GAP JUNCTION STRUCTURES

VI. Variation and Conservation in Connexon Conformation and Packing

LEE MAKOWSKI
Department of Biochemistry, Columbia University, New York, New York 10032

D. L. D. CASPAR, W. C. PHILLIPS, AND T. S. BAKER
Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254

D. A. GOODENOUGH
Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT Correlation of structural changes in isolated gap junctions with the mechanism of channel gating is complicated by the effects of isolation procedures and the lack of a direct functional assay. The effect of variations in the isolation procedure are examined by comparison of the structures of gap junctions isolated by different protocols. X-ray diffraction data from over two hundred specimens are compared to provide a basis for identification of invariant aspects of the connexon structure and variable properties related either to functional switching or experimental modifications. We discuss the relationship between subunit tilt, lattice symmetry and packing, and membrane curvature and demonstrate that membrane curvature may be a natural consequence of the structure of the connexons and the patterns of interactions between them.

INTRODUCTION

Gap junctions are membrane junctions consisting of an array of morphological units that span the plasma membranes of two adjoining cells and the ~30-Å wide extracellular gap between the membranes (Goodenough and Revel, 1970; Caspar et al., 1977; Makowski et al., 1977). Each of the morphological units is composed of twelve copies of the connexin molecule arranged as a pair of hexamers called connexons, one connexon associated with each membrane. A gated aqueous channel extends along the center of the morphological units, connecting the cytoplasms of the two cells and providing a pathway for the transfer of ions and other small molecules between the cells (Bennett, 1973; Bennett and Goodenough, 1978). Changes in connexon structure possibly associated with channel gating have been observed by x-ray diffraction (Makowski et al., 1977; 1982) and three-dimensional image reconstruction from electron micrographs (Unwin and Zampighi, 1980).

Detailed structural studies have concentrated on gap junctions from mouse or rat liver which can be isolated in the form of large two-dimensional crystalline lattices suitable for analysis by x-ray diffraction and Fourier microscopy. Electron micrographs of gap junctions in tissues frozen rapidly to 4°K from the living state (Raviola et al., 1980) show that in gap junctions from mouse liver, the connexons are closely packed but not in crystalline arrays. This indicates that the gap junction lattices studied by electron microscopy and x-ray diffraction are formed as a consequence of the preparative procedure. Treatments known to uncouple cells connected by gap junctions may result in increases in the packing density of membrane particles and curvature of the junction membranes (Peracchia, 1977; Baldwin, 1977; 1979; Raviola and Raviola, 1978; Raviola et al., 1980). Thus the formation of junction lattices suitable for detailed structural studies may be affected by the physiological state of the junction. It is possible that a correlation exists between changes in connexon structure associated with channel gating and changes in lattice packing and membrane curvature. Changes in the lattice packing associated with structural changes have been observed (Makowski et al, 1977; Unwin and Zampighi, 1980). In this paper we demonstrate that membrane curvature may be a natural consequence of gap junction structure when the connexons are closely packed in the junction lattice.

It is difficult to assess the significance of all the modifications and variations in the isolated junctions induced by the preparative procedures. During isolation, the junction protein may be subject to proteolysis by endogenous enzymes; the connexon conformation may be changed by the action of physiological effectors; and the lipid composition and lattice constant of the junction plaques may be altered by the action of detergents. Identification of the

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factors responsible for structural changes requires controlled pairs of experiments in which only a single variable is changed. However, structural variability due to unidentified factors may obscure the effect of some variables and changes in some important experimental variables may result in a disordered junction lattice difficult to characterize structurally. Comparison of x-ray diffraction data accumulated during the past decade from specimens prepared by a variety of methods provides a basis for identifying invariant aspects of the junction structure and the accessible range of structural variation related either to functional switching or to experimental procedures.

Gap Junction Structure

The drawing in Fig. 1 summarizes much of the information available about gap junction structure. The morphological units of the gap junction lattice consist of 12 copies of the connexin molecule arranged as a pair of hexamers (Makowski et al., 1977). The molecular weight of connexin from mouse liver appears to be ~26,000 (Henderson et al., 1979) and from rat liver, ~28,000 (Hertzberg and Gilula, 1979; Nicholson et al., 1981). As illustrated in Fig. 1, the connexin molecules in each connexon are related to one another by a sixfold rotation axis and the two connexons making up a morphological unit are related by twofold axes perpendicular to the sixfold axis and in the center of the gap. Thus, each morphological unit has point group symmetry 622.

