheva, I., Kleinschmidt, T., Aleksiev, B., & Braunitzer, G. (1984) *Hoppe-Seyler's Z. Physiol. Chem. 365*, 885-894.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual*, pp 76-85, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Moore, P. B., & Dedman, J. R. (1982) *J. Biol. Chem. 257*, 9663-9667.
Persson, B., Flinta, C., von Heijne, G., & Jornvall, H. (1985) *Eur. J. Biochem. 152*, 523-527.
Reutelingsperger, C. P. M., Hornstra, G., & Hemker, H. C. (1985) *Eur. J. Biochem. 151*, 625-629.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463-5467.
Saris, C. J. M., Tack, B. F., Kristensen, T., Glenney, J. R., Jr., & Hunter, T. (1986) *Cell (Cambridge, Mass.) 46*, 201-212.
Schlaepfer, D. D., & Haigler, H. T. (1987) *J. Biol. Chem. 262*, 6931-6937.
Schmer, G., & Kreil, G. (1969) *Anal. Biochem. 29*, 186-192.
Shadle, P. J., Gerke, V., & Weber, K. (1985) *J. Biol. Chem. 260*, 16354-16360.
Sudhof, T. C., Ebbecke, M., Walker, J. H., Fritsche, U., & Boustead, C. (1984) *Biochemistry 23*, 1103-1109.
Wallner, B. P., Mattaliano, R. J., Hession, C., Cate, R. L., Tizard, R., Sinclair, L. K., Foeller, C., Chow, E. P., Browning, J. L., Ramachandran, K. L., & Pepinsky, R. B. (1986) *Nature (London) 320*, 77-81.
Weber, K., & Johnsson, N. (1986) *FEBS Lett. 203*, 95-98.
Wold, F. (1981) *Annu. Rev. Biochem. 50*, 783-814.
Young, R. A., & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1194-1198.

Conformational Properties of the Main Intrinsic Polypeptide (MIP26) Isolated from Lens Plasma Membranes†

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ABSTRACT: The conformational properties of the main intrinsic polypeptide (MIP26) isolated from lens plasma membranes were studied by using near- and far-ultraviolet circular dichroism. The far-ultraviolet spectrum of MIP26 solubilized with octyl β-D-glucopyranoside indicates an α-helical content of ~50% and a β-structure content of ~20%. A detergent-free membrane suspension of MIP26 produced a typically distorted far-ultraviolet spectrum which was caused by differential light scattering and absorption flattening. However, decreasing the size of the membrane fragments by sonication produced a far-ultraviolet spectrum free of distortion, and with a rotatory strength profile similar to that obtained for MIP26 solubilized with octyl β-D-glucopyranoside. This implies similar secondary structure properties for the protein in both the suspension and the sugar detergent. The cleavage of MIP26 with *Staphylococcus aureus* protease, which results in removal of a 5-kilodalton peptide and which mimics the age-dependent posttranslational changes that take place in the lens, did not significantly affect the conformation of the core protein as judged by the near-ultraviolet circular dichroism spectra.

The vertebrate eye lens is an avascular tissue with a highly ordered architecture (Maisel et al., 1981; Benedetti et al., 1981). The bulk of the lens is composed of a multitude of elongated, nondividing cells which are called lens fibers. These lens fibers are coupled to one another by many intercellular junctions situated along their plasma membranes (Rae, 1979; Goodenough et al., 1980; Benedetti et al., 1981). The most abundant protein in the lens fiber plasma membrane is an intrinsic membrane protein known as MIP26 or MP26 (Broekhuyse et al., 1976; Benedetti et al., 1981). This protein,

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which has a molecular weight of ~28000, has been the subject of intense investigation in recent years and is believed to be directly involved in forming the communicating channels between lens fibers. During normal aging, MIP is known to undergo posttranslational cleavage and lose a fragment(s) equaling ~5000 daltons. However, the significance of this posttranslational cleavage is not understood. It has been shown that cleavage of MIP26 with V8 *Staphylococcus aureus* protease mimics the natural cleavage occurring in situ (Horwitz & Wong, 1980; Do Ngoc et al., 1985). Recent experiments by several investigators in which purified MIP26 was reconstituted into lipid vesicles support the idea that this protein can form cell to cell channels (Girsch & Peracchia, 1985a,b; Gooden et al., 1985; Nikaido & Rosenberg, 1985; Zampighi et al., 1985). The nucleotide and the deduced amino acid sequence of MIP26 cDNA were recently published, and on the basis of this sequence, a model was contracted. The predicted structure for MIP suggests that the protein traverses the lipid bilayer 6 times with both carboxy and amino termini on the cytoplasmic side (Gorin et al., 1984). The model also suggests that this protein can contribute to the formation of an aqueous channel needed for the formation of a communicating junction.

In this study, we report on some of the secondary and tertiary structural properties of MIP26. Near- and far-ultraviolet circular dichroism was utilized to probe the properties of detergent-solubilized MIP26, detergent-free membrane suspensions containing MIP26, and MIP26 which was cleaved with *S. aureus* protease.

