Isolation and protein composition of gap junctions from rabbit hearts

Chellakere K. MANJUNATH, Gwendolyn E. GOINGS and Ernest PAGE

Department of Medicine, The University of Chicago, 950 East 59th Street, Chicago, IL 60637, U.S.A.

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We have modified a method for isolating gap-junctional membrane from mouse hearts [Kensler & Goodenough (1980) *J. Cell Biol.* **86**, 755–764] to isolate gap junctions of comparable purity from rabbit hearts more rapidly, with better yield, and without resort to non-ionic detergents. Purification was monitored by electron microscopy of thin-sectioned membrane pellets and by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Gap junctions were obtained as vesicles whose mean surface area approximated that of junctions in intact myocardial cells. About 10–20% of the vesicles were ferritin-impermeable. Approx. 125 μ g of membrane protein was obtained per 8 g of rabbit heart. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of purified gap junctions showed five major protein bands of mol.wts. 46 000, 44 000, 33 000, 30 000 and 28 500 that co-purified with the junctions. This protein composition was nearly identical with that published for gap junctions of mouse hearts, and differed markedly from the protein composition of gap junctions from non-excitable cells (lens and liver). The constancy of junctional protein composition between hearts of two different species and its non-identity with that from liver and lens suggest that, although gap-junctional structure in mammalian tissues seems to be remarkably similar by electron-microscopic techniques, junctional-channel protein composition actually varies from tissue to tissue and may be adapted to the permeability requirements of the tissue.

Gap junctions are membrane specializations that mediate cell-to-cell transport of small molecules and ions in many vertebrate and invertebrate tissues (Bennett, 1977; Loewenstein, 1978). Electrophysiological studies on mammalian heart muscle indicate that gap junctions are responsible for the behaviour of heart muscle cells as an electrical syncytium (Déléze, 1970; Page & Shibata, 1981). Gap junctions have been isolated from mammalian non-excitable tissues including liver (Culvenor & Evans, 1977; Ehrhart & Chauveau, 1977; Evans & Gurd, 1972; Finbow *et al.*, 1980; Henderson *et al.*, 1979; Hertzberg & Gilula, 1979) and lens (Dunia *et al.*, 1974; Goodenough, 1979; Takemoto & Hansen, 1981) and shown to contain a major polypeptide of mol.wt. approx. 26 000. Gap junctions from mammalian myocardium have, however, proved more difficult to purify. Recently, Kensler & Goodenough (1980) have isolated gap junctions from mouse hearts without using exogenous proteinases. These workers used the ionic detergents *N*-dodecyl-sarcosine and deoxycholate and the non-ionic detergent Tween-20 to obtain 50–150 μ g of junctional protein from 200 mouse-hearts. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the preparation showed four major protein bands of

mol.wts. 47 000, 33 500, 31 000 and 28 000 that co-purified with the junctions. Since heart muscle, unlike liver or lens, is an electrically excitable tissue, we were interested in determining whether the more complex biochemical composition found by Kensler & Goodenough (1980) in mouse cardiac gap junctions is present in gap junctions from other mammalian hearts with similar electrical properties; i.e., whether the protein compositions of junctions from hearts of different species display a tissue specificity correlated with the physiological role of cardiac gap junctions. This question arises because sequencing studies on gap junctions from liver and lens of the same mammal (the rat) indicate that their primary structures differ greatly (Nicholson *et al.*, 1980).

The small yield of purified gap-junctional membrane obtained by Kensler & Goodenough (1980) from mouse hearts seriously limits further biochemical studies of cardiac gap-junctional peptides. It therefore seemed essential to us to develop a procedure for isolation of gap junctions from the hearts of one or more large laboratory mammals in higher yields. In the present paper we describe an easier and faster method for the isolation of substantially larger amounts of gap junctions from

rabbit hearts by a modification of the method of Kensler & Goodenough (1980) that additionally avoids the use of non-ionic detergents. The results indicate that the protein composition of cardiac gap junctions from different species is tissue-specific and more complex than that for non-excitabile tissues.

An abstract of this work has been published previously (Manjunath *et al.*, 1981).

