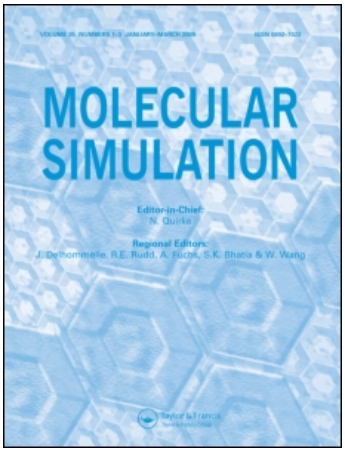

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Spectroscopy-Based Modelling of the 3D Structure of the β Subunit of the High Affinity IgE Receptor

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SPECTROSCOPY-BASED MODELLING OF THE 3D STRUCTURE OF THE β SUBUNIT OF THE HIGH AFFINITY IgE RECEPTOR

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The high affinity IgE receptor, possesses a tetrameric structure. The 243 residue β subunit is a polytopic protein with four hydrophobic membrane-spanning segments, whereas the individual α and γ subunits are bitopic proteins each containing one transmembrane domain in their monomeric form. In the proposed topographical model (Blank *et al.*, 1989), the four transmembrane α helices of the β subunit are connected by three loop sequences.

To study the individual subunits and intact receptor, this membrane protein was divided into domains such as its loop peptides, cytoplasmic peptides and transmembrane helices according to Blank *et al.*, 1989. The 3D structure of the synthesized loop peptides and cytoplasmic peptides were calculated; CD and/or NMR data were used as appropriate to generate the resultant structures which were then used as data basis for the higher level calculations.

The four individual transmembrane helices of the β subunit were characterised, first of all, by mapping the relative lipophilicity of their surfaces using lipophilic probes. A second procedure, docking of the individual helices in pairs, was used to predict helix - helix interactions.

The data on the relative lipophilicity of the surfaces as well as the surfaces that favoured helix - helix interactions were used in combination with the spectroscopy-based structures of the loops and cytoplasmic domains to calculate *via* molecular dynamics, the helix arrangement and 3D structure of the β subunit of the high affinity IgE receptor. In the final analysis, the molecular simulations yielded two structures of the β subunit, which should form a basis for the modelling of the whole high affinity IgE receptor.

Keywords: High affinity IgE receptor; molecular modelling; membrane proteins; lipid - helix interaction; docking

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1. INTRODUCTION

The high affinity IgE receptor (Fc-ε-RI) plays an important role in allergy and inflammation processes. This receptor is a polytopic (multimeric) receptor consisting of four subunits, one α, one β and a γ dimer connected by a disulfide bond (Blank *et al.*, 1989). It is a 7-helix integral membrane protein, with the α and γ chains containing one putative transmembrane (TM) helix and the β subunit having four putative TM helices. The α chain consists of two extracellular immunoglobulin C2 type domains (D1 and D2) and contains the IgE binding site (Sutton and Gould, 1993). It is the interaction of IgE and α subunit of the IgE receptor and the subsequent cross-linking of whole receptors induced by specific antigen, that activates numerous inflammatory cell types.

The β and γ chains play important roles in down stream signal transduction (Sutton and Gould, 1993; Beavan and Baumgartner, 1996). Tyrosine phosphorylation of these subunits was pinpointed as an early biochemical event preceding the activation of protein kinase C and the rise of the intercellular calcium (Paolini *et al.*, 1991). It was found that the fusion proteins containing the Src homology 2 (SH2) domains of Syk and Lyn tyrosine kinases precipitated tyrosine-phosphorylated proteins from RBL-2H3 cell lysates. There was more binding of Syk SH2 to γ subunit than to β subunit of Fc-ε-RI, whereas Lyn SH2 bound only to the β subunit. The SH2-mediated association of these two protein tyrosine kinases with Fc-ε-RI could play an important role in receptor signaling (Kihara and Sigaranian, 1994). The immunoreceptor tyrosine-based activation motifs (ITAM), common to many receptors, were found to interact with SH2 domains (Labadia *et al.*, 1997). The motif detected in the cytoplasmic domain of the γ subunit ([D,E]-X2-Y-X2-L-X7-Y-X2-L) is known also as a Reth motif (Reth, 1989). The motif found twice in the cytoplasmic C-terminal tail of the β subunit was different: [RK]-X(2,3)-[DE]-X(2,3)-Y, than that detected in γ subunit.

