Structure of Chicken Annexin V at 2.25-Å Resolution[†,‡]

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ABSTRACT: The crystal structure of chicken annexin V has been solved by molecular replacement and refined at 2.25 Å. The final *R* factor is 19.7% with good geometry. The chicken annexin V structure is very similar to the human annexin V structure, with four similar domains each containing five helices. The structure includes three calcium ions in domains I, II, and IV, each bound by the characteristic K-G-X-G-T-(38 residues)-D/E motif. In view of the structural similarity between human and chicken annexin V, we suggest that they have a common vital function which developed early in evolutionary history.

The annexins are a family of widely distributed calcium-dependent phospholipid-binding proteins [for reviews, see Geisow et al. (1987), Klee (1988), Burgoyne and Geisow (1989), and Römisch and Paques (1991)]. At least 12 annexins have so far been identified in a wide range of animals, as well as plants and simple eukaryotes.

The complete amino acid sequences of annexins I–XI and intestine-specific annexin have been determined. Each annexin consists of a unique N-terminal tail followed by a core of four (or eight in the case of annexin VI) repeats of a highly conserved 70–80 amino acid sequence, suggesting that they have evolved via gene duplication (Barton et al., 1991). Unlike other calcium-binding proteins, such as troponin C and calmodulin, the annexins do not contain the classical E–F-hand calcium-binding motif (Kretsinger, 1987) and as such constitute a distinct family of calcium-binding proteins. Different annexins from the same species share approximately 50% amino acid similarity, while the same annexin from different mammalian species shows around 98% amino acid identity. The relationships of the primary sequences of the annexins to one another suggest a structural basis for their common and unique characteristics.

Annexins are found in high concentrations [up to 2% of cell protein (Schlaepfer & Haigler, 1990)] in numerous cell types within a given organism. A number of functions have been attributed to the various members of this family, including a role in calcium-dependent exocytosis (Drust & Creutz, 1988; Ali et al., 1989), regulation of membrane–cytoskeleton interactions (Gerke & Weber, 1984), anticoagulant activity (Hauptman et al., 1989), inhibition of both phospholipase A_2 (Wallner et al., 1986; Davidson et al., 1987) and protein kinase C (Schlaepfer et al., 1992), and as calcium-specific voltage-gated channels (Burns et al., 1989; Rojas et al., 1990; Huber et al., 1992). The physiological significance of these activities is not yet clear.

Annexin V is a 36 000-Da member of the family. The complete cDNA sequences of both the human (Funakoshi et al., 1987; Iwasaki et al., 1987) and the chicken (Fernandez et al., 1988, 1990) proteins have been determined. Chicken annexin V (also previously known as anchorin CII) was originally identified as an extracellular glycosylated collagen-binding protein (Mollenhauer & von der Mark, 1983). However, annexin V lacks a signal sequence, and although a proportion of the protein may be secreted (Christmas et al., 1991), the majority of the protein appears to be intracellular.

The only annexin structure to have been solved by X-ray crystallography so far is that of human annexin V (Huber et al., 1990a,b, 1992). We describe here the refined crystal structure of the trigonal form of chicken annexin V and compare it with the refined human annexin V structure.

EXPERIMENTAL PROCEDURES

Chicken annexin V was isolated and crystallized using the method of Boustead et al. (1991). Trigonal crystals of chicken annexin V, space group *R*3, were grown using the vapor diffusion method from a 10 mg mL^{-1} protein solution in 20 mM Tris-HCl (pH 8.0). The precipitant contained 2.1 M ammonium sulfate in 20 mM Tris-HCl (pH 8.0), with 1 M $CaCl_2$ added to give a final calcium concentration of 10 mM. The crystals have unit cell dimensions of $a = b = 99.4$ Å, $c = 96.2$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$ with one molecule per asymmetric unit, giving an estimated solvent content of 52%. This crystal form appeared to be isomorphous to one of the crystal forms of human annexin V grown under similar conditions (Huber et al., 1990a). Human and chicken annexin V sequences are 78% identical.

Diffraction data were measured to 2.25-Å resolution from one annexin crystal measuring 0.20 × 0.15 × 0.10 mm using a Siemens X-100A area detector mounted on a Rigaku RU-200B rotating anode X-ray source operating at 45 kV and 60 mA. A total of 37 651 observations were measured, and the data were processed using the program XDS (Kabsch, 1988) to yield 16 189 unique reflections. The merging *R* factor [$R_{merge} = \Sigma(\|I\| - |I_{av}\|)/\Sigma|I_{av}|$] for the 90% complete data set was 4.5%.