Experimental

Chemicals

Deoxycholate (sodium salt), *N*-dodecylsarcosine (97%), ferritin and all the reagents for electrophoresis were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 'Ultrapure' sucrose was from Schwartz/Mann, Orangeburg, NY, U.S.A. Protein standards for electrophoresis were bought from Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. All other reagents were of analytical grade.

Animals

New Zealand White rabbits of either sex weighing 3–4 kg were used in all the studies.

Preparation of gap junctions

Hearts were excised from rabbits under diethyl ether anaesthesia and placed in cold 1 mM-NaHCO₃, pH 8.2. All subsequent steps were carried out at 0–4°C unless otherwise specified. The procedure was usually applied to one heart but could readily be scaled up to handle up to three hearts. After trimming off the major vessels and fat, the heart tissue was minced and homogenized in 1 mM-NaHCO₃, pH 8.2, with a Virtis homogenizer (model 23, set at maximal speed for 60 s). The initial homogenate (50 ml/heart) was further homogenized with a Tissuemizer (model SDT 100 EN; Tekmar Instruments; set at maximal speed for 30 s). The homogenate was diluted to 500 ml/heart with 1 mM-NaHCO₃, pH 8.2, and filtered through eight layers of cheesecloth to remove shreds of connective tissue. The filtrate was centrifuged at 33 000 *g* for 15 min in a Sorvall SS-34 rotor. The supernatant was discarded and the pellet was rehomogenized with a Dounce homogenizer (pestle B; three to four strokes) in 300 ml of 1 mM-NaHCO₃, pH 8.2. The suspension was centrifuged at 33 000 *g* for 15 min as before and the supernatant was discarded. The resulting pellet was suspended in 200 ml/heart of a solution containing 0.6 M-KI and 6 mM-Na₂S₂O₃ in 1 mM-NaHCO₃, pH 8.2, and stirred overnight in the cold to extract the contractile proteins. The following day, the KI-insoluble material was recovered as a pellet by centrifuging the suspension at 27 000 *g* for 30 min in a Sorvall SS-34 rotor. The pellet was washed three times by re-suspension and re-

centrifugation: once with a solution of 0.6 M-KI/6 mM-Na₂S₂O₃ in 5 mM-Tris (70 ml), pH 9.0, and twice with 5 mM-Tris (300 ml each time), pH 9.0. The final pellet was suspended in 5 mM-Tris, pH 10.0, and made up to 30 ml/heart at room temperature (22°C). The suspension was homogenized briefly (Dounce pestle B; three strokes) and 30 ml of a solution of 0.6% (w/v) *N*-dodecylsarcosine in 5 mM-Tris, pH 10.0, was added to it with stirring. Stirring was continued for 10 min at room temperature. The suspension was then divided equally among three tubes, and discontinuous sucrose gradients were constructed as follows (top to bottom): (1) 20 ml of membrane suspension containing 0.3% (w/v) *N*-dodecylsarcosine; (2) 8 ml of 35% (w/v) sucrose/0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0; (3) 5 ml of 44.5% (w/v) sucrose/0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0. The preparation of the gradients was completed within 20 min at room temperature, and the tubes were centrifuged at 65 000 *g* for 60 min at 15°C in a Beckman SW 25.1 rotor. At the end of the run, the material floating at the 35%/44.5% sucrose interface was collected and diluted to 45 ml with 0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0. Of the diluted solution 15 ml was layered on top of each of three sucrose step gradients made up of 9 ml each of 35% (w/v) sucrose and 0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0, and 44.5% (w/v) sucrose and 0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0. The tubes were centrifuged at 65 000 *g* for 90 min at 15°C in a Beckman SW 25.1 rotor. The 35%/44.5% interface material was collected from the second gradient run and, after dilution with 0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0, subjected to one more gradient run exactly as before. The 35%/44.5% interface material from the final gradient run was diluted 1:1 with 0.3% deoxycholate in 5 mM-Tris, pH 10.0, and centrifuged at 106 000 *g* for 30 min at 15°C in a Beckman 40 rotor to yield the purified gap-junctional pellet, which was washed with 5 mM-Tris, pH 10.0, to remove sucrose and deoxycholate.

