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Lens Cell-to-Cell Channel Protein: I. Self-Assembly into Liposomes and Permeability Regulation by Calmodulin

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Summary. Lens fibers are coupled by communicating junctions which contain a 28-kDalton protein (MIP26) believed to be the main component of the cell-to-cell channel. To study the permeability properties and regulation of these channels, an *in vitro* system has been developed in which MIP26 isolated from calf lens is incorporated into liposomes and the resulting channels are studied spectrophotometrically by a swelling assay. Liposome vesicles were prepared using a sonication/resuspension method. Incorporation efficiency was monitored by freeze-fracture. Vesicles were resuspended in 6% Dextran T-10. Assay buffer was identical, except for isotonic substitution of sucrose for T-10. MIP26-incorporated (but not control) vesicles swell under isotonic conditions indicating sucrose entry (via channels) followed by water to maintain osmotic balance. In the absence of calmodulin, calcium ion has no effect on channel permeability. On the contrary, vesicles prepared with equimolar amounts of MIP26 and CaM do not swell in the presence of calcium ion, indicating that the channels can be closed. Addition of EGTA to these vesicles reinitiates swelling—evidence that the channel gating mechanism is reversible. Magnesium ion has no effect on either type of vesicle.

Key Words lens gap junctions · lens channel protein (MIP26) · communicating junctions · calmodulin and gap junctions · reconstitution of lens junction channels · cell-to-cell channel gating · liposome reconstitution

Introduction

The contiguous cells of most tissues exchange protoplasmic molecules as large as one kDalton (Flagg-Newton, Simpson & Loewenstein, 1979) via cell-to-cell channels housed in the intramembrane particles of gap junctions (communicating junctions) (Peracchia, 1984*a*). It is generally agreed that these channels close in response to an intracellular increase in either $[Ca^{++}]$ or $[H^+]$ (Loewenstein, 1981; Turin & Warner, 1977, 1980; Spray, Harris & Bennett, 1981; Spray, Stern, Harris & Bennett, 1982), the result being cell-to-cell uncoupling. With uncoupling, changes in the structure of communicating junctions have been observed. These changes are characterized by an increase in the tightness and regularity (crystallization) of the particle array and possibly a decrease in particle size (Peracchia, 1984*a*; Peracchia & Bernardini, 1984).

In spite of our knowledge of uncoupling agents, the uncoupling mechanism is still poorly understood. A recent hypothesis has proposed the involvement of calmodulin (CaM) in the uncoupling mechanism (Peracchia, Bernardini & Peracchia, 1981, 1983). This hypothesis, which is an extension of the calcium hypothesis (Loewenstein, 1981), is based on the observation that CaM inhibitors such as trifluoperazine (TFP) (Peracchia et al., 1981, 1983) or calmidazolium (CDZ) (Peracchia, 1984*b*) effectively protect amphibian embryonic cells from uncoupling by CO_2 (lowered pH_i) and on evidence that CaM binds to lens (Welsh et al., 1981, 1982; Hertzberg & Gilula, 1981) and liver (Hertzberg & Gilula, 1981) gap junction proteins. Also consistent with this hypothesis is the observation that internally perfused crayfish axons lose the capacity to uncouple with increased Ca_i^{++} (Johnston & Ramón, 1981). This observation was interpreted to indicate the presence of a soluble uncoupling intermediate.

A conventional approach for studying the uncoupling mechanism employs intact cells in which channel permeability regulation is probed electrophysiologically. The whole-cell approach, however, has many obvious limitations. To address the problem in a simpler and more direct way we have developed an *in vitro* system in which the gap junction protein (MIP26), isolated from bovine lens fibers, is incorporated into liposomes. Both the permeability characteristics and regulatory mechanism of the resulting channels are studied spectrophotometrically by the swelling assay of Luckey and Nikaido (1980).

The present data show that MIP26, incorporated into liposomes, forms channels permeable to sucrose but not to Dextran. These channels close in the presence of calcium-activated CaM but not with

Ca^{++} alone. A preliminary account of this study has been published (Girsch & Peracchia, 1983).