The structure was solved by molecular replacement using the full coordinates of human annexin V at 2.1 Å (Huber et al., 1990b) as a search object. These coordinates were translated so that their center of gravity was at the origin. The rotation and translation calculations were performed using

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[‡] The coordinates of chicken annexin V have been deposited with the Protein Data Bank and assigned the code 1ALA.

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the PROTEIN crystallographic package (Steigemann, 1974). Cross-rotation searches were performed in Patterson space with the Patterson maps calculated from 3- to 8-Å resolution. The limits were chosen to omit low-resolution terms, including a large solvent contribution, and high-resolution terms influenced by the fine structural details. The initial rotational search (±90° in steps of 5° for ψ, θ, and ϕ) produced an orientation with the highest correlation coefficients at the origin. A finer search around the origin (±3° in 1° steps for ψ, θ, and ϕ) produced a peak at (0.0, −1.0, −0.5) having a solution 9.1 σ above background (the next highest peak in the same solution was 7.1 σ). Translational searches with and without the rotation applied were carried out using data between 3- and 8-Å resolution. The best solutions had values of 26.3σ and 25.5σ above background for rotated and unrotated coordinates, respectively, in positions corresponding to the original location of the human annexin coordinates before they were translated, so that their center of gravity was at the origin. These rotational and translational searches confirmed that the human and chicken annexin V crystals were isomorphous.

Because the human and chicken systems proved to be isomorphous, the human coordinates were used in their original location in the cell. The initial R factor [$R_{factor} = \sum(||F_{obsd}| - |F_{calcd}||)/\sum|F_{obsd}|$] calculated using the human annexin coordinates with calcium ions and water molecules removed was 40% for all data between 8 and 2.5 Å.

The chicken annexin V structure was refined using restrained least-squares techniques implemented in EREF (Jack & Levitt, 1978) (the first four cycles) and PROLSQ (Hendrickson, 1985) (43 cycles). The refinement was punctuated by four manual interventions using FRODO (Jones, 1978) on an Evans and Sutherland PS330 graphics system. The manual interventions were used to incorporate the chicken amino acid sequence, rebuild sections of the model, and introduce calcium ions and water molecules as the refinement progressed.

RESULTS

Electron density maps were first calculated after four cycles of refinement. Comparison of these maps with the chicken annexin V sequence immediately indicated that we had crystallized annexin V and not annexin IV as we previously reported (Boustead et al., 1991). A number of examples served to confirm that we were dealing with annexin V. For instance, residue 12 is a phenylalanine in all of the known annexin V sequences, while the corresponding residue in annexin IV sequences is an alanine. We clearly had electron density for an aromatic residue at this position (Figure 1). The refinement converged after 47 cycles. The R factor for the refined chicken annexin V structure that includes three calcium ions and 82 water molecules is 19.7% for all data between 10 and 2.25 Å. The mean temperature factor over all atoms is 19.4 Å^2. The standard deviations from ideal geometry are shown in Table I. A Luzatti plot (not shown) indicates that the error in the final refined coordinates is 0.22 Å.

The chicken annexin V structure is very similar to the human structure (Huber et al., 1900b), as expected given the degree of identity (78%) between the sequences (Figure 2a) and the fact that the crystals are isomorphous. Substitutions occur throughout the chain, in both α-helical and loop regions. Like human annexin V, the chicken protein consists of an N-terminal tail and four similar domains (I–IV). Each domain contains the annexin repeat consisting of five helices (A–E) arranged in a four-helix bundle, with a fifth helix (C) linking the antiparallel helix–turn–helix substructures A–B and D–E

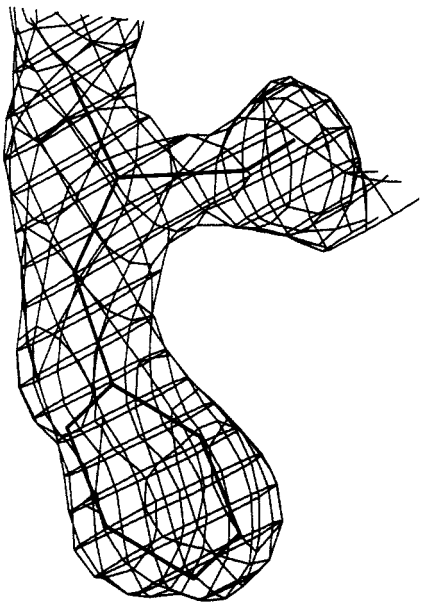

FIGURE 1: Electron density and structure (boldface) at residue Phe 12. The map was calculated from the molecular replacement solution after the initial four cycles of least-squares refinement. This $2F_o - F_c$ map is contoured at 3σ and clearly shows density for an aromatic ring and not an alanine.