Materials and Methods

Purification of Lens Membranes. Lens membrane suspensions were isolated as described previously (Bok et al., 1982; FitzGerald et al., 1983). Briefly, bovine lenses were homogenized in 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl)[1]/0.1 M NaCl, pH 7.8, buffer. The homogenate was centrifuged at 40000*g* for 30 min, and the soluble proteins were discarded. The pellet was washed 6–8 times by resuspending with homogenization in the same buffer and repelleting the insoluble fraction by centrifugation. The insoluble fraction was then rehomogenized in the same buffer containing 7 M urea to remove cytoskeletal and peripheral membrane proteins. The membrane-rich fraction was pelleted by first diluting the sample 1:4 with Tris-HCl buffer without urea and then centrifuging the suspension at 40000*g* for 30 min. This urea treatment was repeated 6 times. Urea was removed from the insoluble fraction by twice suspending the pellet in the Tris-HCl buffer. The urea-treated pellet was further purified by rehomogenization in 0.1 N NaOH according to the procedure of Russell et al. (1981), and the remaining insoluble material was used for the extraction of MIP26.

Extraction and Purification of MIP26. The sodium hydroxide washed membrane-enriched pellet was extracted with 20 mM Tris buffer, pH 8, containing 2 mM EDTA and 2% octyl β-D-glucopyranoside. The detergent-solubilized proteins were then chromatographed on a Pharmacia CL-6B (1.5 cm × 80 cm) gel filtration column equilibrated with 20 mM Tris-HCl buffer, pH 8, containing 1% octyl β-D-glucopyranoside. In a typical experiment, 6–8 mg of total protein

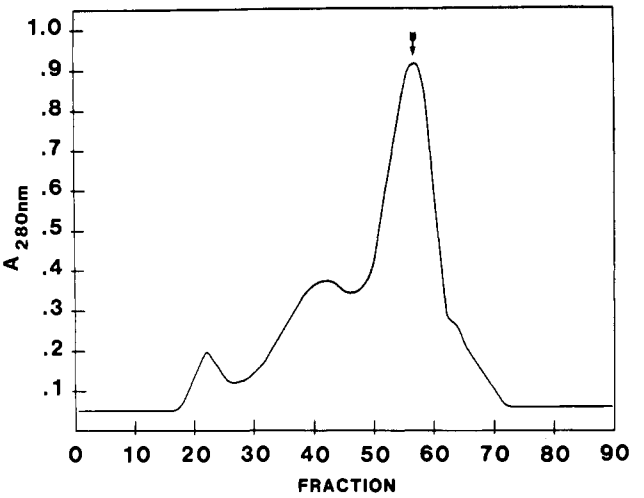

FIGURE 1: Gel filtration elution profile of lens membrane proteins extracted with octyl β-D-glucopyranoside. The proteins were chromatographed on a 1.5 × 80 cm column of Pharmacia CL-6B. Monomeric MIP26 was obtained by collecting the major peak fractions (fractions 55–58).

was applied to the column with a flow rate of 0.15 mL/min. Fractions of 1.5 mL were collected. Purified MIP26 solubilized in SDS was prepared by using preparative SDS gel electrophoresis and chromatography on a hydroxylapatite column as described previously (Bok et al., 1982).

For spectroscopic studies of MIP26 in 2,2,2-trifluoroethanol, sodium hydroxide washed enriched membrane pellets were suspended in 100% of 2,2,2-trifluoroethanol. The suspension was then centrifuged at 15000*g* for 15 min. This procedure selectively extracted MIP26, and the clear supernatant contained purified MIP26 as judged by SDS–polyacrylamide gel electrophoresis. Limited proteolysis of MIP26 in detergent solutions using *Staphylococcus aureus* protease V8 was performed as described previously (Horwitz & Wong, 1980).

Membrane suspensions for circular dichroism spectroscopy were prepared by resuspending the sodium hydroxide washed material in 20 mM Tris-HCl, pH 8, buffer and sonicating the preparation with a Braun Sonic 1510 sonicator equipped with a titanium needle probe. A power level of 40 W was used. Sonication was performed by placing ~5 mg of membrane material in 0.5 mL of Tris-HCl buffer in a glass test tube immersed in water with crushed ice. The sonicator was turned on for bursts of 20 s, for a total time of 5 min. The sonicated sample was then centrifuged at 40000*g* for 30 min, and the supernatant was used for analysis.

The concentration of purified MIP26 in SDS and in octyl β-D-glucopyranoside was determined spectrophotometrically using $E_{1\%}^{280nm}$ = 16.7 which was obtained by amino acid analysis. Solubilizing MIP26 in 2,2,2-trifluoroethanol resulted in hypochromism. The absorbance ratio at 280 nm for MIP26 solubilized in 2,2,2-trifluoroethanol and SDS was 0.71.

Circular dichroism spectra were obtained on a modified Beckman CD spectrophotometer coupled to a signal averager as described previously (Horwitz et al., 1979). Each spectrum was the average of 16 or 32 scans. For far-ultraviolet measurements, cells with a path length of 0.1 and 0.2 mm were used. Base lines were obtained by using indoleacetic acid having absorption profiles similar to that of MIP26.

Electron microscopy of sonicated and nonsonicated membrane preparations was performed by fixing the preparations in 2% glutaraldehyde/0.1 M phosphate, pH 7.3, for 2 h and further fixing in 1% osmium tetraoxide for 1 h. Pellets were dehydrated in a graded ethanol series and embedded in Araldite 502. Sections were prepared and viewed on Form-

[1] Abbreviations: CD, circular dichroism; SDS, sodium dodecyl sulfate; $C_{12}E_8$, *n*-dodecyl octaethylene glycol monoether; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; kDa, kilodalton(s).