The intercellular channel has an average radius of ~10 Å along most of its length. At the cytoplasmic surfaces of the membranes the channel is broader. It appears to have a funnel-shaped entrance that narrows from an average radius of 25 Å at the level of the cytoplasmic surface of the bilayer to ~10 Å radius at a point 15 Å below the bilayer surface (Makowski et al., 1983).

The gap junction protein appears to be divided into two domains (Makowski et al., 1983). One domain, with a

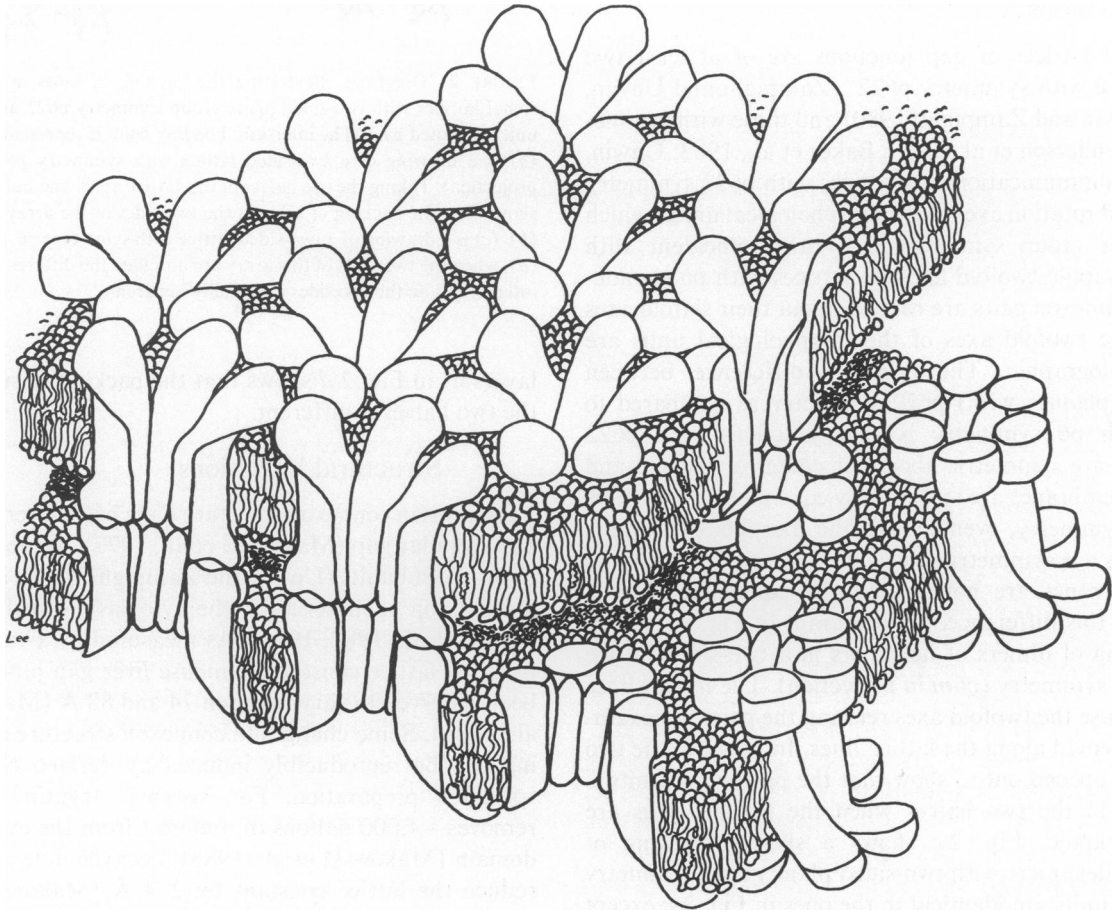

FIGURE 1 Drawing of the structure of gap junctions isolated from mouse liver as deduced by x-ray diffraction. The gap junction units are made up of twelve copies of the connexin molecule arranged into two hexamers (connexons), one associated with each membrane. Each connexin molecule is divided into two domains. The trans-membrane domain spans one bilayer and half of the extracellular gap. The cytoplasmic domain is tightly associated with the lipid polar head groups on the cytoplasmic surface of the membrane. An aqueous channel extends along the center of the connexons. At the cytoplasmic surface of the membrane the channel is ~25 Å in radius, narrowing to ~10 Å radius ~15 Å inside the membrane. One of the gating structures responsible for the closing of the channel is apparently located at the base of the funnel-shaped opening, ~50 Å from the center of the gap.