Table I: Final Refinement Statistics

	target	standard deviation
distances (Å)		
bond lengths	0.02	0.019
bond angles	0.04	0.066
dihedral angles	0.05	0.90
calcium–protein distances	unrestrained	0.640
planar groups	0.02	0.014
chiral volumes (Å^3)	0.15	0.235
nonbonded contacts (Å)		
single torsion contacts	0.30	0.214
multiple torsion contacts	0.30	0.256
possible hydrogen bonds	0.30	0.377
torsion angles (deg)		
peptide planes	3	3.447
staggered	15	23.75
orthonormal	20	26.41

(Figure 3). In domain III the fourth (D) helix (residues 222–225) is a 3_{10}-helix rather than an α-helix. The helix lengths, determined using the criteria of Kabsch and Sander (1983), vary between 7 and 16 residues.

Figure 4 shows a stereo ribbon drawing (Flower, 1991) of the molecule. A total of 69% of the polypeptide chain is α-helix. The N-terminal 16 residues and the linker between domains II and III (12 residues) form the only extended nonhelical sections. Residues 1–2 and 318–321 are not visible in the final electron density maps. Figure 5 is a Ramachandran plot (Ramachandran et al., 1963) of the refined chicken annexin structure. With the exception of Gln 134, which falls within generous accepted areas (Morris et al., 1992), dihedral angles for all non-glycine amino acids lie within the energetically preferred zones, documenting the predominantly α-helical conformation of this protein. Six well-defined non-glycine residues have ϕ,ψ angles in the left-handed helical region of the map. These residues are conserved between species and have similar conformations in human annexin V.

A total of 57 residues have ϕ,ψ angles that fall into the β-structure region of the Ramachandran plot. Of these, 39 are located around the loops containing the three calcium sites and on either side of the 3_{10}-helix (D) in domain III. The

a

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                    10        20        30        40        50        60        70        80        90       100
                    ----------|---------|---------|---------|---------|---------|---------|---------|---------
CHICKEN    1 -  100 MAKYTRGTVTAFSPFDARADAEALRKAMKGMGTDEETILKILTSRNNAQRQEIASAFKTLFGRDLVDDLKSELTGKFETLMVSLMRPARIFDAHALKHAI
                    *  ***** *  ** ***** ******* ***** **  **** *******  ********** ********** * * ** * *  **  ****
HUMAN      1 -   99 -AQVLRGTVTDFPGFDERADAETLRKAMKGLGTDEESILTLLTSRSNAQRQEISAAFKTLFGRDLLDDLKSELTGKFEKLIVALMKPSRLYDAYELKHAL
                    ----------  --------  ---------------  --------  ----------  ----------
                              IA        IB          IC        ID        IE        IIA

                   110       120       130       140       150       160       170       180       190       20-0
                    ----------|---------|---------|---------|---------|---------|---------|---------|---------
CHICKEN  101 -  200 KGAGTNEKVLTEIIASRTPAEVQNIKQVYMQEYEANLEDKITGETSGHFQRLLVVLLQANRDPDGRVEEALVEKDAQVLFRAGELKWGTDEETFITILGT
                    ************** ***** *  *****  **   ***   * ***  ** ************    ** **  *** ** ********** **** **
HUMAN    100 -  199 KGAGTNEKVLTEIIASRTPEELRAIKQVYEEEYGSSLEDDVVGDTSGYYQRMLVVLLQANRDPDAGIDEAQVEQDAQALFQAGELKWGTDEEKFITIFGT
                    ----------  ----------  ---------  ----------  ----------  ----------
                              IIB       IIC       IID       IIE             IIIA        IIIB

                             210       220       230       240       250       260       270       280       290       300
                    ---------|---------|---------|---------|---------|---------|---------|---------|---------|---------
CHICKEN  201 -  300 RSVSHLRRVFDKYMTISGFQIEETIDRETSGDLEKLLLAVVKCIRSVPAYFAETLYYSMKGAGTDDDTLIRVMVSRSEIDLLDIRHEFRKNFAKSLYQMI
                    ******* ************************ ** ******* *** *** ****** ******** ********** ** ******* *** **
HUMAN    200 -  299 RSVSHLRKVFDKYMTISGFQIEETIDRETSGNLEQLLLAVVKSIRSIPAYLAETLYYAMKGAGTDDHTLIRVMVSRSEIDLFNIRKEFRKNFATSLYSMI
                    ----------  ----  ----------  ----------  ----------  ----------  ----------  ------
                              IIIC      IIID      IIIE        IVA         IVB         IVC         IVD