Isolation of non-junctional membranes contaminating the junctional pellet

The contaminating non-junctional membranes were isolated exactly as the gap junctions, except that (a) sucrose solutions of 31.5% (w/v) sucrose/0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0, and 35% (w/v) sucrose/0.3% (w/v) deoxycholate in 5 mM-Tris, pH 10.0, were employed in the discontinuous density gradients; and (b) the membranes were recovered from the 31.5%/35% sucrose interface.

Ferritin-labelling of isolated gap junctions

A purified gap-junctional pellet was prepared

EXPLANATION OF PLATE 1

Section through a pellet of the material from the interface between 31.5% (w/v) sucrose/0.3% (w/v) deoxycholate and 44.5% (w/v) sucrose/0.3% (w/v) deoxycholate

Profiles of vesicles of non-junctional membrane are seen lying between and within profiles of gap-junctional vesicles. Note (a) typical five-layered appearance of the gap-junctional membranes where the plane of section cuts across the membrane; (b) broadening of the gap-junctional membrane profile where membrane was sectioned across the face or tangentially; and (c) apparent defects in continuity of gap-junctional vesicles. The bar represents 0.2 μm .

EXPLANATION OF PLATE 2

Electron micrographs of thin sections of a gap-junctional membrane pellet and of a pellet of contaminating non-junctional membranes

(a) shows profiles of non-junctional membrane vesicles retained by 35% (w/v) sucrose/0.3% (w/v) deoxycholate. The bar represents 0.3 μm . (b) shows profiles of gap-junctional vesicles from the final fraction obtained after removal of most of the contaminating non-junctional membranes shown in (a). The bar represents 0.5 μm . (c) shows profiles of gap-junctional vesicles that were incubated with ferritin before fixation. Note that the ferritin is located between and within vesicular profiles, but that the interior of the profile at upper right appears devoid of ferritin. The bar represents 0.2 μm .

from one rabbit heart as described previously. The supernatant was carefully removed and replaced with 8 ml of 5 mM-Tris, pH 10.0, containing 200 μ g of ferritin. The tube was centrifuged at 106 000 *g* for 30 min at 15°C in a Beckman 40 rotor to yield a ferritin pellet superimposed on the gap-junctional pellet. Immediately after the run the pellet was treated in the tube with 3% glutaraldehyde at room temperature for 30 min and processed further for electron microscopy.

Electron microscopy of pellets

Membrane pellets were fixed at 20°C for 30 min by immersion in 3% glutaraldehyde buffered to pH 7.4 with 150 mM-sodium cacodylate. After fixation, the pellets were rinsed four times for 15 min at 0°C by immersion in 150 mM-sodium cacodylate, pH 7.4, then post-fixed for 20 min at 0°C with 32.8 mM-OsO₄ buffered to pH 7.4 with 50 mM-sodium cacodylate. After post-fixation, the pellets were again rinsed four times for 15 min at 0°C in 150 mM-sodium cacodylate, then processed for embedding in Epon, thin sectioning, and staining with uranyl acetate and lead by conventional procedures (Stewart & Page, 1978). The sections were examined and photographed in a Siemens Elmiskop 1A electron microscope operated and calibrated as previously described (Stewart & Page, 1978).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed in 1.5 mm thick slab gels with 5% stacking gels and 12.5% separating gels. The discontinuous buffer system described by Laemmli (1970) was used. Samples were dissolved in 2% sodium dodecyl sulphate/5% 2-mercaptoethanol at 22°C for 1 h. The following proteins and molecular weights were used as molecular-weight markers: phosphorylase *b* (mol.wt. 94 000); bovine serum albumin (67 000); ovalbumin (43 000); carbonic anhydrase (30 000); soya-bean trypsin inhibitor (20 100); α -lactalbumin (14 400).

Protein estimation

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

The end product of the isolation procedure was a pellet highly enriched in gap junctions. The typical yield of this membrane fraction was 125 μ g of protein per rabbit heart, i.e., per 8 g of starting material.