The basic structural building-block in plasma membrane proteins of both prokaryotic and eukaryotic cells is the apolar, often slightly amphiphatic transmembrane α helix. For multi-spanning membrane proteins with most of their mass buried within the bilayer in the form of transmembrane helices, the insertion event is decisive. Most prediction of the topology and structure of receptors used hydrophobicity analysis algorithms, in which the amino acid sequence was scanned to locate segments rich in apolar residues. The most suitable helix–helix packing arrangements were then sought using rules derived from helical soluble proteins and by rotating the

helices such that the most hydrophobic sides faced the lipids (von Heijne, 1992) and to dock TM helices (Vakser, 1996). Homology modelling based upon membrane proteins with known structure was also used (Habibi-Nezhad *et al.*, 1996). While the apolar surfaces of the transmembrane helices provided a good interface for the membrane lipids, it was believed that the hydrophilicity of the polar amino acids within a helix should favour the spontaneous formation of helical bundles within which polar surfaces interacted and were removed from the lipid environment (Rees *et al.*, 1989). It was proposed that the structure of short extramembraneous loops connecting contiguous helices should be predictable using libraries of known loop-structures (Rooman *et al.*, 1989), biophysical data of peptides with the same sequence of these loops and molecular mechanics and dynamics calculations of the loops.

Since the size of the receptor subunits as well as the existence of both lipophilic and hydrophilic domains in the same subunit have not yet permitted direct experimental structure determination, characterisation of the receptor subunits by cutting them into smaller domains or structural units is the methods of choice since the anisotropic environment of membrane receptors leads to their natural division in terms of environment. Thus each membrane receptor, including the high affinity IgE receptor, can be classified into extracellular, transmembrane and cytoplasmic domains. This domain approach to structure determination of large proteins has been pioneered by Campbell and workers (Baron *et al.*, 1991) and used by others (Anderson *et al.*, 1992; Musco *et al.*, 1995). The novel method of cutting large protein into small to medium size peptides was used to determine the secondary structure of vaccinia virus thymidine kinase, porcine adenylate kinase and yeast guanylate kinase (Behrends *et al.*, 1996; Behrends *et al.*, 1997). Using a similar approach several domains of the high affinity IgE receptor have been studied. Structural information for the α subunit has so far been limited to modelling studies of the extracellular portion of based on homologous proteins of known structure (Padlan and Davies, 1986; Padlan and Helm, 1993; McDonnell *et al.*, 1996), or the NMR studies of the extracellular Fc-ε-RI α chain mimic peptides (McDonnell *et al.*, 1997). The structural information of the rest of the receptor was studied by Gibbons and co-workers; antibody studies (Gao *et al.*, 1993, 1994); the spectroscopic studies of the cytoplasmic part of the γ subunit (Anderson *et al.*, 1992; Anderson *et al.*, 1994/95), spectroscopic studies of the cytoplasmic portion of the α subunit (Thomas *et al.*, 1993; Zloh *et al.*, 1997) and spectroscopic studies of the cytoplasmic C-terminal domain of the β subunit (Zloh *et al.*, 1994a; Zloh *et al.*, 1994b). Although some structural information has been

obtained, the structure of Fc-ε-RI and mechanism of the signal transduction has yet to be precisely identified. The model of the β subunit was previously proposed (Zloh *et al.*, 1995), but this model was preliminary, since only a small number of experimental and theoretical constraints were available to be incorporated in the short molecular dynamics simulations.

The motif or domain approach was therefore used to calculate the 3D structure(s) of the β subunit. Specifically, the β subunit calculations were carried by adding the following data:

1. CD and prediction based structures of the loop peptides;
2. CD and prediction based structures of the cytoplasmic tails;
3. NMR-based structures of loop 1–2 and loop 2–3 (Zloh *et al.*, 1996; Zloh *et al.*, 1998);
4. Calculation of the hydrophobic moments;
5. Dodecane mapping of the relative lipophilicity of surfaces of the individual TM helices (Zloh and Gibbons, 1996);
6. Palmitic fatty acid mapping of the relative hydrophobicity of surfaces of the individual TM helices;
7. Docking of TM helices in pairs and mapping surfaces favourable to helix–helix interactions.