                             310       320
                    ---------|---------|
                    ---  ----------
CHICKEN  301 -  322 QKDTSGDYRKALLLLCGGDDE
                      ****** ******** **
HUMAN    300 -  320 KGDTSGDYKKALLLLCGEDD
                    ---  ----------
                         IVE
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b

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                                10        20        30        40        50        60        70
                                ----------|---------|---------|---------|---------|---------|---------
Domain I        17 -  86  -----ARADAEALRKAMKGMGTDEETILKILTSRNNAQRQEIASAFKTLFGRDLVDDLKSELTGKFETLMVSLMR
                                  |**|**  *|** **|*||   ** **  *  *  *   | | ||  * *   *  *  *| * * *
Domain II       88 -  158 ----ARIFDAHALKHAIKGAGTNEKVLTEILASRTPAEVQNIKQVYMQEYEANLEDKITGETSGHFQRLLVVLLQ
                              | |    *||** *  |  ** |*      |   **      |*  *  ****    **  |
Domain III     169 -  246 EALVEKDAQVLFRAGELK-WGTDEETFITILGTRSVSHLRRVFDKYMTISGFQIEETIDRETSGDLEKLLLAVVKCIRS
                              * ***  * *    **   *                 *   ****  * **
Domain IV      247 -  317 ---SVPAYFAETLYYSMKGAGTDDDTLIRVMVSRSEIDLLDIRHEFRKNFAKSLYQMIQKDTSGDYRKALLLLCGGDDE
                                @@@@@                                          #
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FIGURE 2: (a) Alignment of chicken and human annexin V sequences with helices indicated by dashed horizontal bars and labeling. (b) Sequence alignment (Parry-Smith & Attwood, 1990) of the four domains of chicken annexin V. The * indicates identity to a residue in an adjacent sequence, while | indicates identity to a residue in a remote sequence. The residues of the calcium-binding loop are marked with @ and the calcium-binding D/E is marked with a #.

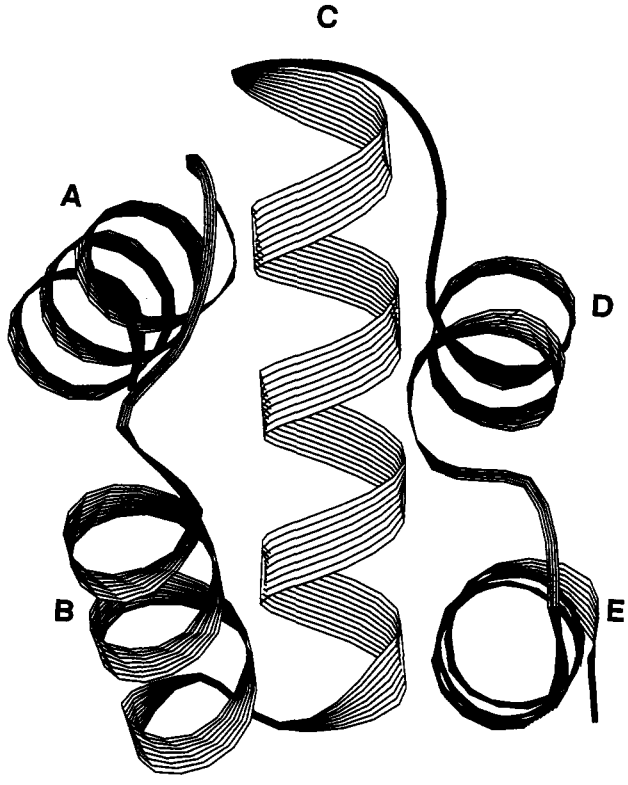

FIGURE 3: Ribbon diagram showing the annexin fold. It comprises five helices (A–E), with the C helix linking the two helix–turn–helix pairs A–B and D–E.

N-terminal region and linker region between domains II and III have almost continuous β-strand conformation.

Twelve Asp, Glu, and Arg residues have accessibilities close to zero. Ten of these residues form internal salt bridges, six

FIGURE 4: Stereo ribbon diagram of chicken annexin V. The black circles represent the calcium ions in domains I, II, and IV.

of them with the other buried residues. Of the 10 salt bridges, four are interdomain with the other six being intradomain. They are summarized in Table II, together with their salt-bridging partners. Asp 303 is a calcium ion ligand in domain IV. Arg 50 forms a water-mediated salt bridge to Glu 278. All of these buried residues are conserved in all of the known annexin V sequences, as are the residues with which they form salt bridges.

Least-squares fitting of the main chain atoms of the human and chicken annexins gives a root-mean-square deviation of 0.3 Å, which is indicative of the similarity of the human and chicken structures.