Materials and Methods

MIP26

Young bovine eyes were obtained fresh from the local abattoir. Lens junction protein was isolated by a modified Russell (Russell, Robison & Kinoshita, 1981) procedure. The method involves stripping fiber membranes of all soluble and extrinsically bound protein by successive washes of buffer, urea, and 0.1 N NaOH. In most experiments, after the NaOH wash, the membrane pellet was extracted for 24 hr with stirring at 15°C in 2% octyl-POE (octylpolyoxyethylene). The supernatant was decanted after clarification (20 min at 48,000 × *g*) and stored as 200-μl aliquots in plastic vials at 4°C. MIP26 solutions precipitate irreversibly when frozen. Homogeneity of MIP26 was checked by means of SDS-PAGE and found to be 90–95% pure. In some experiments, homogeneous MIP26 was used as obtained by preparative electrophoresis. A 0.5-ml aliquot of 2% SDS-extracted MIP26 was mixed with sucrose/tracking dye and electrophoresed at 120 VDC on 5-mm SDS-PAGE slab gel (12.5%). A central 1-cm vertical strip was excised and developed in 1 M KCl (5 min) to resolve the major horizontal band (MIP26). The band was cut from the slab gel by reference to the KCl strip. Gel pieces were placed in the sample cup of an ISCO Model 1750 Electroelution Concentrator in 0.1% octyl-POE, 5 mM PO_4, pH = 6.8. Tank buffer was double concentration. Samples were electroeluted for 3 hr at 7 mA and collected by micropipette as 0.5-ml aliquots from the concentrator nipple. Yields were better than 80%. In all cases non-MIP26 fractions were saved for use as vesicle controls.

Lipids

Brain phospholipids (B1627, Sigma, St. Louis, MO) were stirred under N_2 in acetone for 1 hr to remove neutral lipids, taken to dryness and resolubilized in $CHCl_3$/MeOH at 3 : 1. Final concentration was 200 μg/μl. Samples were stored in liquid N_2 to retard oxidation.

Preparation of Vesicles

A 60-μl aliquot (~7 μmol) of phospholipids was added to a conical test tube and taken to dryness by nitrogen stream. After addition of 200 μl of H_2O, 50-μl aliquots of either non-MIP26 fractions, MIP26 or MIP26/CaM at 1 : 1 (5 mg/ml) were added to the lipid suspension, vortex-mixed and sonicated for 30 min at 5°C (Ladd Research Industries, Model G-80-80-1 bath sonicator). Samples were again taken to dryness and redissolved in 1 ml of 6% Dextran T-10, 5 mM Tris, pH 7.4, and sonicated for 8 min until translucent.

Permeability Assay

Changes in the scattering cross-section of vesicle suspensions were used as a measure of vesicle size, as modified from the technique described by Luckey and Nikaido (1980). Either a Gilford Model 210 single-beam or Perkin-Elmer 3A double-beam spectrophotometer was used with full scale set between 0.1–0.2 OD units and wavelength at 500 nm. Scattering changes were recorded by strip chart with accuracy better than 1 mOD unit. Vesicle aliquots of 50 μl were mixed with 1 ml of the appropriate buffer and placed in the cell holder; elapsed mixing time was less than 3 sec.

Osmolarity

The approximate osmolarity of T-10, sucrose, and other buffers was measured using a Wescor Model 5100C vapor pressure osmometer. Osmotic test solutions of 50% hypotonic sucrose and 50% hypertonic sucrose were also prepared.

Electron Microscopy

Vesicle samples were mixed with equal amounts of 6% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.4, and allowed to stand for 30 min. The crosslinked vesicles were centrifuged at 48,000 × *g* and stored under 0.1 M cacodylate buffer, pH 7.4. Freeze-fracture replicas of the vesicle pellets were prepared on a Balzers BAF 301 freeze-etch unit as previously described (Peracchia & Peracchia, 1980*a*) and examined with an AEI EM 801 electron microscope.

pH

pH was measured before and after additions to vesicle preparations in the cuvette using a Fisher Accumet model 620 with Ingold Combination microtip electrode 6025-04.

Calcium

Total calcium was determined using a Perkin-Elmer 305B atomic absorption spectrophotometer with auto zero and auto sampling attachment. All buffers and samples were prepared in Nalgene and/or polycarbonate labware. Calcium ion values obtained in micromolar: water (0.35–0.45), sucrose buffers (4.5–8.8), T-10 (9.0–12.0), MIP stock (3.5–4.8), and CaM stock (2.0).

Chemicals

Tris, EGTA, and cacodylate were obtained from Sigma, St. Louis, MO. Sucrose was supplied by J.T. Baker, Phillipsburg, NJ. Dextran T-10 was purchased from Pharmacia, Uppsala, Sweden. Glutaraldehyde was supplied by Electron Microscopy Sciences, Fort Washington, PA. Purified calmodulin was obtained from M.J. Welsh, University of Michigan, Ann Arbor, MI.

Results

MIP26

Figure 1*a* shows the electrophoretic profile of octyl-POE-extracted MIP26 with reference to suitable

standards. MIP26 was adjudged to be better than 90% pure by silver stain density. Figure 1*b* illustrates the use of 1 M KCl to reversibly precipitate SDS-proteins for identification, without use of stains.