Table I: Estimates of α-Helix and β-Structure of MIP26 in Various Solvents

	solvent[a]	$\Delta\epsilon_{208} \times 10^{-3}$	α (%)[b]	β (%)[d]	α (%)[c]	β (%)[d]
MIP26	octyl β-D-glucopyranoside	1.53	53	20	49	23
	SDS	1.60	55	18	43	27
	2,2,2-trifluoroethanol	1.97	71	6	55	18
	sonicated suspension	1.51	52	20	49	23
MIP22[e]	octyl β-D-glucopyranoside	1.39	59	15	58	16

[a] The buffer used was 20 mM Tris-HCl, pH 8. [b] Percent of α-helix was determined from the CD spectra at 208 nm according to Greenfield and Fasman (1969). [c] Percent of α-helix was determined from the CD spectra at 222 nm according to Wu et al. (1981) using an α-helix basis function with an average chain length of 25 residues. [d] Percent of β-strand was estimated according to the equation $f_\beta = (-0.729 \pm 0.080)f_H + (0.583 \pm 0.162)$ which was derived by Siegel et al. (1980), and which is based on the correlation between the amount of β-strand and helix for 60 reference proteins. [e] The number of residues in MIP22 was assumed to be 216 (see text).

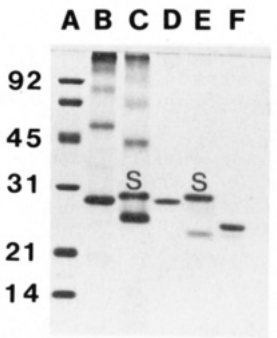

FIGURE 2: SDS–polyacrylamide gel electrophoresis of various preparations of MIP26. Column A, standard proteins. Column B, MIP26 purified by preparative SDS gel electrophoresis followed by chromatography on a hydroxylapatite column. Column C, proteolysis by *S. aureus* protease of SDS-purified MIP26; S denotes the *S. aureus* protein band. Column D, fractions (55–58) of MIP26 solubilized in octyl β-D-glucopyranoside and chromatographed on a CL-6B column as shown in Figure 1. Column E, proteolysis by *S. aureus* protease of the MIP26 preparation described in column D. Column F, molecular weight standard marker (bacteriorhodopsin, M_r 26 000).

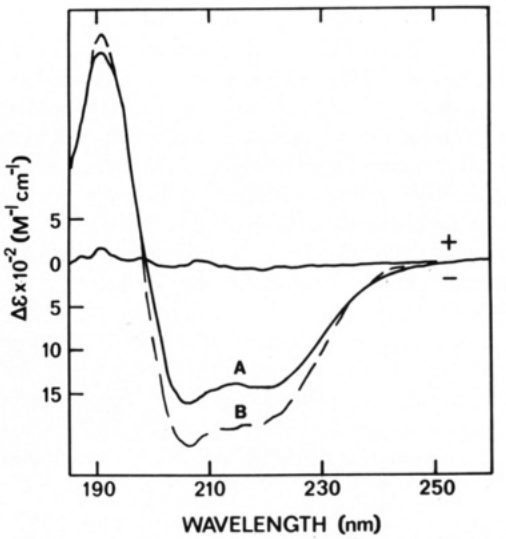

FIGURE 3: Far-ultraviolet CD spectra of MIP26. Curve A, MIP26 solubilized and purified in octyl β-D-glucopyranoside. Curve B, MIP26 solubilized in SDS. The path length was 0.2 mm. Each curve represents the average of 16 scans.

var/carbon-coated grids in a JEOL 100 C electron microscope.

Results

Protein Purification. The gel filtration elution profile of lens membrane proteins extracted with octyl β-D-glucopyranoside is shown in Figure 1. Highly purified monomeric MIP26 was obtained by collecting the major peak fractions (fractions 55–58). The peaks appearing in fractions 22 and 41 consisted mainly of multimeric aggregates of MIP26. As we have shown previously, MIP26 formed multimeric aggregates with increased purification and concentration (Horwitz & Wong, 1980; Bok et al., 1982). The SDS–polyacrylamide gel electrophoresis of monomeric MIP26 purified on the CL 6B column is shown in Figure 2 (lane D).

The SDS–polyacrylamide gel electrophoresis profile of MIP26 which was extracted with 1% SDS and purified by preparative SDS gel electrophoresis followed by chromatography on a hydroxylapatite column is shown in Figure 2 (lane B). As expected, this sample contains significant amounts of multimeric aggregates of MIP26 (Horwitz & Wong, 1980; Bok et al., 1982). To obtain a monomeric sample, this material was chromatographed on the CL-6B column which was equilibrated with buffer containing 1% SDS instead of the 1% octyl β-D-glucopyranoside. The elution profile obtained with SDS was similar to that shown in Figure 1. Only the monomeric peak fraction was collected, and gel electrophoresis of this fraction yielded a single band identical with that shown in Figure 2 (lane D).