Figure 6 shows the variation of average temperature factor for a residue with residue number. In general, the areas with the lowest temperature factors correspond to the helical segments of the polypeptide. Residues at the N-terminus in the linker and in the interhelical loops have larger temperature factors. The temperature factors in domain III are particularly

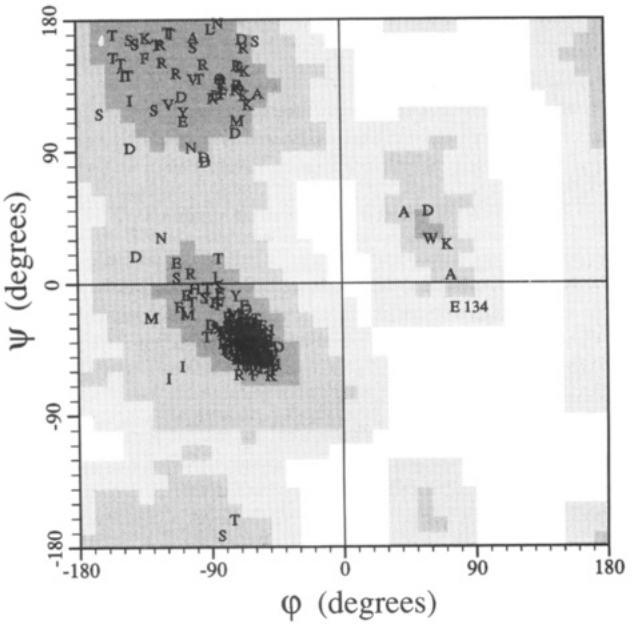

FIGURE 5: Ramachandran map for chicken annexin V structure excluding all glycine and proline residues.

Table II: Charged Residues Buried within the Structure and Their Salt-Bridging Partners

buried residue	salt-bridging partner	domain to domain[a]
Asp 20	Arg 45	I–I
Asp 68	Arg 63	I–I
Asp 92	Arg 117	II–II
Glu 112	Arg 117	II–II
Glu 112	Arg 271	II–IV
Arg 117	Asp 92	II–II
Arg 117	Glu 112	II–II
Asp 175	Arg 201	III–III
Asp 266	Lys 76	IV–I
Arg 271	Glu 112	IV–II
Arg 276	Asp 280	IV–IV
Asp 280	Arg 276	IV–IV
Arg 50	Glu 278	
Arg 50	(via water)	I–IV
Asp 303	(calcium ligand)	

[a] Roman numerals indicate which domains are linked by a particular salt bridge.

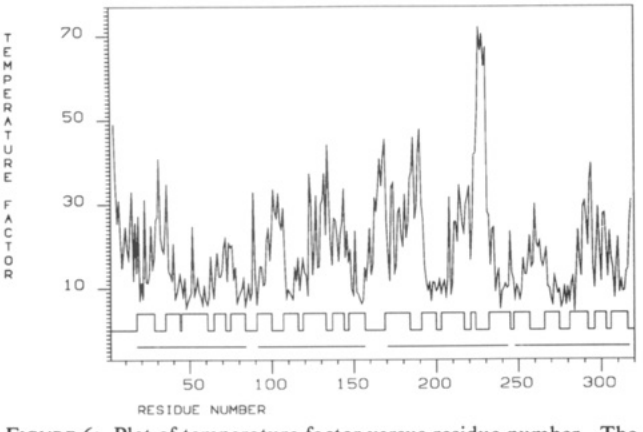

FIGURE 6: Plot of temperature factor versus residue number. The horizontal lines indicate the positions of the four domains while the blocks show the helices A–E in each domain.

high. This may well be due to the lack of a calcium-binding site which helps to stabilize the other domains of the protein.

Calcium is generally coordinated by seven oxygen ligands, approximately located at the vertices of a pentagonal bipyramid (Strynadka & James, 1989). This type of ligand geometry is observed in domains I, II, and IV, which each bind a calcium ion (Figure 7). In domains I and II only five of the possible seven ligation sites are occupied. In domain IV a water molecule occupies a sixth site. The sequences of the four domains are aligned in Figure 2b. Three ligands are provided by the carbonyl groups of residues in the loop between helices A and B. Two further ligands are provided by the carboxylic acid moiety of either an Asp or Glu residue, 44 amino acids downstream from the first loop ligand. The sixth and seventh coordination sites can accommodate water molecules. In the chicken crystal structure, the sixth ligand is only visible in domain IV and the seventh is not seen at all. All of the calcium sites in the human model have electron density for water molecules at the sixth and seventh coordination sites, although it is weaker in some cases than others (Huber et al., 1992). The average calcium–oxygen distance is 2.52 Å for chicken and 2.42 Å for human annexin V.