MIP26 Vesicles

In freeze-fracture replicas, MIP26 reconstituted vesicles range in size from 0.1 to 0.8 μm (Fig. 2). The majority of the vesicles were less than 0.4 μm in diameter and appeared unilamellar in cross fractures (Fig. 2), while larger vesicles often appeared to be multilamellar. Both the concave and the convex fracture faces of most vesicles contain intramembrane particles. Most of the particles are 8.5 nm in size but occasionally particles as small as 6.5 nm are seen. The same range of particle size is observed in gap junctions of intact lens fibers (Fig. 2, *inset*). In heavily incorporated vesicles, the particles tend to aggregate (Fig. 3) forming groupings reminiscent of the disordered particle arrays of intact junctions (Fig. 2, *inset*). Vesicle preparations are stable for at least 8 hr, but begin to deteriorate 12–24 hr after sonication. In these experiments vesicle samples were used within 2 hr of preparation.

Permeability Assay

Figure 4 shows the results obtained when plain (protein-free) vesicles prepared in 6% Dextran T-10 are placed in buffered sucrose solutions of different molarities. When external sucrose molarity is less than internal T-10 Dextran molarity, a net influx of H_2O occurs causing vesicle swelling and hence a decrease in scattering cross-section. Under sucrose hyperosmotic conditions the vesicles shrink while scattering increases. Before beginning the experiments the approximate osmolarities of sucrose and T-10 buffers are determined by vapor pressure measurements. Sucrose buffer molarity is then adjusted empirically until light scattering remains constant and hence iso-osmotic conditions prevail. This particular sucrose buffer could then be used with all vesicle types of one batch, but could not be used with vesicle preparations from a different batch. Vesicle preparations placed in hypotonic sucrose exhibit rapid loss of optical density (swelling). The ΔOD observed is roughly proportional to initial vesicle concentration.

Permeability of MIP26 Vesicles

Figure 5 shows the results of placing MIP26-incorporated vesicles, prepared in 6% Dextran T-10, into

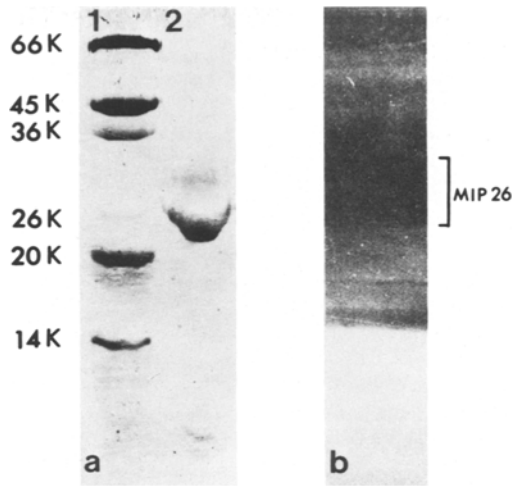

Fig. 1. (*a*): Electrophoretic profile of octyl-POE extracted MIP26 from base-washed lens junction membranes. SDS-PAGE, 3% stacking gel, in 0.125 M Tris, 0.1% SDS, pH = 6.8; 12% running gel, 0.325 M Tris, 0.1% SDS, pH = 8.8; tank buffer, 0.025 M Tris, 0.192 M glycine, 0.05% SDS, pH = 8.3 (standards lane 1, sample lane 2). (*b*): Preparative slab gel of MIP26 preparation illustrating use of 1-M KCl to delineate the 26K band (Rf $\simeq$ 0.45). Horizontal band protein is removed and concentrated by electroelution

iso-osmotic sucrose. As can be seen in trace #3, an immediate decrease in scattering cross-section commences, presumably due to vesicle swelling as sucrose enters. Iso-osmotic conditions are confirmed, since unincorporated vesicles from the same batch are optically stable in sucrose solution (trace #1). The decrease in scattering is not due to aggregation, disintegration, or other phenomena of dilution/mixing since pipetting the MIP26-vesicles into iso-osmotic Dextran T-10 buffer produces a stable optical density trace. When MIP26-incorporated vesicles are placed in hypertonic sucrose there is an initial shrinkage indicated by increased light scatter, followed by swelling at the usual rate. Unincorporated vesicles continuously shrink (Fig. 4, trace #1). The observation of swelling under hypertonic conditions is additional evidence for vesicle porosity.

When incorporated vesicles are placed in calcium-containing solutions (1×10^{-5} M $[Ca^{++}]$) no change in swelling rate is observed (trace #2). Additional Ca^{++} has no effect on swelling, but above 1 mM causes an increase in scattering. MIP26 vesicles placed in solutions in which magnesium is substituted for calcium also fail to show a change in swelling rate.