molecular weight of ~15,000, spans the bilayer and half of the gap and is contained largely within a radius of 30 Å from the sixfold axis. Three-dimensional reconstructions of negatively stained junctions (Unwin and Zampighi, 1980) and three-dimensional electron-density maps calculated from x-ray diffraction data[1] indicate that this domain is tilted 5–20° relative to the membrane perpendicular. Within the bilayer, this transmembrane domain appears to have a high proportion of β-sheet conformation with the strands of the β-sheet running approximately parallel to the membrane surfaces (Makowski et al., 1982). The second domain is somewhat smaller and occupies the cytoplasmic surface of the gap junction membrane. It appears to be relatively labile, being sensitive to digestion by trypsin (Makowski et al., 1983) and perhaps other proteases. This cytoplasmic domain is closely associated with lipid polar head groups and extends from distances of >20 Å from the sixfold axis, forming a funnel-shaped entrance to the narrow transmembrane channel.

Symmetry

Hexagonal lattices of gap junctions are of at least two types; those with symmetry p622[1] (Zampighi and Unwin, 1979; Unwin and Zampighi, 1980) and those with symmetry p6 (Henderson et al., 1979; Baker et al., 1983; Unwin, personal communication). In lattices with p622 symmetry the twofold rotation axes of the morphological units (which have point group symmetry 622) are coincident with crystallographic twofold axes. In lattices with p6 symmetry, the connexon pairs are rotated about their sixfold axes so that the twofold axes of the morphological units are noncrystallographic. The principal difference between (junction plaques with) p622 symmetry as compared to those with p6 symmetry is that junctions with p622 symmetry are symmetric about the center of the gap and the two membranes are structurally equivalent. In a lattice with p6 symmetry, even though the morphological units themselves are symmetric about the center of the gap, the two membranes are not structurally equivalent. Fig. 2 illustrates this difference. The diagram in Fig. 2 *a* shows the packing of dimers of hexamers in a two-sided lattice with p622 symmetry (p6*m* in projection). The mirror lines arise because the twofold axes relating the pairs of hexamers are oriented along the lattice lines. In Fig. 2 *b*, the two layers are opened out to show that the packing of units is the same in the two halves when the twofold axes are crystallographic. Fig. 2 *c* shows a skewed packing of dimers of hexamers with two-sided plane group symmetry p6. These units are identical to the ones in Fig. 2 *a* except that they have been rotated in the lattice so that their twofold axes are noncrystallographic. Opening out the two layers as in Fig. 2 *d* shows that the packing of subunits in the two halves is different.

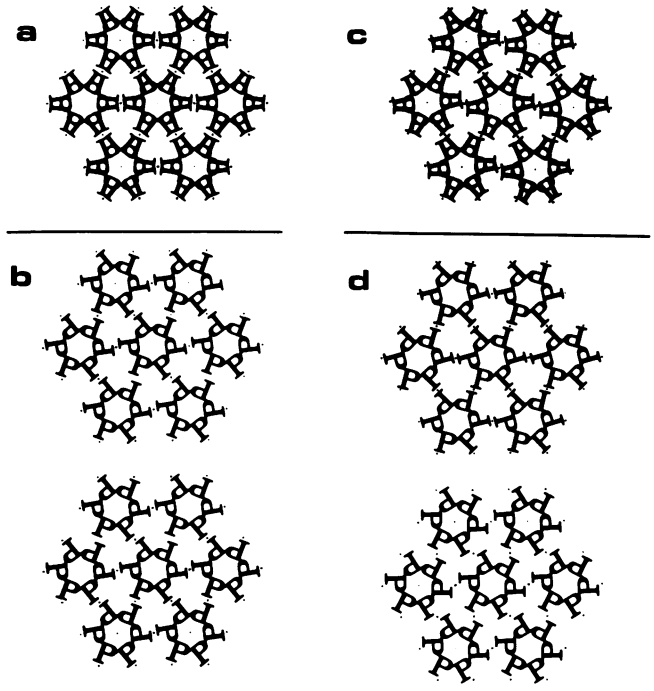

FIGURE 2 Diagrams illustrating the packing of units in two-dimensional lattices with two-sided plane group symmetry p622 and p6. Each unit is denoted by *P*. The inter-unit bonding point is represented by a dot. (*a*) is a drawing of a two-sided lattice with symmetry p622 (p6m in projection). Taking the two halves of this lattice apart and unfolding them shows that the packing of units in the two sides of the array is the same (*b*). (*c*) is a drawing of a two-sided lattice with symmetry p6. As shown in (*d*), when the two sides of this array are unfolded the different patterns of interactions on the two sides are readily apparent.