In our initial experiments we prepared gap

junctions using step gradients containing 31.5% (w/v) sucrose/0.3% (w/v) deoxycholate and 44.5% (w/v) sucrose/0.3% (w/v) deoxycholate solutions. Electron microscopy of the resulting gap-junctional pellets showed substantial contamination with non-junctional membrane (Plate 1). Attempts to remove these contaminants with 1.0 M-urea, or with the non-ionic detergents Triton X-100 (0.1–0.3%) and Tween-20 (0.3%) were unsuccessful. The gap-junctional membranes could, however, be readily separated from the contaminating non-junctional membranes by increasing the concentration of the sucrose solution from 31.5% (w/v) to 35% (w/v); under these conditions the non-junctional membranes were retained, whereas the gap junctions passed through. Plate 2(a) illustrates the appearance of the non-junctional membranes retained by 35% sucrose. This fraction consisted of single-walled vesicles of unknown origin. Plate 2(b) is a section through the gap-junctional pellet prepared with 35%/44.5% (w/v) sucrose gradients. In this thin-sectioned material, the junctional profiles were predominantly closed. Where the section cut across the vesicular membrane, the micrograph shows the pentalaminar (and occasionally heptalaminar) structure characteristic for gap junctions prepared in this way (Bennett, 1977). Where sections passed parallel to the plane of the membrane, the profile broadens and appears fuzzy; higher magnifications of such across-the-face sections show the well known subunit structure of the gap junctions.

Inspection of Plates 1 and 2(b) indicates multiple small gaps in the gap-junctional membrane profiles. These gaps could be either defects in the membrane produced by the preparative procedure (e.g., by the detergents) or areas where the vesicular membrane passes out of the plane of the section. To determine whether actual defects existed in the vesicular surface, we exposed the vesicles to ferritin before fixation. Plate 2(c) shows that most of the vesicles were penetrated by ferritin, hence defects must have existed in the vesicular membranes. However, about 10% of the total junctional vesicle profiles contained no ferritin, suggesting that some of the vesicles had intact surfaces.

We were interested in determining whether the surface areas of the isolated gap-junctional vesicles were similar to the areas of the gap junctions from intact mammalian ventricular myocardial cells *in situ*. Measurement of the perimeters of the apparently closed gap-junctional profiles gave a mean length ($\pm$ S.E.M.) of $2.50 \pm 0.05 \mu\text{m}$ ($n = 178$ profiles). This mean value was doubtless substantially smaller than the true mean 'equatorial' perimeter of the vesicles due to the well-known Wicksell (1925) effect. Recognizing that the vesicles were neither perfectly spherical nor ellipsoidal, and that the mean surface areas even of true spheres or

ellipsoids are systematically underestimated by the surface areas of their junctional profiles, we can nevertheless calculate, as a lower limit, a minimal mean vesicular surface area. The minimal mean surface area thus obtained (assuming spherical

shape) was $2.0\mu\text{m}^2$, corresponding to a minimal mean vesicle volume of $0.26\mu\text{m}^3$. The calculated surface area falls well within the range of gap-junctional areas observed in intact ventricular myocytes (Shibata *et al.*, 1980).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide gels of purified gap junctions showed five major protein bands with mol.wts. 46 000, 44 000, 33 000, 30 000 and 28 500 (Fig. 1a). Minor bands of both higher and lower molecular weights were also present, the most prominent being at mol.wts. 62 000, 55 500, 35 500 and 31 000. Although the samples were solubilized for 1 h at 22°C with 2% sodium dodecyl sulphate and 5% 2-mercaptoethanol, considerable amounts of protein remained at the top of the stacking gel and at the interface between the stacking and separating gels. The minor bands in the low-molecular-weight region may represent proteolytic products. These faint bands varied somewhat in intensity from preparation to preparation and were not altered by incorporating the proteinase inhibitors *p*-hydroxymercuribenzoate and phenylmethane-sulphonyl fluoride in the isolation media.

Fig. 1(b) shows the sodium dodecyl sulphate/polyacrylamide-gel-electrophoretic profile of the gap-junctional preparation isolated using 31.5%/44.5% (w/v) sucrose gradients. The predominantly non-junctional membranes were separated from this preparation by a single passage through a 31.5%/35% (w/v) sucrose gradient. The presumptive gap-junctional fraction [i.e., the fraction that passed through 35% (w/v) sucrose, gel B] showed major protein bands of mol.wts. 62 000, 55 500, 46 000, 35 500, 33 000, 30 000 and 28 500 respectively. The non-junctional material that was retained at the interface between 31.5% and 35% (w/v) sucrose (gel A of Fig. 1b, corresponding to Plate 2a) showed predominant bands at mol.wts. 62 000 and 55 500 and only a faint band at mol.wt. 46 000. The bands at mol.wts. 35 500, 33 000, 30 000 and 28 500, which co-purify with the gap junctions, were conspicuously faint or absent from this predominantly non-junctional material.