Figure 6 shows optical density recordings of MIP26 vesicle suspensions in sucrose, prepared from octyl-POE extracted MIP26 (trace #2), MIP26

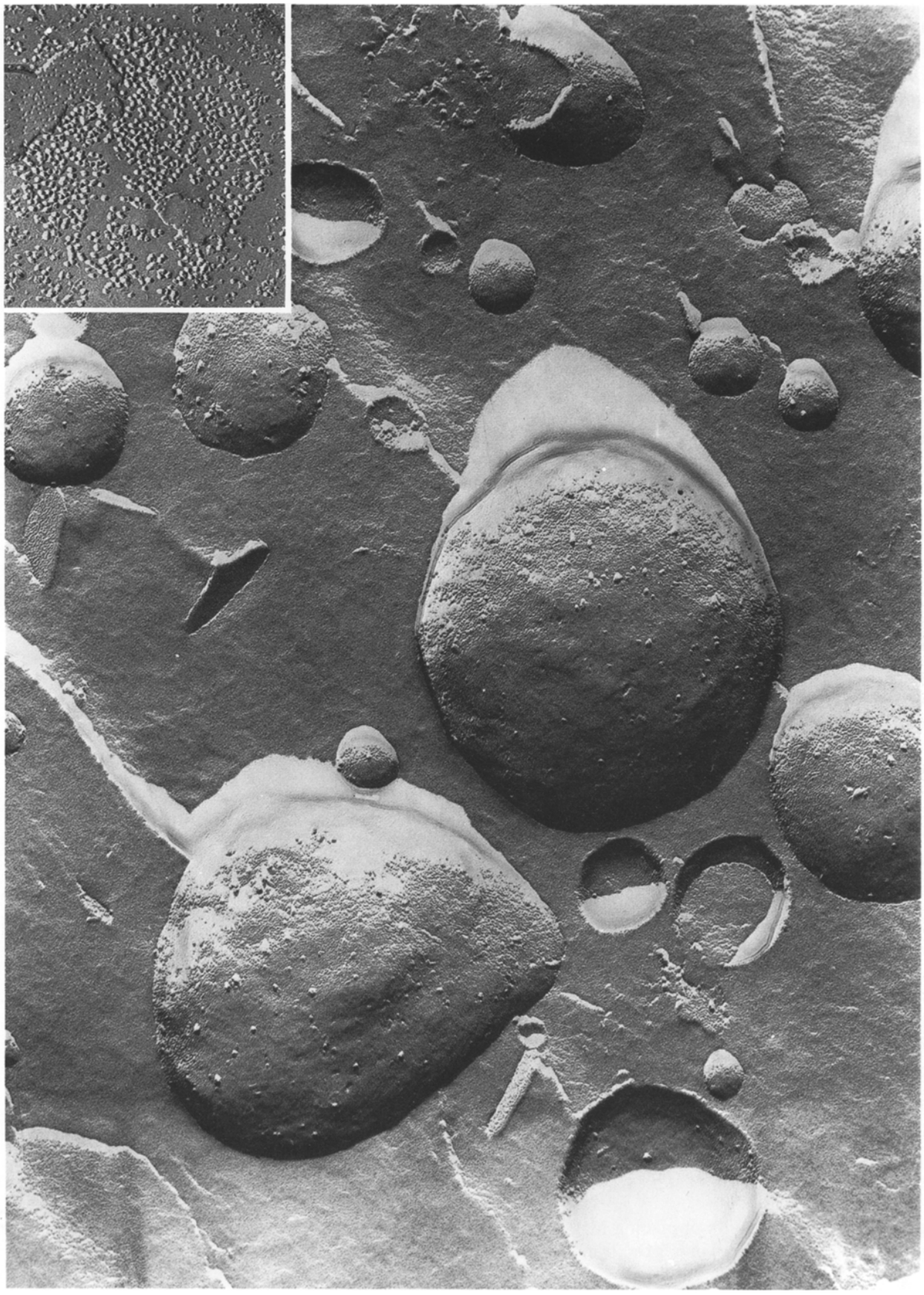

Fig. 2 (*facing page*). Freeze-fracture replica of MIP26 reconstituted vesicles. The vesicles range in size from 0.1–0.8 μm. Intramembrane particles and pits can be seen on both the convex and concave surfaces of most vesicles, regardless of size. The particles vary in size from 6.5 to 9 nm but most commonly measure ~8.5 nm. Similar particle size range is observed in gap junctions of intact lens fibers (*inset*). ×111,400