MIP26 is known to be fully soluble in chloroform/methanol (1:1 v/v), and extraction of enriched urea-washed lens membranes with chloroform/methanol yields a highly purified MIP26 preparation (Alcala et al., 1975; Broekhuyse et al., 1976). We have found that 2,2,2-trifluoroethanol is as efficient a solvent as chloroform/methanol in selectively solubilizing and extracting MIP26 from lens membrane suspensions. The SDS–polyacrylamide gel electrophoresis profile of MIP26 extracted with 2,2,2-trifluoroethanol produced a single band identical with that shown in Figure 2 (lane D).

Far-Ultraviolet CD of MIP26. The far-ultraviolet CD spectrum of MIP26 isolated and purified with octyl β-D-glucopyranoside is shown in Figure 3 (curve A). The CD profile with negative minima around 208 and 222 nm suggests that the secondary structure of MIP26 is similar to that of proteins that are rich in separate α-helix and β-sheet regions (Manavalan & Johnson, 1983). Estimating the percent α-helix according to the method of Greenfield and Fasman (1969) yielded a value of 53% whereas estimating the helicity by the method of Yang and co-workers (Chen et al., 1974; Wu et al., 1981) yielded a value of 49% (Table I). MIP26 solubilized in SDS produced a far-ultraviolet CD spectrum which was similar to that shown in Figure 3 (curve A).

Solubilization of MIP26 in 100% trifluoroethanol resulted in a significant increase in the intensity of the CD spectra as shown in Figure 3 (curve B). The estimated percent helicity of MIP26 in this solvent is given in Table I. Whereas the helical content of proteins can be calculated from CD spectra within a few percent accuracy, estimates of β-sheet content are not as reliable. This is especially true for membrane proteins where reference values from protein with known structures are yet not available [see, for example, Jap et al. (1981)]. We therefore estimated the β-structure content of MIP26 according to the method of Siegel et al. (1980) which is based on the correlation between the amount of β-strand and α-helix obtained from 60 reference water-soluble proteins

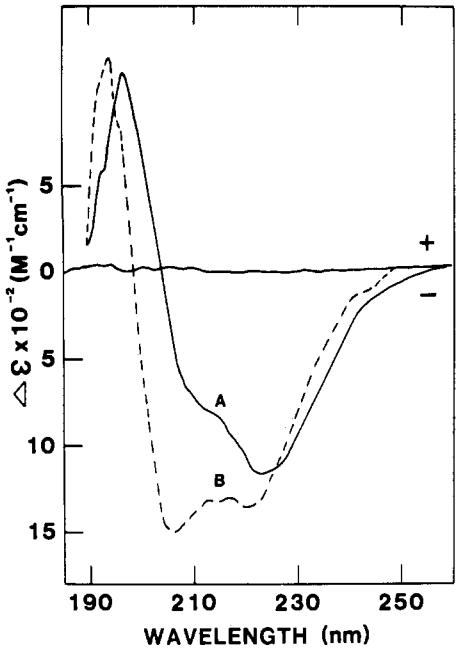

FIGURE 4: Far-ultraviolet CD spectra of membrane suspensions. Curve A, spectra of purified membrane suspensions of vesicles with linear dimensions up to 1.3 μm. Curve B, CD spectra of sonicated membrane suspensions. The path length was 0.2 mm. Each spectrum was obtained by averaging 16 scans.

with known X-ray structure (see Table I).

Far-Ultraviolet CD of Membrane Suspensions. The far-ultraviolet CD spectra of purified lens membrane suspensions which were not treated with any detergent or organic solvent are shown in Figure 4 (curve A). The profile obtained is similar to that seen with many other membrane proteins in which circular differential light scattering and absorption flattening produce a distorted spectrum. Electron microscopy of this membrane suspension shows a heterogeneous population of vesicles with linear dimensions up to ~1.3 μm (Figure 5A). When such a preparation was sonicated as described under Materials and Methods, the size of the vesicles was reduced by about a factor of 10, and typical vesicles in their longest dimension were 0.1–0.2 μm (Figure 5B). The CD spectrum of the sonicated material is shown in Figure 4 (curve B). This spectrum is similar to that obtained for MIP26 solubilized in octyl β-D-glucopyranoside or SDS. Estimation of the percent α-helix from the CD intensity at 208 or 222 nm yielded values of 52% and 49%, respectively (Table I).

Near-Ultraviolet CD Spectra of MIP26. The near-ultraviolet CD spectra of MIP26 solubilized in SDS, trifluoroethanol, and octyl β-D-glucopyranoside are shown in Figure 6. In octyl β-D-glucopyranoside (Figure 6C), a tryptophan vibronic transition is evident at 295 nm (Strickland, 1974). The broad band at 270 nm reflects contribution from both tryptophan and tyrosine residues, and the fine structure below 270 nm is generated by the phenylalanine residues (Horwitz et al., 1969). Whereas the general near-ultraviolet CD profile of MIP26 in trifluoroethanol (Figure 6B) and SDS (Figure 6A) is similar to that obtained in octyl β-D-glucopyranoside, the rotatory strength in these two solvents is significantly lower.