Discussion

Because of the relatively small content of gap junctions in the heart (Stewart & Page, 1978; Page & Shibata, 1981), the purification of substantial quantities of this specialized membrane requires a large amount of starting material. The expensive isolation procedure developed for mouse heart (Kensler & Goodenough, 1980) does not, however, meet this need, since even 200 mice do not yield junctional membrane in sufficient quantities for more

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of purified gap junctions (a) and combined gap-junctional fraction and the non-junctional membrane contaminants (b)

(a) Purified gap junctions were prepared by centrifuging *N*-dodecylsarcosine-treated membranes successively on three discontinuous density gradients made with 35% (w/v) sucrose and 44.5% (w/v) sucrose solutions containing 0.3% (w/v) deoxycholate. The sample was dissolved in 2% sodium dodecyl sulphate/5% 2-mercaptoethanol in 62.5 mM-Tris, pH 6.8, at room temperature for 1 h. It was then electrophoresed on 5% stacking and 12.5% separating gels. (b) Purified gap junctions were prepared by centrifuging *N*-dodecylsarcosine-treated membranes on discontinuous density gradients made with 31.5% (w/v) and 44.5% (w/v) sucrose solutions containing 0.3% (w/v) deoxycholate. The material floating at the interface between 31.5 and 44.5% sucrose was collected and diluted 1:1 with 0.3% (w/v) deoxycholate. The suspension was layered on 35% (w/v) sucrose containing 0.3% (w/v) deoxycholate and centrifuged at 65 000 *g* for 1 h at 15°C to obtain gap-junctional pellet (gel B). The non-junctional membranes were recovered from the sample at the 35% sucrose interface and diluted with 0.3% (w/v) deoxycholate. After layering on a 31.5% (w/v) sucrose/0.3% (w/v) deoxycholate solution, the suspension was centrifuged at 65 000 *g* for 1 h at 15°C to yield the non-junctional membrane pellet (gel A). The samples were treated exactly as in (a).

extensive biochemical and structural studies. These considerations prompted us to prepare gap junctions from rabbit hearts, in which we had previously estimated the content of gap-junctional membrane by morphometric techniques (Stewart & Page, 1978; Page & Shibata, 1981).

The application of the method of Kensler & Goodenough (1980) for mouse hearts to rabbit hearts gave a very low yield of gap junctions; the final product consisted of very small junctional fragments that were grossly contaminated with amorphous material. We were able to identify the steps responsible for these problems in Kensler and Goodenough's procedure and to modify them successfully as follows. (a) When applied to rabbit hearts, the use of KI-containing sucrose density gradients after extraction of the contractile proteins (Kensler & Goodenough, 1980) reduced the yield of the junctions because material that accumulated at the lower (non-junctional) interfaces acted as a pseudo-filter, owing to the large amount of particulate material present at this stage. Partial solubilization of the membranes with *N*-dodecylsarcosine before density-gradient centrifugation resulted in higher yields of junctional membrane. (b) We have found that pelleting of the *N*-dodecylsarcosine-resistant material by centrifugation, and resuspension of the pellet before loading it on the sucrose gradients [the procedure used by Kensler & Goodenough (1980) for mouse hearts] fragmented the junctions into small pieces. The amorphous material that contaminated the resulting junctional pellets could not be removed by treatment with the non-ionic detergents Tween-20 (0.3% w/v) or Triton X-100 (0.1–0.3%, w/v). By directly loading the *N*-dodecylsarcosine-treated membranes on sucrose gradients we obtained greater yields of much larger and more highly purified junctional fragments. (c) Although the use of non-ionic detergents did not improve either the yield or the purity of the junctions, incorporation of the ionic detergent sodium deoxycholate in the sucrose solutions effectively prevented the aggregation of membranes at the sucrose interfaces. Prolonged exposure to 0.3% deoxycholate did not alter either the electron-microscopic appearance or the gel-electrophoretic pattern of the isolated junctions. Moreover, unlike the non-ionic detergents, the ionic detergents that we have employed (*N*-dodecylsarcosine and sodium deoxycholate) can be removed by dialysis (Allen *et al.*, 1980).