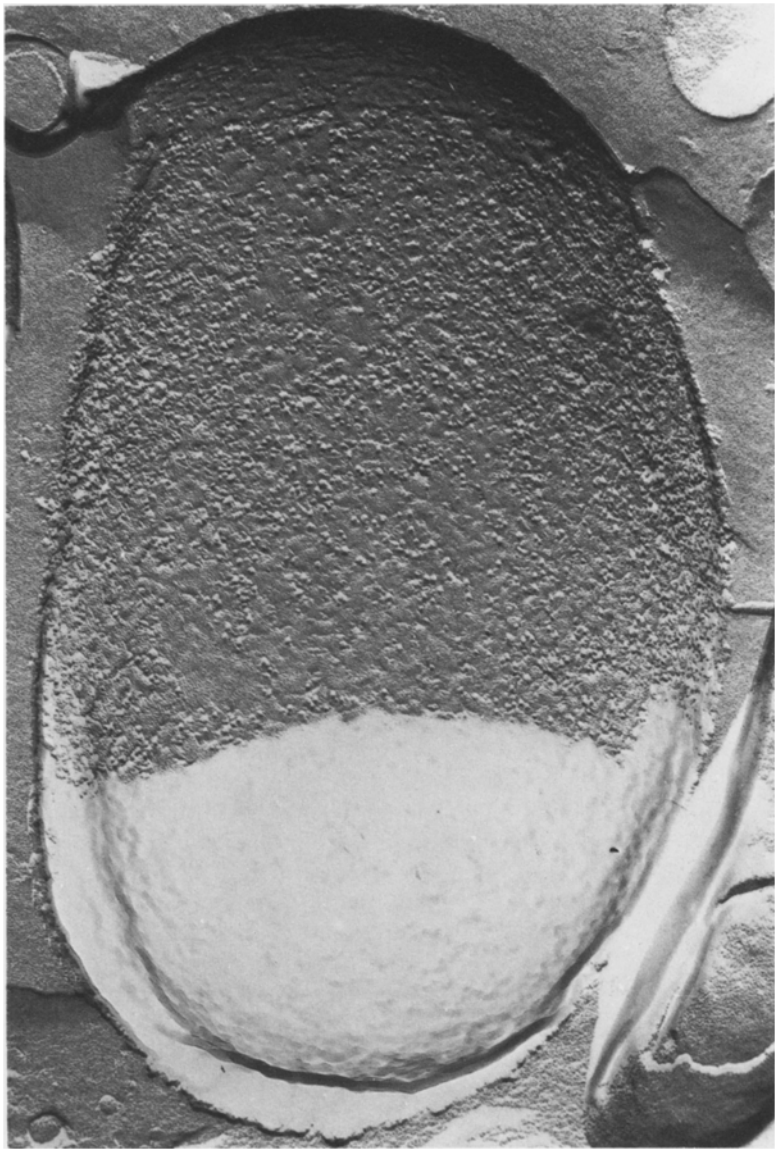

Fig. 3. Freeze-fracture replica of an MIP26 reconstituted vesicle. In some experiments large vesicles can be seen which are heavily incorporated. In these vesicles most of the particles and pits are grouped into disordered arrays similar to those of intact junctions (Fig. 2, *inset*). ×88,000

obtained by electroelution from slab gel both without (trace #3) and with (trace #1) a 1-min boiling prior to electrophoresis. The octyl-POE preparation and the electroeluted preparation exhibit similar swelling kinetics. The preboiled sample, however, exhibits no channel-forming capacity.

Permeability of MIP26-CaM Vesicles

Vesicles were prepared as before, but equimolar amounts of homogeneous CaM and MIP26 were included in the reconstitution mix. Figure 7 shows the results obtained when MIP26-CaM reconstituted vesicles are placed in iso-osmotic sucrose solution. Trace #1 shows that MIP26-CaM vesicles also swell, indicating the presence of channels, but the rate of sucrose influx is less than that of MIP26 vesicles. Unlike MIP26 vesicles, however, the addition of MIP26-CaM vesicles to iso-osmotic sucrose solution containing 1×10^{-5} $[Ca^{++}]$ (trace #2) results in no swelling indicating that the channels are closed. When a 10 μl aliquot of chelator (EGTA,

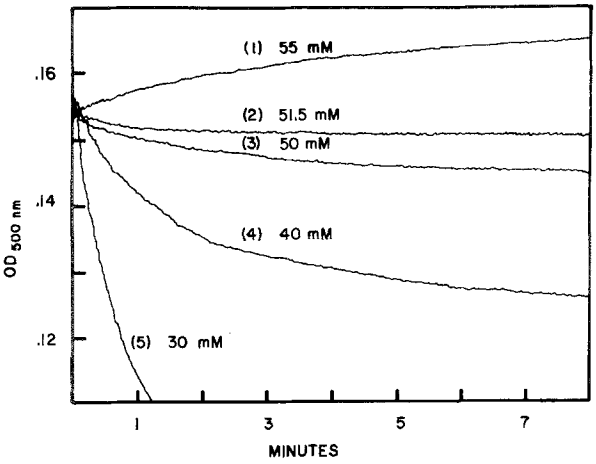

Fig. 4. Adjustment to iso-osmotic conditions. Plain (protein-free) liposomes prepared in 6% Dextran T-10 are placed in sucrose buffers of various molarities. Size increase/decrease is monitored by scattering cross-section. Under iso-osmotic conditions net flux of water is zero, and vesicle size remains constant (trace #2). This osmotic parameter changes with each set of vesicle preparations but is identical for all preparations within the same batch