Conformational Properties of MIP26 Cleaved with S. aureus Protease. It has been shown previously that MIP26 lost about 3–4 kDa upon incubation with *S. aureus* protease in SDS. We have found that in nonionic detergents such as octyl β-D-glucopyranoside, or in *n*-dodecyl octaethylene glycol monoether ($C_{12}E_8$), a somewhat larger segment (5 kDa) was cleaved. Figure 2 shows the *S. aureus* cleaved MIP26 in SDS and in octyl β-D-glucopyranoside (compare lane C with lane E). On the basis of the mobilities observed, we can estimate that approximately 5 kDa was cleaved by *S. aureus* protease of MIP26 solubilized in octyl β-D-glucopyranoside. Similar results were observed with MIP26 solubilized in $C_{12}E_8$.

Circular Dichroism of S. aureus Cleaved MIP26. The far-ultraviolet CD spectrum of MIP26 cleaved by *S. aureus* in octyl β-D-glucopyranoside was similar in its profile to that shown for the native protein (Figure 3, curve A). Estimation of the α-helical content showed it to increase to about 59% (see Table I). A comparison of the near-ultraviolet CD spectrum of the cleaved MIP26 with the native proteins is shown in Figure 7. While small intensity differences between the two preparations are seen, these changes cannot be considered as being significant. Thus, it appears that cleaving 5 kDa from MIP26 had only a minimal effect on the aromatic amino acid conformation as judged by the near-ultraviolet CD measurements.

DISCUSSION

It is well established that CD spectra of membrane proteins in suspension can be grossly distorted because of artifacts arising from circular differential scattering and absorption flattening (Schneider et al., 1970; Urry et al., 1970; Maestre et al., 1971; Litman, 1972; Bustamante et al., 1983; Glaeser & Jap, 1985; Urry, 1985). These distorted spectra can lead to erroneous estimations of the secondary structural parameters of the proteins studied. Several experimental methods for dealing with differential scattering and absorption flattening have been used. Reduction of the particulate size by sonication (Schneider et al., 1970; Litman, 1972), use of high cell concentration in very short light-path cuvettes (Schneider & Harmatz, 1976), and increasing the solid angle of detection for collecting more scattered light, as well as fluorescence-detected CD (Reich et al., 1980), are the major techniques which are utilized for measuring CD spectra of membrane proteins. Recently, a new method was introduced in which membrane particles are incorporated into small unilamellar vesicles in order to minimize absorption flattening and differential light scattering (Mao & Wallace, 1984). However, at present, there is some controversy as to the validity of this approach (Glaeser & Jap, 1985; Wallace & Teeters, 1987). For MIP26, the decrease by sonication in particle sizes to ~0.1–0.2 μm and the use of a relatively small path-length cell (0.1–0.2 mm) have eliminated the distortion in the CD spectra.

Girsch and Peracchia (1985a,b), who recently studied the conformational changes in MIP26 following incorporation into liposomes, reported that MIP26 possesses about 37% of α-helix conformation. This value is significantly lower than the value we obtained in the present work which indicates that sonicated detergent-free MIP26 possesses about 50% of an α-helical conformation (Table I). Inspection of the CD spectra reported by Girsch and Peracchia and the conditions by which MIP26 was prepared [see Figure 8 in Girsch and Peracchia (1985b)] suggest that scattering did interfere with their CD measurements. The authors state that the micellar dispersions of MIP26 were only semistable and tended to precipitate. In addition, they used a relatively long path-length sample cell (2.0 mm). All of the above-mentioned conditions will contribute to scattering artifacts which, in general, will give lower values for the α-helical content.

Detergents are commonly used to solubilize membrane proteins, and many membrane proteins have been solubilized with various detergents without the loss of biological activity (Tanford & Reynolds, 1976). Sugar-based detergents and poly(oxyethylene)-based detergents are at present the most