DISCUSSION

The initial identification of the purified protein as the chicken homologue of mammalian annexin IV was based on their similarities in M_r, p*I*, and elution position in ion-exchange chromatography. These properties provide a useful means of identifying mammalian annexins IV and V (Creutz et al., 1987; Boustead et al., 1988). However, despite its closer similarity in p*I* to mammalian annexin IV (p*I* 5.6), this structural study and subsequent partial amino acid sequence analysis (Boustead et al., 1992) have now identified the purified chicken protein as the homologue of mammalian annexin V (p*I* 5.0).

Human and chicken annexin V are, so far, the only annexins whose three-dimensional structures have been solved. As expected given the high degree of identity between the amino acid sequences (78%), the tertiary structure of the chicken protein is closely related to that of human annexin V, being made up of an N-terminal tail followed by four domains, each containing five α-helices arranged as a right-handed superhelix (Huber et al., 1990a,b, 1992). The domains are positioned side by side such that the molecule is approximately disc-shaped, with convex and concave surfaces to the disc. The calcium-binding sites are located on three projecting loops on the convex side of the molecule in domains I, II, and IV (see Figure 7). The convex surface is proposed to interact with the lipid bilayer (Huber et al., 1990a,b). The N-terminal tail, which is the most variable region of the annexins, is situated on the concave surface. In annexin II, the N-terminal tail is the site of attachment of the small subunit p11 (Johnsson et al., 1986, 1988). The binding of this subunit regulates various properties of annexin II, such as binding to phospholipids and cytoskeletal proteins (Powell & Glenney, 1987; Glenney et al., 1987). The N-terminal tail of annexins frequently contains phosphorylation sites, and it may play a regulatory role in all annexins.

An interesting result of the comparison of the human and chicken structures is the occurrence of a number of the amino acid sequence differences as sterically compensating pairs. Where a large side chain is replaced by a smaller one, a second substitution replacing an adjacent small side chain with a larger one occurs. The clearest example involves residues 282 and 286. Residue 282 in human annexin V is a phenylalanine, while it is a leucine in chicken annexin V; to compensate for this change, the lysine at position 286 in the human annexin becomes a histidine in the chicken protein. This change avoids the formation of a hole or cavity within the structure. A number of examples of compensatory changes