Structural Variations

Variations in connexon structure have been observed in the extracellular gap (Makowski et al., 1977), in the degree of tilt of the subunits (Unwin and Zampighi, 1980) and in the distribution of material on the cytoplasmic surface (Makowski et al., 1982, 1983). As measured by x-ray diffraction, the lattice constant of mouse liver gap junctions has been observed to vary between 74 and 88 Å (Makowski et al., 1982). Some changes in connexon structure and packing can be reproducibly induced by defined changes in specimen preparation. For instance, trypsin treatment removes ~4,000 daltons of material from the cytoplasmic domain (Makowski et al., 1983); deoxycholate appears to reduce the lattice constant by 2–4 Å (Makowski et al., 1982); and dialysis in distilled water for several days alters the tilt of the connexon subunits (Unwin and Zampighi, 1980). However, structural differences are also seen among specimens prepared by identical protocols (Makowski et al., 1977). Lattice constant differences of 2–4 Å are commonly observed among specimens prepared according to the same procedure. This is comparable to the

[1]Makowski, L., D. L. D. Caspar, D. A. Goodenough, and W. C. Phillips. 1983. Three-dimensional structure of gap junctions. Manuscript in preparation.

consistent differences seen, for instance, between controlled pairs of specimens isolated with and without deoxycholate. When the magnitude of structural variation is comparable to the structural change induced by a change in protocol, the positive identification of a structural effector requires the repeated observation of consistent structural changes between controlled pairs of specimens.

SPECIMEN PREPARATION

Comparison of Isolation Procedures

Details of the isolation procedure influence the extent of proteolytic cleavage of the junction protein, the regularity of the hexagonal junction lattice, and the curvature of the junction membranes. Because there is no functional activity that can be assayed in isolated junctions, the criteria for satisfactory purification have been chemical homogeneity and morphological regularity. Isolation of gap junctions from mouse or rat liver involves initial isolation of a plasma membrane fraction in low-ionic–strength carbonate buffer followed by detergent treatment to solubilize nonjunctional membranes (Goodenough, 1974; Henderson et al., 1979; Hertzberg and Gilula, 1979; Fallon and Goodenough, 1981; Nicholoson et al., 1981). Most x-ray diffraction experiments have used junctions lightly fixed with gluteraldehyde. No structural differences between fixed and unfixed specimens have been identified by x-ray diffraction. However, because use of gluteraldehyde may result in a closing of the membrane channel (Spray et al., 1981), it is possible that most of the junctions we have studied by x-ray diffraction have been in the closed, high resistance state.

The initial x-ray diffraction studies (Caspar et al., 1977; Makowski et al., 1977) used mouse liver gap junctions isolated by a procedure (refered to below as protocol I) requiring exogenous collagenase and hyaluronidase to remove contaminants (Goodenough et al., 1974). In these preparations, the apparent molecular weight of connexin in SDS was ~20,000 daltons. Interpretation of electron density profiles calculated from x-ray diffraction patterns from these junctions (Makowski et al., 1977) indicated that there were 23,000–28,000 daltons of protein per connexin molecule present in these junctions. Comparison of these results with the 26,000–28,000 molecular weight of unproteolyzed connexin suggests that although the junction protein was nicked by the action of proteases, most of the junction protein remained associated with the isolated junction plaques. Lattice constants of gap junctions isolated by this procedure varied from 80–88 Å. Treatment of these isolated junctions with trypsin led to the formation of junction vesicles with sharply curved surfaces (Goodenough, 1976) and very highly ordered crystalline lattices (Makowski et al., 1977).

A second protocol (protocol II) was developed (Fallon and Goodenough, 1981) that utilized the detergent Brij 58 and required no exogenous proteases. The apparent molecular weight of connexin in junctions isolated by this procedure remained 21,000 daltons. Interpretation of electron density profiles calculated from x-ray diffraction patterns from these specimens (Makowski et al., 1983) indicates a total protein mass of ~24,000 daltons per connexin molecule. Lattice constants of junctions isolated by variations of this protocol varied from 74 to 88 Å. Treatment with trypsin removed ~4,000 daltons of protein mass per connexin molecule (Makowski et al., 1983) but no increase in lattice order or membrane curvature resulted from this treatment.

Fig. 3 shows electron-density profiles of gap junctions isolated by protocol I and protocol II. The two profiles are very similar in most features. The electron density profile of the specimen isolated by protocol I (---) has a slightly broader bilayer with slightly less density projecting into the cytoplasm. These features may correspond to differences in lipid composition and degree of proteolysis in the two specimens. Recent x-ray diffraction studies of unproteolyzed gap junctions isolated from rat liver have demonstrated that except for differences near the cytoplasmic surface the membrane profile is not substantially altered by the proteolysis.