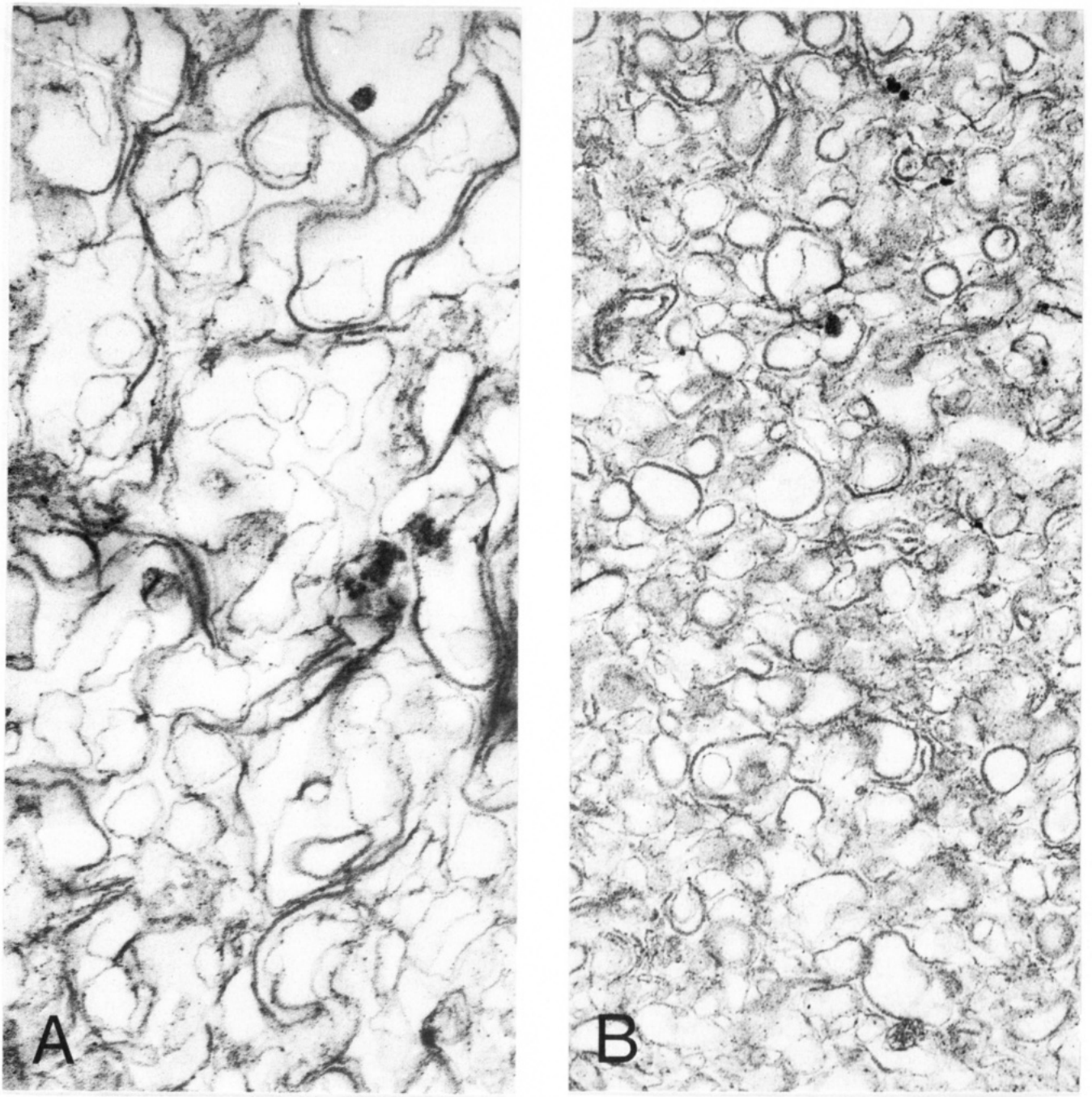

FIGURE 5: (A) Electron micrograph of lens fiber membrane sample prior to sonication. The membrane profiles consist of a heterogeneous population of vesicles with linear dimensions exceeding 1 μm. (B) Membrane sample after sonication. Average vesicle size is decreased by an order of magnitude. Magnification 38000×.

commonly used (Baron & Thompson, 1975; McCaslin & Tanford, 1981a,b; Robinson et al., 1985). Our observation that the far-ultraviolet CD spectrum of detergent-free sonicated vesicles rich in MIP26 is similar to the spectrum of MIP26 solubilized in octyl β-D-glucopyranoside implies that our membrane preparations did not contribute significantly to scattering artifacts. It also strongly suggests that the far-ultraviolet CD spectra in octyl β-D-glucoside or in $C_{12}E_8$ represent the native secondary structure. Since MIP26 is soluble in pure organic solvents such as chloroform/methanol and 2,2,2-trifluoroethanol, it was of interest to monitor the conformational properties of this protein with such a solvent. Trifluoroethanol is known to stabilize and induce the formation of α-helix, and, indeed, the CD spectrum of MIP26 in this solvent shows an increase in the amount of α-helix (see Figure 3 and Table I).

Even though the far-ultraviolet CD spectra of MIP26 are nearly identical for the detergent-free suspension and the detergent-solubilized sample, we cannot assume that the tertiary structure of MIP26 is the same in both cases, nor can we assume that it is the same in the different detergents. McCaslin and Tanford (1981b) have shown that rhodopsin, when solubilized in $C_{12}E_8$, or in sodium cholate, possesses the same native absorption spectrum as well as the same ultraviolet CD spectrum, indicating that the secondary structure in both detergents is similar. However, the rhodopsin preparation in $C_{12}E_8$ irreversibly loses its ability to recombine with 11-*cis*-retinal while in sodium cholate 11-*cis*-retinal does recombine with opsin. This suggests that conformational changes were induced by $C_{12}E_8$ and not by sodium cholate. Unlike rhodopsin or other membrane-bound enzymes such as cytochrome *c* oxidase or $(Na^+ + K^+)$-ATPase where the detergent effects

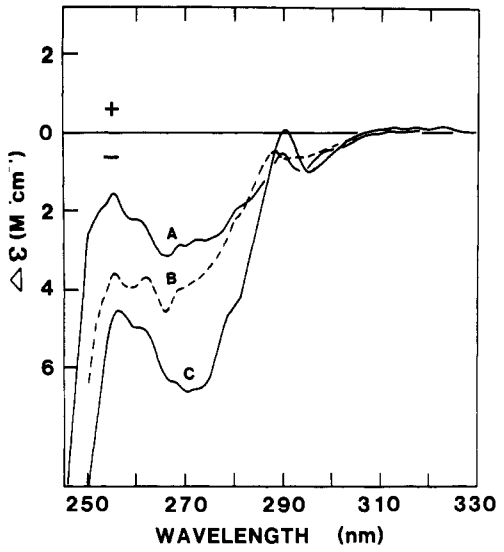

FIGURE 6: Near-ultraviolet CD spectra of MIP26. Curve A, MIP26 solubilized in SDS. Curve B, MIP26 solubilized in trifluoroethanol. Curve C, MIP26 solubilized in octyl β-D-glucopyranoside. The path length was 10 mm. Each spectrum represents the average of 32 scans.