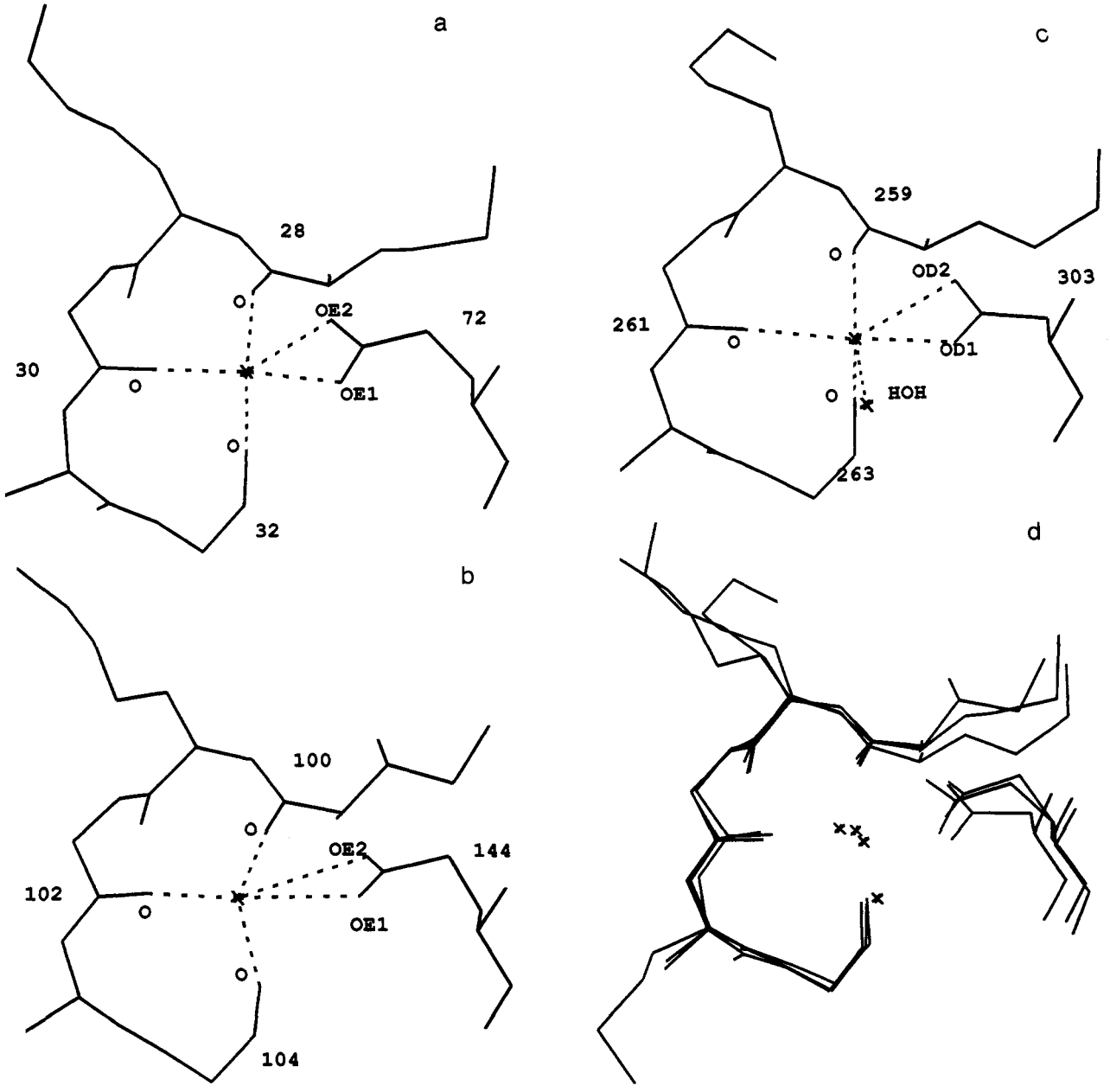

FIGURE 7: Calcium-binding sites in chicken annexin V: (a) domain I; (b) domain II; (c) domain IV. Residue numbers and atom names are included. (d) Superposition of the three sites based on the three carbonyl oxygen ligands.

Table III: Residues which Form Sterically Compensating Pairs with the Amino Acids of Each Residue in Both Human and Chicken Annexin V

residue no.	human	chicken	residue no.	human	chicken
11	D	A	46	S	N
86	K	R	88	S	A
135	S	A	140	D	K
247	I	V	251	L	F
282	F	L	286	K	H

in the structure of annexin V are listed in Table III. Similar changes have been observed in other proteins and utilized in protein design (Cohen & Parry, 1990). The ultimate consequence of such compensatory changes is to maintain a correctly folded, densely packed protein structure. Clearly, there are important evolutionary implications in such changes.

The solution of the structures of human and chicken annexins confirms the significance of the sequence motif K-G-X-G-T-(38 residues)-E/D as the calcium-binding site. This sequence occurs in domains II and IV in all annexins sequenced to date. It is also found in domain I in some annexins (including annexin V, but not annexins I or II), but it has not been observed in domain III of any annexin sequenced so far. The sequence and structure of domain III in annexin V differ considerably at residues 186–189 from the pattern of domains I, II, and IV (see Figure 2b), and it is unable to bind calcium.

The calcium-binding site of annexins is distinct from the E–F-hand type characteristic of the calmodulin family. Biochemical evidence and structural considerations suggest a close proximity of the phospholipid-binding sites to the calcium-binding sites in annexins (Glenney et al., 1987; Taylor & Geisow, 1987; Huber et al., 1990b). The only known calcium-binding site structurally related to that of annexins is found in phospholipase A_2. However, apart from the calcium-binding site, there is no overall sequence homology between the two proteins. In addition, phospholipase A_2 differs from annexins in the number of residues between the calcium-binding loop and the carboxylic acid (Huber et al., 1992).

Both annexins and phospholipase A_2 bind to phospholipids in a calcium-dependent manner and, by analogy, annexins may interact with phospholipids at the calcium-binding sites in a manner similar to phospholipase A_2 (Huber et al., 1990b).

The incomplete ligation of the calcium ions is in marked contrast to the human structure where all three calcium ions are fully ligated. It is possible that a more complete picture of the calcium coordination could be obtained following further refinement at higher resolution. The apparent incomplete ligation may be due to the presence of disordered water molecules. The chicken annexin V crystals were grown from a calcium-containing mother liquor whereas the human crystals had calcium soaked into them, but it is not clear why this should have any effect on the final coordination of the calcium ions. Another possibility is that traces of phospholipid have been left in the chicken preparation and these are bound to the calcium ions but that they are disordered and therefore not visible in electron density maps.