RESULTS

Morphological Regularity

Among junction plaques in a single specimen a high degree of morphological regularity and homogeneity is usually observed. Fig. 4 is an x-ray diffraction pattern from a specimen of gap junctions isolated by protocol II. The lattice constant of this specimen is 78 Å and the lattice is very highly ordered, with equatorial lattice sampling being observable to at least 9 Å spacing. This indicates that most if not all of the junction lattices in the specimen are highly ordered with almost identical lattice constants. Along the meridian it is possible to see continuous diffraction to at least 10 Å spacing. This indicates that the pair of membranes is well-ordered in the direction perpendicular to

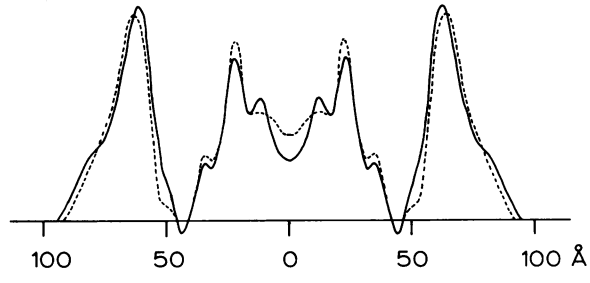

FIGURE 3 Comparison of the electron density profiles of specimens isolated by protocol I (---) (specimen E153 [cf. Makowski et al., 1977]) and by protocol II (—) (specimen I150 [cf. Makowski et al., 1983]). The two electron density profiles are very similar except for small differences in the width of the bilayers and the distribution of density on the cytoplasmic surfaces of the membranes. Both profiles were calculated using meridional data extending to 11 Å spacing.

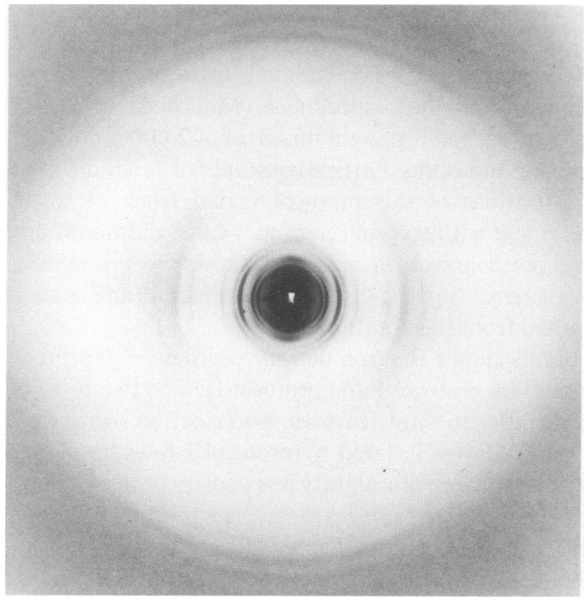

FIGURE 4 X-ray diffraction pattern from isolated mouse liver gap junctions (specimen L68). The meridian extends vertically above and below the center of the pattern and the equator to the right and left of the center. Sharp reflections along the equator index on a hexagonal lattice with lattice constant of 78 Å. Continuous diffraction along the meridian arises from the electron density contrast in the direction perpendicular to the membrane surfaces and can be used for the calculation of an electron density profile similar to those in Fig. 3. Strong diffraction from the β-sheet conformation can be seen at ~4.7 Å spacing along the meridian at the top and bottom of this diffraction pattern.

their surfaces with the pair separation varying by not more than a few angstroms throughout the specimen. Centered at about 4.7 Å spacing on the meridian there are three to five sharp diffraction fringes. This has been interpreted as arising from β-sheet conformation in the connexin protein subunits (Makowski et al., 1982). A spacing of about 1/60 Å between fringes is consistently observed. This modulation arises from the regularity on spacing and extent of the β-sheet domains. The sharpness of these fringes indicates that the β-sheet domains in the two connexons making up a morphological unit are within about an ångström or less of being the same distance apart in all the junctions of the specimen.