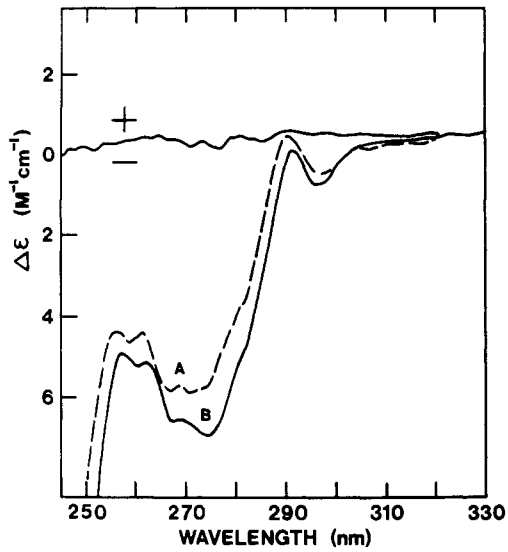

FIGURE 7: Near-ultraviolet CD spectra of *S. aureus* cleaved MIP26. Curve A, MIP26 which was cleaved with *S. aureus* protease in octyl β-D-glucopyranoside. Curve B, native MIP26 in the same buffer. The path length was 10 mm. Each curve represents the average of 32 scans.

can be evaluated (Robinson et al., 1985; Huang et al., 1985), we do not yet have a functional assay for MIP26 in detergent solutions.

Near-ultraviolet CD which is generated by the aromatic amino acids and disulfide bonds is a sensitive indicator of the tertiary structure of proteins (Strickland, 1974). The near-ultraviolet CD spectra of MIP26 solubilized in octyl β-D-glucopyranoside are significantly different from the spectra obtained in SDS or 2,2,2-trifluoroethanol, suggesting a different microenvironment or different conformation of the aromatic amino acid residues in the different solvents. That these are significant differences in tertiary structure for MIP26 solubilized in SDS as compared to octyl β-D-glucopyranoside is also evident from the limited proteolysis studies with *S. aureus* V8 protease. This protease, which is highly specific and cleaves only the carboxyl-terminal side of either aspartic acid or glutamic acid, removed a 3–4-kDa peptide of MIP26 solubilized in SDS. For MIP26 which is solubilized in octyl β-D-glucopyranoside, *S. aureus* V8 protease removes a ~5-kDa peptide (Figure 2, lanes C and E). Several studies using limited proteolysis, as well as antibodies made against synthetic peptides of MIP26, have shown that the "core" protein is protected and is buried in the lipid bilayer, whereas the amino and carboxy termini appear to be exposed (Takemoto et al., 1981; Keeling et al., 1983; Paul & Goodenough, 1983; Takemoto & Takehana, 1986). Sequence analysis of peptide fragments has shown that *S. aureus* V8 protease removes 16 residues from the amino terminus (cleavage at Glu_{16}) as well as a larger peptide from the carboxy terminus (Do Ngoc et al., 1985). Although sequence analysis of the carboxy-terminus fragments has not yet been performed, based on the fragment size and the available sequence (Gorin et al., 1984), it is very likely that in SDS *S. aureus* protease cleaves MIP26 at Glu_{245}, whereas in the sugar-based detergent, the cleavage is at Glu_{232} (Gorin et al., 1984; Do Ngoc et al., 1985).

The observation that the near-ultraviolet CD spectrum of MIP22 is very similar to that of the native protein suggests that the contribution to the optical activity in the near-ultraviolet is generated mainly by the core protein and that the removal of 5 kDa from the carboxy and amino termini does not affect the conformation of the core protein as judged by CD. The near- and far-ultraviolet CD data on MIP26 and MIP22 strongly support the recently proposed structure of this protein which was based on cDNA cloning (Gorin et al., 1984). This model envisions MIP26 to contain six transmembrane helices and to have both its carboxy and amino termini on the cytoplasmic side of the fiber cell. The removal of 16 residues from the amino terminus and 31 residues from the carboxy terminus is expected in this model to increase helicity to approximately 60%, which is in excellent agreement with our observed CD data. Further studies are needed to substantiate this model and to elucidate the mechanisms by which this protein may serve as a communicating channel.

Registry No. CF_3CH_2OH, 75-89-8; octyl β-D-glucopyranoside, 29836-26-8.

REFERENCES

Alcala, J., Liesk, N., & Maisel, H. (1975) *Exp. Eye Res. 21*, 581–595.

Baron, C., & Thompson, T. L. (1975) *Biochim. Biophys. Acta 382*, 276–285.

Benedetti, E. L., Dunia, I., Ramaekers, F. C. S., & Kibbelaar, M. A. (1981) in *Molecular Biology of the Eye Lens* (Bloemendal, H., Ed.) pp 137–188, Wiley, New York.

Bok, D., Dockstader, J., & Horwitz, J. (1982) *J. Cell Biol. 92*, 213–220.