The omission of the sucrose/KI gradients and loading of *N*-dodecylsarcosine-solubilized membranes directly on deoxycholate-containing sucrose gradients both reduced the time of preparation (2 days for our complete procedure, compared with 3 days for the mouse hearts used by Kensler and Goodenough). In addition, our procedure yielded

larger junctions in considerably higher amount [typically 125 µg from an 8 g heart, compared with 50–150 µg from 16 g mouse hearts (Kensler & Goodenough, 1980)]. The total yield could be further increased by scaling up the preparation to handle two or more rabbit hearts.

Properties of gap junctions isolated from rabbit hearts

Most of the gap junctions in our preparation exhibit vesicular profiles. It has been suggested (Goodenough, 1976) that isolated gap junctions form vesicles as a result of proteolysis. However, in our preparations we find minimal evidence of proteolysis as judged by the intensity of the low-molecular-weight bands on electrophoretic gels. Moreover, the mean area of the curved surface of the vesicles is roughly comparable with the area of gap junctions in intact myocardial cells, an improbable result if extensive fragmentation due to proteolysis had taken place. The formation of gap-junctional vesicles without evidence of proteolysis has also been observed in the junctions isolated from mouse hearts by Kensler & Goodenough (1980). Our experience indicated that gap junctions form vesicles only if they are isolated as large sheets of membrane; smaller fragments of junctional membrane do not vesiculate, presumably because mechanical constraints on the degree of curvature that their surfaces can undergo prevent them from forming closed structures.

Gel-electrophoretic analysis of our junctional preparations showed five major protein bands corresponding to mol.wts. 46 000, 44 000, 33 000, 30 000 and 28 500. In addition the gels yielded four distinct minor bands at mol.wts. 62 000, 55 500, 35 500 and 31 000. Comparison of the gel profiles of gap junctions and non-junctional membranes contaminating the final junctional pellet showed that bands corresponding to mol.wts. 62 000 and 55 000 were non-junctional. It was, however, unclear whether the bands corresponding to mol.wts. 35 500 and 31 000 were minor gap-junctional components or non-junctional contaminants.

The molecular weights obtained for the gap-junctional peptides in our preparations from rabbit hearts are very similar to those reported by Kensler & Goodenough (1980) for mouse hearts. Our results agree with their conclusion that the electrophoretic patterns of isolated gap junctions from mammalian hearts differ from liver and lens junctions, although the homology between different bands cannot be ruled out without sequencing.

This conclusion is also consistent with the rather surprising results of partial sequencing studies on gap-junctional proteins from rat lens and liver (Nicholson *et al.*, 1980; Hertzberg *et al.*, 1982). These studies have shown essentially no homologies

in primary structure between these two junctional proteins from liver and lens of the same animal, although both proteins have similar molecular weights and form structures with very similar morphology. It is therefore of particular interest to examine the protein composition of gap junctions from additional tissues like heart muscle. Moreover, our findings that the gel-electrophoretic pattern of rabbit heart gap junctions is essentially identical with that of mouse heart gap junctions indicates that the compositional differences between junctions from different mammals may be small compared with differences between junctions from different tissues of the same animal. That is what one might expect if gap-junctional protein composition was adapted to meet the junctional permeability requirements of particular cell types and tissues.

Our studies on isolated rabbit heart gap junctions, like other published studies on isolated gap junctions, present the problem of accounting for the multiple gel-electrophoretic bands that co-purify with the junctions. The literature on isolated gap junctions has considered this problem in terms of proteolysis and heat-induced peptide aggregation. In our experiments on rabbit cardiac gap junctions, although we have avoided heating the preparation, we nevertheless obtained proteins with mol.wts. 44 000 and 46 000.