Correlation of Lattice Constant with Membrane Pair Separation

Changes in the conformation of the gap junction protein were observed within the extracellular gap using x-ray diffraction from gap junctions isolated by protocol I (Makowski et al., 1977). Changes in the lattice constant of the hexagonal junction lattice were correlated to changes in the membrane pair separation as measured by the position of the third zero of intensity of meridional diffraction in these specimens. Although potentially affected by other variations in membrane structure, the position of this zero of intensity is a sensitive measure of the center-to-center separation of the two membranes making up the junctional plaque. The center-to-center separation between membranes is roughly 3/2 D, where D is the spacing of the zero (in $Å^{-1}$) (see Kirschner and Caspar, 1972). As the lattice constant decreased from 87 to 82 Å, the apparent membrane pair separation decreased from ~86 to 82 Å. This decrease in pair separation appears to be a result of a decrease in the width of the extracellular gap while the widths of the bilayers remained constant. As gap width decreased the cross-sectional area of the protein in the gap increased, so that the volume of protein in the gap remained unchanged. Although variations in protocol I were attempted, no experimental variable was identified as responsible for the observed structural variations.

The position of the third zero of meridional intensity is plotted in Fig. 5 as a function of lattice constant for 33 specimens isolated by variations of protocol I (•) and 198 specimens isolated by variations of protocol II (○). The mean zero position for specimens isolated by protocol I was 0.01794 ± 0.00018 $Å^{-1}$ (95% confidence limits) with a standard deviation of 0.00050 $Å^{-1}$. The samples appear to exhibit a decrease in zero position with increasing lattice constant. The mean zero position of specimens with lattice constant <84.0 Å is 0.01284 ± 0.00024 $Å^{-1}$. The mean for specimens with a lattice constant of 85.0 Å or greater is 0.01764 ± 0.00031 $Å^{-1}$. For this relatively small sample of specimens the data indicate a significant correlation of meridional zero position with lattice constant.

For specimens isolated by variations of protocol II no such correlation is observed. No significant variation in the mean position of the third meridional node is observed for any lattice constant between 74 and 88 Å. For 198 specimens, the mean is 0.01768 ± 0.00006 $Å^{-1}$. Comparison of these figures with those calculated from protocol I would indicate that the two samples are representative of different populations. However, examination of Fig. 5 suggests that the specimens isolated by protocol I may be a subpopulation of the population represented by the sample of specimens isolated by protocol II. Inevitably, small samples may occasionally exhibit a statistically significant correlation that does not, in fact, exist in the sampled

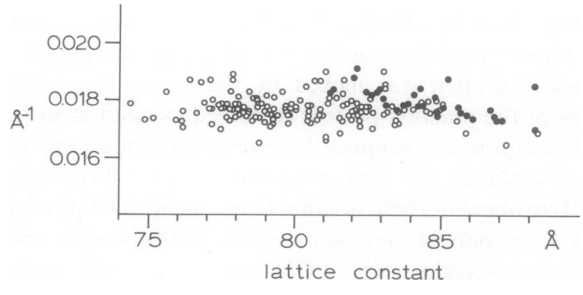

FIGURE 5 A plot of the position of the third zero of meridional intensity as a function of lattice constant. • indicate specimens isolated by variations in protocol I and ○ indicate specimens isolated by variations in protocol II.

population (e.g., Diaconis and Efron, 1983). It is possible that the apparent correlation of lattice constant and membrane pair separation evident in the positions of solid circles (•) in Fig. 5 is a sampling artifact. However, the differences in isolation protocol suggest that the two populations are different and a statistical analysis of the data demonstrates that it is unlikely that the populations are the same. Both samples represent subpopulations of the structural states accessible to isolated mouse liver gap junctions. Distribution of membrane pair separation (measured from third meridional node position) is approximately Gaussian with a standard deviation of ~2.8% independent of the lattice constant for the 19% range of variation from 74 to 84 Å.

Structural Variability

The distribution of lattice constants and apparent membrane pair separations represented by the data from 231 specimens in Fig. 5 are indicative of real structural variations. Errors in measurement of the position of the third meridional node and lattice constant are ~1%. The standard deviation in the position of the meridional node for the 231 specimens is 2.8%, which is significantly greater than the experimental errors. The variation in these measurements, therefore, indicates significant differences in membrane pair separation. The data in Fig. 5 indicate a variation in membrane pair separation from about 80 Å to over 90 Å. These variations may be due not only to movement of the lipid bilayers but also to changes in connexon structure or lipid composition. Some of the structural variability apparent in this data arose from controlled changes in specimen preparation made in attempts to improve specimen orientation or designed to test the effect of particular treatments on gap junction structure. However, differences between specimens prepared by repetition of the same procedures were often as large or larger than the differences between pairs of specimens from the same isolation that had been treated differently.