Broekhuyse, R. M., Kuhlman, E. D., & Stols, A. L. H. (1976) *Exp. Eye Res. 28*, 365–371.

Bustamante, C., Tinoc, I., & Maestre, M. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3568–3572.

Chen, Y. H., Yang, J. T., & Chau, K. H. (1974) *Biochemistry 13*, 3350–3359.

Do Ngoc, L., Paroutand, P., Dunia, I., Benedetti, E. L., & Hoebeke, J. (1985) *FEBS Lett. 181*, 74–78.

FitzGerald, P. G., Bok, D., & Horwitz, J. (1983) *J. Cell Biol. 97*, 1491–1499.

Girsch, S. J., & Peracchia, C. (1985a) *J. Membr. Biol. 83*, 217–225.

Girsch, S. J., & Peracchia, C. (1985b) *J. Membr. Biol. 83*, 227–233.

Glaeser, R. M., & Jap, B. K. (1985) *Biochemistry 24*, 6398–6401.

Gooden, M., Rintoul, M., Takehana, M., & Takemoto, L. (1985) *Biochem. Biophys. Res. Commun. 128*, 993–999.

Goodenough, D. A., Dick, J. S. B., II, & Lyons, J. E. (1980) *J. Cell Biol. 86*, 576–589.

Gorin, M. B., Yancey, B. S., Cline, J., Revel, J.-P., & Horwitz, J. (1984) *Cell (Cambridge, Mass.) 39*, 49–59.
Greenfield, N., & Fasman, G. D. (1969) *Biochemistry 8*, 4108–4116.
Horwitz, J., & Wong, M. M. (1980) *Biochim. Biophys. Acta 622*, 134–143.
Horwitz, J., Strickland, E. H., & Billups, C. (1969) *J. Am. Chem. Soc. 91*, 184–190.
Horwitz, J., Bullard, B., & Mercola, D. (1979) *J. Biol. Chem. 254*, 350–355.
Huang, W.-H., Kakar, S. S., & Askari, A. (1985) *J. Biol. Chem. 260*, 7356–7361.
Jap, B. K., Maestre, M. F., & Hayward, S. B. (1983) *Biophys. J. 43*, 81–89.
Keeling, P., Johnson, K., Sas, D., Klukas, K., Donahue, P., & Johnson, R. (1983) *J. Membr. Biol. 74*, 217–228.
Litman, B. J. (1972) *Biochemistry 11*, 3243–3247.
Maestre, M. F., Gray, D. M., & Cook, R. B. (1971) *Biopolymers 10*, 2537–2553.
Maisel, H., Harding, C. V., Alcala, J. A., Kuszak, J., & Bradley, R. (1981) in *Molecular Biology of the Eye Lens* (Bloemendal, H., Ed.) pp 49–85, Wiley, New York.
Manavalan, P., & Johnson, W. C., Jr. (1983) *Nature (London) 305*, 831–832.
Mao, D., & Wallace, B. A. (1984) *Biochemistry 23*, 2667–2673.
McCaslin, D. R., & Tanford, C. (1981a) *Biochemistry 20*, 5207–5212.
McCaslin, D. R., & Tanford, C. (1981b) *Biochemistry 20*, 5212–5221.
Nikaido, H., & Rosenberg, E. Y. (1985) *J. Membr. Biol. 85*, 87–92.
Paul, D. L., & Goodenough, D. A. (1983) *J. Cell Biol. 96*, 633–638.
Rae, J. L. (1979) *Curr. Top. Eye Res. 1*, 37–90.
Reich, C., Maestre, M. F., Edmondson, S., & Gray, D. M. (1980) *Biochemistry 19*, 5208–5213.
Robinson, N. C., Neumann, J., & Wiginton, D. (1985) *Biochemistry 24*, 6298–6304.
Russell, P., Robinson, G., & Kinoshita, J. (1981) *Exp. Eye Res. 32*, 511–516.
Schneider, A. S., & Harmatz, D. (1976) *Biochemistry 15*, 4158–4162.
Schneider, A. S., Schneider, M. J. T., & Rosenheck, K. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 793–798.
Siegel, J. B., Steinmetz, W. E., & Long, G. L. (1980) *Anal. Biochem. 104*, 160–167.
Strickland, E. H. (1974) *CRC Crit. Rev. Biochem. 2*, 113–175.
Takemoto, L. J., & Takehana, M. (1986) *Exp. Eye Res. 43*, 661–667.
Takemoto, L. J., Hansen, J. S., & Horwitz, J. (1981) *Comp. Biochem. Physiol., B: Comp. Biochem. 68B*, 101–106.
Tanford, C., & Reynolds, J. A. (1976) *Biochim. Biophys. Acta 457*, 133–170.
Urry, D. W. (1985) in *Modern Physical Methods in Biochemistry* (Neuberger & Van Deenen, Eds.) pp 275–346, Elsevier, Amsterdam.
Urry, D. W., Massoti, L., & Krivacic, J. (1970) *Biochem. Biophys. Res. Commun. 41*, 521–524.
Wallace, B. A., & Teeters, C. L. (1987) *Biochemistry 26*, 65–70.
Wu, C.-S. C., Ikeda, K., & Yang, J. T. (1981) *Biochemistry 20*, 566–570.
Zampighi, G. A., Hall, J. E., & Kreman, M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8468–8472.