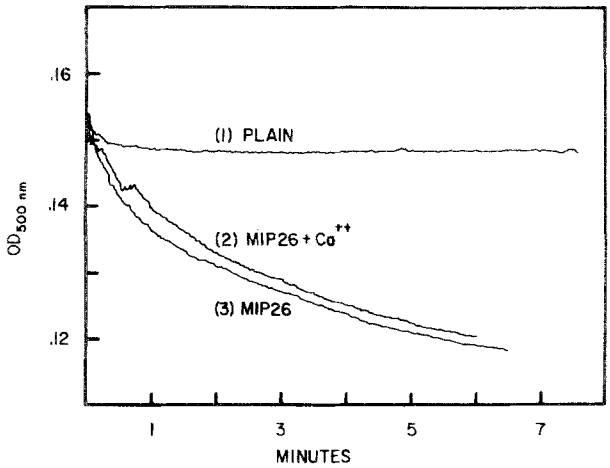

Fig. 5. MIP26 incorporation. Plain vesicles exhibit no swelling (trace #1), while those that contain MIP26 are quite permeable when placed in iso-osmotic sucrose. The incorporated vesicles apparently now contain channels large enough to allow influx of sucrose (trace #3). Addition of $CaCl_2$ to 1×10^{-5} M $[Ca^{++}]$ does not change swelling rate (trace #2)

final conc. = 5×10^{-4} M) is added to the cuvette from trace #2, swelling resumes, indicating sucrose influx (trace #3); this process of opening and closing could be repeated for several cycles until salting out of the vesicles occurs.

When equimolar CaM is added to preformed MIP26 vesicles in sucrose solution already containing Ca^{++} (1×10^{-5} M), swelling of the vesicles continues with only a slight decrease in sucrose influx (trace #4). Increasing $[Ca^{++}]$ by one or two orders does not impede swelling. $[Mg^{++}]$ as high as 1 mM does not close MIP26-CaM vesicles channels, but precipitation occurs at higher concentrations. Control vesicles prepared using CaM alone, or CaM in combination with lens H_2O-soluble fraction, urea-soluble fraction, or detergent-washed membrane fraction show no permeability under any of the conditions discussed (*not shown*).

Discussion

This paper describes a method for incorporating lens junction protein into vesicles and a means to measure the permeability properties of the channels obtained. MIP26 vesicles are readily permeable to sucrose, even in the presence of Ca^{++}, Mg^{++} or H^+. Conversely, vesicles formed with equimolar MIP26 and CaM can be reversibly closed by Ca^{++}, consistent with the CaM hypothesis for the gating of communicating channels (Peracchia et al., 1981, 1983).

The lens provides an excellent source for the isolation and *in vitro* reconstitution of cell-to-cell channel proteins since over 50% of the fiber-fiber appositional surface is junctional (Broekhuyse, Kuhlmann & Winkens, 1979), allowing high yields of MIP26 by a simple procedure. MIP26 has been recognized as the main lens junction protein by immuno-electronmicroscopy, using ferritin-labeled polyclonal antibodies (Bok, Dockstader & Horwitz, 1982). Recently, however, this finding has been questioned by Paul and Goodenough (1983). Using affinity-purified antibodies to MIP26, they found no labeling of the fiber-fiber junction. A preliminary study using monoclonal antibodies to MIP26, however, has reconfirmed the localization of label at the junctional membrane site (Sas & Johnson, 1983).

Lens fiber junctions, although structurally (Peracchia & Peracchia, 1980*a,b*; Zampighi et al., 1982), chemically (Nicholson et al., 1981, 1983), and immunologically (Hertzberg, Anderson, Friedlander & Gilula, 1982) different from other gap junctions, are believed to function as communicating junctions as they allow cell-to-cell diffusion of ions and dyes (Rae, 1979), can be uncoupled by cell injury in the presence of Ca^{++} (Bernardini, Peracchia & Venosa, 1981) or treatment with 2,4-dinitrophenol (Rae, Thompson & Eisenberg, 1982) and crystallize *in vitro* with divalent ions or hydrogen ion (Peracchia & Peracchia, 1980*a,b*). Moreover, lens junction crystallization is inhibited by a CaM inhibitor (Peracchia et al., 1981).