It is difficult to identify the causes of structural variations among specimens prepared by nominally identical procedures. The quality of x-ray diffraction patterns such as that in Fig. 4 demonstrates the structural homogeneity of single specimens and indicates that different specimens prepared following as closely as possible the same procedures may not have the same structures. Differences in lattice constant are indicative of differences in lipid content, which may be induced by slight variations in detergent treatment. Differences in the position of the third meridional node most likely indicate some change in connexon structure leading to change in membrane pair separation. The data plotted in Fig. 5 represent the range of structures accessible to gap junctions isolated from mouse liver. They provide a context in which to assess the statistical significance of small structural variations observed between controlled pairs of specimens isolated with a difference in one experimental variable.

Treatments that gave rise to statistically significant structural changes consistently in several experiments included digestion with trypsin; the addition of tannic acid; and the addition of thorazine. Trypsin caused a change in the electron density distribution at the cytoplasmic surface, but no consistent change in the lattice constant (Makowski et al., 1983). Both tannic acid and thorazine appeared to increase the lattice constant slightly and to alter the membrane profile. Addition of either deoxycholate or Brij 58 to the isolation protocol caused a decrease in lattice constant of 2–4 Å, presumably by removal of lipid. The presence of EGTA in the initial homogenization caused a rearrangement of mass on the cytoplasmic surface. This may have been caused by a conformational change in the protein or by a change in the degree of proteolysis. In single experiments addition of bromelain and of imidazole was correlated to structural changes large compared with variations among specimens prepared by the standard procedures. No consistent significant structural changes were observed on addition of $BaCl_2$, NaCl, Ca^{++}, $LaN0_3$, $HgNO_3$ or acetone.

Membrane Curvature is a Natural Consequence of Connexon Structure

Gap junction plaques that are curved, annular, or vesicular have been observed under many conditions. They appear to be a common morphological feature of some tissues (see e.g., Bennett and Goodenough, 1978). It has been suggested (Peracchia, 1977; Raviola and Raviola, 1978; Raviola et al., 1980) that membrane curvature may be a consequence of uncoupling (closing of the junction channels) during preparation of tissues for ultrastructural studies. Usually curvature of the junction appears to be accompanied by a decrease in the center-to-center packing of connexons in the lattice (Bennett and Goodenough, 1978).

The symmetric nature of the gap junction structure and physiology appeared to be inconsistent with the generation of membrane curvature. Junction curvature implies nonequivalence of the two membranes making up the junction. Inasmuch as the junction morphological units are made up of two equivalent halves—two hexamers related by twofold axes in the center of the gap—it seemed unlikely that their structure could lead directly to membrane curvature. However, a structural asymmetry between the two junction membranes is generated when the 622-point group symmetry of the morphological units is not reflected in the symmetry of the gap junction lattice. As shown in Fig. 2, when the gap junction lattice symmetry is p6, the packing of connexons in the two membranes is nonequivalent.

Consider the photographs of the model in Fig. 6. These show planar lattices of morphological units with lattice symmetries p622 and p6. When there are no strong forces tending to push the units closer together the lattice will