The center of the chicken and human annexin V molecules is a pore lined by four helices (from domains II and IV) and containing hydrophilic residues. This is more often seen in integral membrane proteins than in water-soluble proteins, which normally have hydrophobic cores (Freemont et al., 1992). Moreover, a chain of water molecules, observed in several different crystal forms of human annexin V (Huber et al., 1992), runs through the channel. This structure has several features in common with ion-channel-forming proteins, and a role for annexin V as an ion channel has been proposed (Huber et al., 1990a,b, 1992). This is consistent with earlier electrophysiological studies on annexin VII (Burns et al., 1989) and annexin V (Rojas et al., 1990) which showed that both proteins can form voltage-gated Ca^{2+} channels in artificial lipid membranes. The N-terminal tail of annexin V lies close to the central pore on the concave face of the molecule. By analogy with the voltage-gated K^+ channel (Miller, 1991), it could play a role in physically blocking and inactivating the central channel.

The 12 buried charged residues in chicken annexin V are all conserved in human annexin V, suggesting the vital importance of these residues to the function of the protein. Moreover, six of these residues are located in the central hydrophilic pore of the molecule, and all six form internal salt bridges. This is particularly interesting with respect to the model for an annexin V ion channel, which has been constructed on the basis of electrostatic calculations of an annexin V–lipid model (Huber et al., 1992; Karshikov et al., 1992). In this model, annexin V is partially inserted into the membrane, perturbing the lipid bilayer, in keeping with surface pressure measurements of annexin V binding to phospholipid layers (Newman et al., 1989). An electrostatic gradient induced by annexin V at the protein–membrane interface could produce a membrane pore by electroporation (Neumann, 1988). The channel in the center of the annexin V molecule is proposed to be gated by two salt bridges, each of which may exist in an open or closed conformation, giving rise to one open and three closed states of the channel. The residues which may be involved in the salt bridges in human annexin V are Arg 271, Glu 112, Arg 117, Asp 92, Arg 276, and Asp 280 (Huber et al., 1992; Karshikov et al., 1992), all of which are present in the chicken protein. Opening of the channel would involve the formation of salt bridges with alternative residues. The model would allow opening of the channel without a large conformational change in the molecule (fitting with results from electron image analysis described below) or a large change in electrostatic free energy.

Electron image analysis of two-dimensional crystals of human annexin V bound to phospholipids (Mosser et al., 1991) has provided further structural information which is also likely to apply to the homologous chicken annexin V protein. In the presence of calcium, human annexin V molecules bound to lipid layers are organized as trimers in a triskelion-like formation. A comparison of three-dimensional crystals of annexin V obtained from aqueous solution (Huber et al., 1990a,b) with the two-dimensional crystals of lipid-bound annexin V shows the two structures to be closely correlated (Brisson et al., 1991). This suggest that annexin V does not undergo a major conformational change on binding to membranes, and this is taken into account in the model described for ion-channel formation. These studies also show that the central polar pore of the molecule is perpendicular to the plane of the membrane as are, approximately, most of the axes of the helices making up the four domains. Both of these characteristics are typical of integral membrane proteins. Annexin V fits into the category of amphipathic proteins, being water soluble but also interacting with membranes.

It is possible that the trimeric form may be the active form of annexin V. The calcium-dependent binding of human annexin V to lipid vesicles can induce the formation of planar facets on the vesicle, and it has been suggested that this may be due to the formation of a large array of clustered annexin V trimers (Andree et al., 1992). Interestingly, annexin VI also displays a trimeric formation in two-dimensional crystals on lipid layers (Newman et al., 1989), and it has recently been proposed that this trimeric form may be involved in a possible mechanical mechanism for binding to and severing the stalks of coated pits, leading to the formation of coated vesicles (Lin et al., 1992).

Several other roles have been proposed for annexin V on the basis of experiments performed in vitro. These include inhibition of phospholipase A_2 (Pepinsky et al., 1988) and anticoagulant activity (Grundmann et al., 1988). The physiological significance of these functions is not known, and these effects may be a result of the calcium-dependent interaction of annexin V with phospholipids rather than a result of specific properties of the protein. However, a recent report of the inhibition of protein kinase C by annexin V suggests that this function is a direct effect of the protein (Schlaepfer et al., 1992). The interpretation of the various possible functions of annexin V is further complicated by its widespread tissue distribution (Pepinsky et al., 1988) and its multiple subcellular locations. Annexin V is found as a membrane-associated (Spreca et al., 1992), cytosolic (Spreca et al., 1992; Koster et al., 1992), nuclear (Koster et al., 1992), and extracellular (Pfaffle et al., 1988; Christmas et al., 1991) protein. Recently, there has also been a report of a form of annexin V which can only be extracted in the presence of Triton X-100 (Bianchi et al., 1992). It is not yet clear whether the structure of this form differs from that of the EGTA-extractable form of annexin V reported here.

Increasing information on the physiological function of annexin V will allow further correlations to be made with the structures of the chicken and human proteins. The degree of structural conservation between these two evolutionarily distant species suggests that they have a common vital role which developed relatively early in evolutionary history.

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