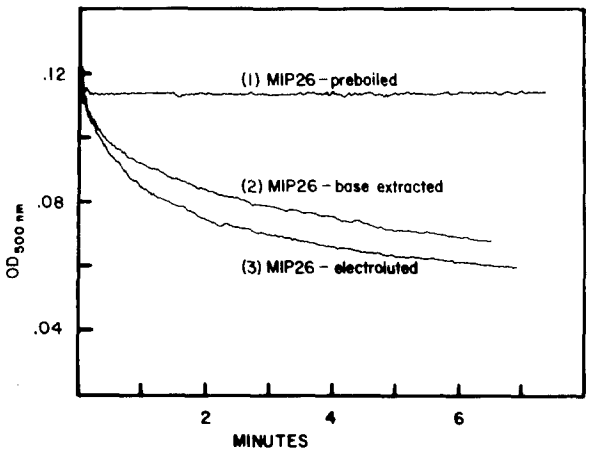

Fig. 6. Permeability of three types of MIP26 vesicles. In trace #1, SDS-extracted MIP26 is boiled for 1 min at 100°C before preparative electrophoresis and electroelution. Trace #2 shows swelling profile in vesicles prepared from base-washed octyl-POE extracted MIP26. Trace #3 shows swelling from MIP26 obtained after preparative electrophoresis and electroelution.

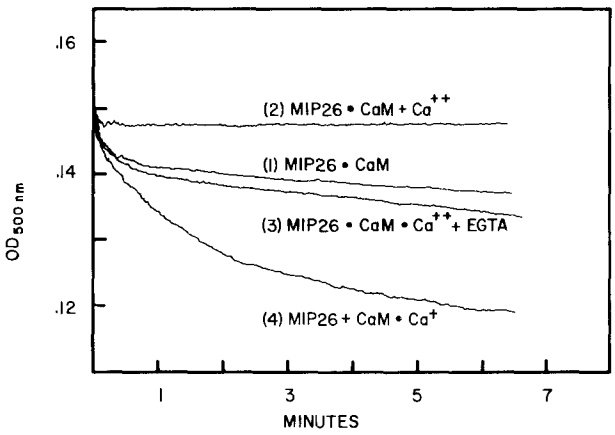

Fig. 7. Permeability of MIP26-CaM vesicles. MIP26-CaM vesicles exhibit swelling under iso-osmotic conditions, indicating presence of channels (trace #1). When an aliquot of vesicles is placed in an identical sucrose buffer, but one containing 1×10^{-5} M [Ca^{++}], swelling is not observed (trace #2); the channels are closed. Addition of 5×10^{-4} M EGTA to the Ca^{++}-occluded vesicles causes reopening and the resumption of swelling (trace #3). Adding CaM and Ca^{++} to preformed MIP26 vesicles does not prevent sucrose influx (trace #4)

Reconstitution and Symmetry

MIP26 is a very hydrophobic protein, thus the thermodynamic driving force favoring partition into the lipid phase is apparently quite high. The detergent octyl-POE has a high critical micellar concentration (CMC) and also expedites transfer of MIP26 from micellar to liposomal carrier. Sodium dodecyl sulfate, although an excellent extractant, made incorporation difficult because of its very low CMC and hence resistance to removal by dialysis or dilution (Peracchia & Girsch, 1983). Octyl-β-D-glucoside has been used in MIP26 extraction (Horwitz & Bok, 1983), but in our preparations channel protein yields were low with this detergent.

Channel-forming capability is solely a property of MIP26 protein. Since insertion and self-association to form channels occurs with equal facility in both electroeluted MIP26 and octyl-POE preparations, we may conclude that neither small cofactors nor minor protein components are a prerequisite for reconstitution. Interestingly, however, boiling in SDS eliminates completely channel-forming capacity. This is somewhat surprising, since MIP26 is quite resistant to extremes of pH, salts, organic solvents, and strong ionic detergents. Thermal denaturation is apparently irreversible.

The mechanism of insertion remains unknown. If the MIP26 protein is always inserted from the same end, incorporation into the bilayer should proceed equally well from either the inside or outside of the vesicle. In fact, particles can be seen on both the concave and convex surface of the vesicles. This, of course, assumes that the fracture properties of the particles, by analogy with intact junctions, are indicative of the particle orientation. Indeed, if this were the case, particles seen on the convex vesicle surface should have their cytoplasmic end in the luminal compartment and vice versa, because in intact junctions the large majority of particles fracture with the protoplasmic leaflet (P face). If the particles, as it seems, have symmetrical orientation, one would expect that CaM added to the external medium would only interact with junctional proteins whose cytoplasmic ends were directed outward. Indeed, when Ca^{++}-activated CaM was added to preformed MIP26 vesicles sucrose entry was not prevented but the rate of swelling was decreased, suggesting that some of the junctional proteins have their CaM receptor sites in the vesicle lumen and thus are inaccessible to external CaM.