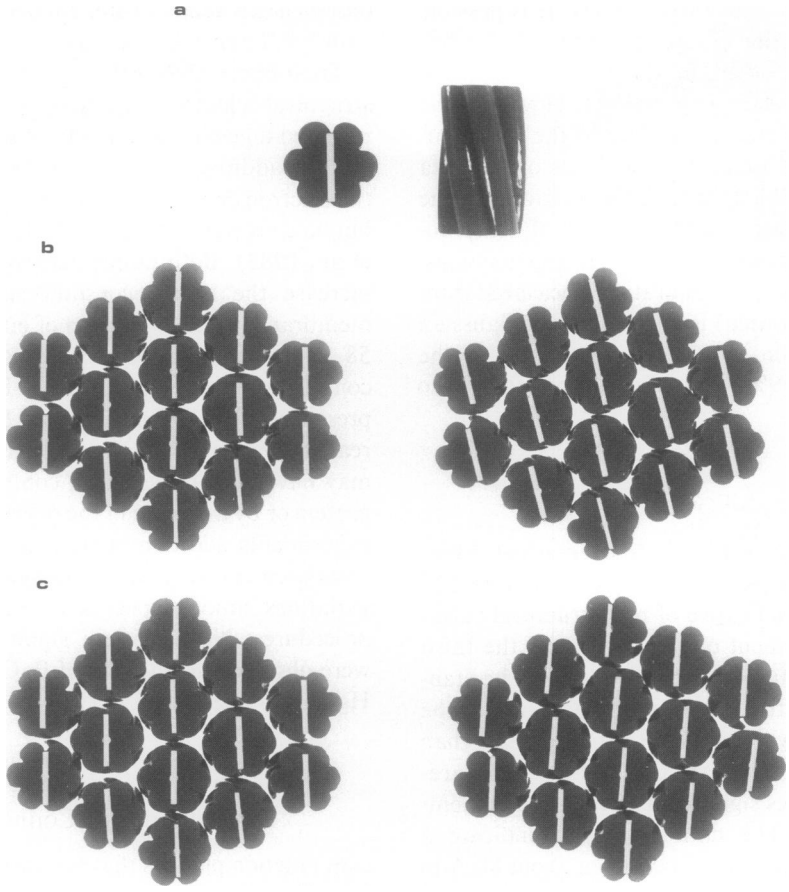

FIGURE 6 Lattices with symmetry p6 and p622. The model morphological units *a* were chosen to have proportions and tilt of subunits similar to the gap junction units. White lines correspond to the direction of one of the twofold axes of the units present at the center of the units (corresponding to the center of the gap). The lattices in *b* are arranged with p622 symmetry (*left*) and p6 symmetry (*right*). Turning these lattices over *c* shows that the packing of subunits is the same on the two surfaces of the lattice with p622 symmetry but different on the two surfaces of the lattice with p6 symmetry (cf. Fig. 2).

remain approximately planar even when its symmetry is p6. When a force acts to push the morphological units closer together it is possible to decrease the surface area of both membranes only so far. After that, the area of one membrane can be decreased only at the expense of some increase in the area of the other membrane. The result is a bistable system in which the gap junction surface is curved in one of two possible directions, either one being more stable than a flat membrane. This is demonstrated in the photographs of the model in Fig. 7.

In Fig. 7, the model morphological units are being pushed together by an external force (applied by elastic bands). The twisting of the subunits around their sixfold axes prevents close packing of both surfaces. One surface packs very tightly as seen in Fig. 7 *a*; the other (shown in Fig. 7 *b*) is much more open. This differential packing results in a curvature of the model as seen in the edge-on view in Fig. 7 *c*.

The nature of the force leading to the close packing of connexons and causing membrane curvature is not obvious. Uncoupling agents may induce membrane curvature by a direct effect on connexon conformation leading to both the closing of the connexon channel and an increase in the affinity of connexons for one another. However, close packing of the units giving rise to membrane curvature could also be caused by indirect effects mediated through other cellular systems and leading to a decrease of the gap junction plaque area.

SUMMARY

Gating of the gap junction channel responsible for control of intercellular communication is accomplished by changes of connexon structure presumably triggered by the interaction of physiological effectors with the connexons. Correlating structural changes in isolated junctions with the gating mechanism is complicated by the effects of preparative procedures and the lack of a direct functional assay. Comparison of the x-ray diffraction and electron microscope data we have accumulated over the past decade from specimens prepared by a variety of methods provides a basis for identifying invariant aspects of the gap junction

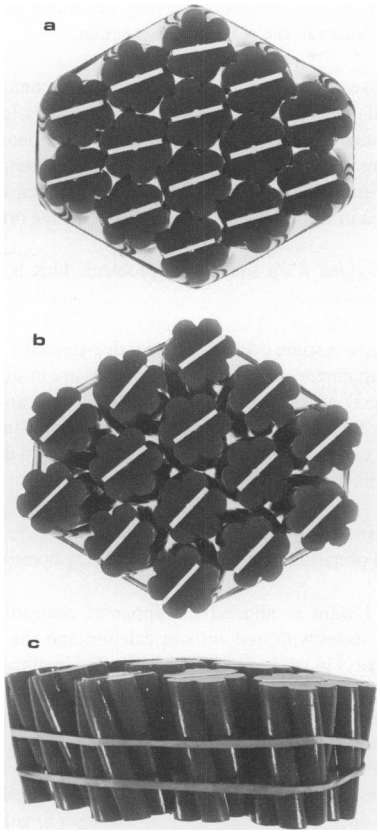

FIGURE 7 Curved lattices produced by close packing of symmetric morphological units. The area of the lattice is minimized by the pressure of two elastic bands. The top *a* and bottom *b* surfaces of the curved lattice have very different packing densities. The side view *c* shows the curvature of the lattice induced by the close packing of model units.

structure and variable properties related either to functional switching or experimental modifications.

Changes in connexon structure appear to affect lattice packing and symmetry and membrane curvature. Curvature of membrane pairs connected by morphological units that are symmetric about the center of the gap implies different packing of the connexons in the two membranes of the junction. This occurs in flat junctional membranes with p6 symmetry. In a closely packed membrane lattice the tilting of protein subunits in the connexons may give rise to membrane curvature.

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