We have considered the following four causes for the multiple bands that co-purify with rabbit cardiac gap junctions in the range of mol.wts. between 28 500 and 46 000. The causes are not mutually exclusive. They include (a) the possibility that gap-junctional peptides may be non-identical, i.e., that there may be more than one type of peptide making up the junctional channel, and (b) the possibility that heterogeneous gap-junctional peptides may reflect the presence of gap junctions originating from different cell types in heart muscle. Although gap junctions in the mammalian heart are predominantly localized to myocardial cells, they are also present in cardiac conduction tissue (Mobley & Page, 1972), vascular smooth-muscle cells (Somlyo, 1980), and vascular endothelium (Simionescu *et al.*, 1975). Other causes include (c) co-purification of non-gap junctional contaminants that may migrate electrophoretically with the junctional peptides, e.g., cytoskeletal peptides that may be components of the fascia adherens, as previously suggested by Kensler & Goodenough (1980) and (d) limited proteolytic degradation involving only small terminal segments of gap-junctional peptides. This possibility cannot be ruled out with certainty for any of the published work on the isolated gap junctions. For example, the observations by Kensler & Goodenough (1980) that the band at mol.wt. 47 000 was sometimes absent from their preparations might be explained if, in their preparations, the proteins of mol.wts.

28 000–33 000 were produced by a limited proteolytic cleavage of the proteins of mol.wts. 44 000–46 000.

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References

- Allen, T. M., Romans, A. Y., Kercret, J. & Segrest, J. P. (1980) *Biochim. Biophys. Acta* **601**, 328–342
- Bennett, M. V. L. (1977) *Handb. Physiol. Sect. 1: Nerv. Syst. Revis. Ed.* 357–416
- Culvenor, J. G. & Evans, W. H. (1977) *Biochem. J.* **168**, 465–481
- Délèze, J. (1970) *J. Physiol. (London)* **208**, 547–562
- Dunia, I., Ghosh, S., Benedetti, E. L., Zweers, A. & Bloemendal, H. (1974) *FEBS Lett.* **45**, 139–144
- Ehrhart, J. C. & Chauveau, J. (1977) *FEBS Lett.* **78**, 295–299
- Evans, W. H. & Gurd, J. W. (1972) *Biochem. J.* **128**, 691–700
- Finbow, M., Yancey, S. B., Johnson, R. & Revel, J. P. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 970–974
- Goodenough, D. A. (1976) *J. Cell Biol.* **68**, 220–231
- Goodenough, D. A. (1979) *Invest. Ophthalmol. Vis. Sci.* **81**, 1104–1122
- Henderson, D., Eibl, J. & Weber, K. (1979) *J. Mol. Biol.* **132**, 193–218
- Hertzberg, E. L. & Gilula, N. B. (1979) *J. Biol. Chem.* **254**, 2138–2147
- Hertzberg, E. L., Anderson, D. J., Friedlander, M. & Gilula, N. B. (1982) *J. Cell Biol.* **92**, 53–60.
- Kensler, R. W. & Goodenough, D. A. (1980) *J. Cell Biol.* **86**, 755–764
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Loewenstein, W. R. (1978) in *Membrane Physiology* (Andreoli, R. E., Hoffman, J. F. & Fanestil, D. D., eds.), pp. 335–356, Plenum, New York
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Manjunath, C. K., Goings, G. E. & Page, E. (1981) *J. Cell Biol.* **91**, 100a
- Mobley, B. A. & Page, E. (1972) *J. Physiol. (London)* **220**, 547–563
- Nicholson, B. J., Hunkapiller, M. W., Hood, L. E. & Revel, J. P. (1980) *J. Cell Biol.* **87**, 200a
- Page, E. & Shibata, Y. (1981) *Annu. Rev. Physiol.* **43**, 431–441
- Shibata, Y., Nakata, K. & Page, E. (1980) *J. Ultrastruct. Res.* **71**, 258–271
- Simionescu, M., Simionescu, M. & Palade, G. E. (1975) *J. Cell Biol.* **67**, 863–885
- Somlyo, A. B. (1980) *Handb. Physiol. Sect. 2: Vascular Smooth Muscle*, 33–68
- Stewart, J. & Page, E. (1978) *J. Ultrastruct. Res.* **71**, 258–271
- Takemoto, L. J. & Hansen, J. S. (1981) *Biochem. Biophys. Res. Commun.* **99**, 324–331
- Wicksell, S. D. (1925) *Biometrika* **17**, 84–99