Channel Formation and Kinetics

As with insertion, the mechanism of MIP26 subunit assembly remains unknown. The proteins are apparently able to self-associate, orient correctly, and bind in spite of pretreatment with 8 M urea, a caustic wash and strong detergent. Moreover, the process of self-assembly, at least in the vesicles, requires no other cofactors, relying only on the interaction among subunits and/or phospholipids.

The reconstituted channels can easily pass hydrated sucrose (radius 5.2 Å, Schultz & Solomon, 1961) establishing a lower limit for their pore diameter. Functional reconstitution of lens MIP26 into liposomes has also been demonstrated by Nikaido and Rosenberg, 1984.

Permeability Control

Vesicles prepared with MIP26 alone apparently have channels configured in a permanently open state. It may be that conditions exist which close MIP26 channels in the absence of CaM, but our present data indicate that in this *in vitro* system they cannot be closed unless CaM is present. Unwin and Ennis (1984) have shown that the liver connexons change as a result of a Ca^{++}-induced radial displacement of the subunits, measured by low-dose microscopy and X-ray diffraction. However, the small changes observed have not been directly linked to permeability, as the channel patency was not demonstrated in any of the conditions tested.

The simple addition of CaM to the reconstitution mixture produces channels in which gating can be readily and reversibly demonstrated. This gating mechanism is operant with only three components in the vesicle preparation: MIP26, CaM, and phospholipids. Thus, at least in this *in vitro* system all the information to produce gating arises from the tertiary and quaternary interactions of the two proteins and the lipid matrix. In intact cells the gating mechanism could be as simple as in the vesicles but other components may be involved.

Although our data support the hypothesis that Ca^{++}-CaM closes the channels by causing conformational changes in MIP26 (Girsch & Peracchia, 1984), the possibility that the channels are closed by nonspecific mechanisms cannot be discarded. For example, CaM could be incorporated into the MIP26 channels as they form, and the change in CaM conformation, when Ca^{++} is added, may block the channels in a nonspecific manner. Alternatively, Ca^{++}-activated CaM may bind to some components of the MIP26 channel not exposed *in vivo* and thus close the reconstituted channels. However, our data (Girsch & Peracchia, 1984) showing specific changes in both the fluorescence and circular dichroism of isolated MIP26-CaM in both the presence and absence of Ca^{++}, suggest that nonspecific interactions are less likely to be the cause of gating.

It is interesting to note that the swelling rates of MIP26-CaM vesicles, although somewhat variable, are slower than those of comparable MIP26 vesicles. It may be that the two types of vesicles differ in their incorporation efficiency or in the permeability of the reconstituted channel. Alternatively, the presence of CaM may confer a different conformation to the MIP26 subunits even in the open configuration. Finally, it may be that background levels of Ca^{++} could be sufficient to occlude some of the channels. In order to preclude the latter possibility, attempts were made to prepare the MIP26-CaM vesicles in the presence of EGTA. These preparations, however, were unstable and precipitation occurred, possibly due to the absence of Ca^{++}, which is known to stabilize phospholipids. In any event, addition of EGTA to preformed MIP26-CaM vesicles did not restore swelling rate to that of the companion MIP26 vesicles, suggesting that the slower swelling rate is not due to contaminant Ca^{++}.

Preliminary experiments indicate that lowering pH is associated with channel occlusion in MIP26-CaM vesicles, but not MIP26 vesicles. Since the assay buffer system contains neither added Ca^{++} nor Ca^{++} chelator, these data cannot distinguish between a direct H^+ effect or a displaced Ca^{++} effect. Experiments using carefully prepared Ca^{++}-EGTA buffers at several pH values are in progress.

In conclusion, this study shows that purified MIP26 self-assembles into channels large enough to permit transit of sucrose. The channels close in the presence of Ca^{++}, only when CaM is included in the reconstitution mixture. These results are consistent with the hypothesis of a CaM involvement in the regulation of cell coupling (Peracchia et al., 1981, 1983) and show the potential of utilizing an *in vitro* system for studying cell-to-cell communication. This model system may prove useful in generating new criteria for studying the functional properties of communicating junctions and for comparing junctions from various sources. However, one should keep in mind that the data presented here were obtained in a cell-free system, and the channel properties of intact cells may be different.

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