The sophistication of the molecular mechanics and dynamics calculations of the 3D structure of the whole β subunit (243 residues) was enhanced with the availability of the above data. In the final analysis, the calculations yielded two subunit structures probably more realistic than those which would be calculated purely by a homology modelling based on the *Bacteriorhodopsin* crystal structure or using only pure prediction methods. The calculated structures of the β subunit will be used in the process of the modelling of the whole high affinity IgE receptor.

2. METHODS

2.1. Docking of the TM Helices into Four Helix Bundles

All calculation described in this section were performed on the SGI O2 workstation with 64 MB RAM and 6 GB hard disk. The TM helices of the β subunit were constructed with the ideal α helix geometry ($\psi = -57°$ and $\phi = -47°$). In this work the version 6.3 and 6.4 of SYBYL were used. The GRAMM software was used for the low resolution docking of the TM helices (Vakser, 1995a; Vakser, 1996b; Vakser, 1996). Parameters for the low

resolution docking were: the matching mode = helix, grid step = 4.1, angle for the rotation = 20°, representation = all, potential range type = grid step, repulsion = 11, attraction double range = 0 and number of the produced low energy structures = 10. The relative orientation of the TM helices was the same as in the topographical model as defined by Blank *et al.*, 1989.

The analysis of the resulting structure was performed with NMRCLUST and Webmol (Walter, 1997) software. Hydrophobic moments in units of hydrophobicity values for TM helices were calculated according to Eisenberg *et al.*, 1982.

The procedure for the docking of the TM helices into a four helix bundle for the-subunit was chosen in such way to explore possible combinations of the helix-helix packing. In the first instance, all possible combinations of the TM helix pairs were used (B1-B2; B1-B3; B1-B4; B2-B3; B2-B4 and B3-B4). For every one of these 6 pairs, 10 dimeric structures were obtained which were then divided into clusters using NMRCLUST. The representative (lowest energy) structures of each cluster were chosen to add a third helix, so that three helix bundles were formed. For example, some bundles were listed: B1-B2-B3; B1-B2-B4; B1-B4-B2; B1-B4-B3; B3-B2-B4; *etc.* (the order of helices in the bundles corresponded to the order in which helices were added). The clusters of each three helix bundle were obtained and representative structures were chosen for further docking. The fourth (missing helix) was docked to each structure and four helix bundles were obtained. Some were listed in this example: B1-B2-B3-B4; B1-B2-B4-B3; B1-B3-B2-B4; B1-B3-B4-B2; *etc.* No distance or any other constraints were used during this docking procedure. The bundles were analysed for the correct orientation. The NMRCLUST program was used to choose the representative bundles.

The helix packing parameter, the crossing angle (ω) values were calculated as a dihedral angle defined by four points; one at the end of each helix axis, and two the two points of closest approach on the helix axis by WebMol software (Walter, 1997). A positive crossing angle represents the left-handed supercoil helix pair, and a negative value is the right-handed supercoil helix structure.

2.2. Molecular Modelling of the β Subunit. Model I-Use of the Docked Four-helix Bundle and the Addition of the Loopsand Tails in Two Stages

All calculation described in this section were performed on the SGI O2 workstation with 64 MB RAM and 6 GB hard disk. The simulated

annealing/molecular dynamics protocol (SA/MD) was applied to 12 different four helix bundles with added connecting loop sequences. The TM helices were constructed in the same manner as described in the previous section. The versions 6.3 and 6.4 of SYBYL [Tripos Association, 1994–1997] were used. The described SA/MD protocol was modified from Kerr *et al.*, 1994, and was originally used to model ion channels and it is summarised in Table I. The loops between helices and parts of N- and C-terminal tails were constructed, based on the available structural data (NMR based structures for loop 1–2 and loop 2–3 were described previously (Zloh *et al.*, 1996; Zloh *et al.*, 1998); secondary structure prediction data for loop 3–4) using biopolymer module of SYBYL. The structures were converted into X-PLOR PDB and PSF files. Those loops were manually docked into the four helix bundle. The 10 residues were added at both N- and C-terminal corresponding to the sequence of the β subunit. These residues were added to prevent electrostatic interaction of the terminal groups in the four helix bundle. The simulated annealing was performed on such truncated models of the β subunit. The cycle of the initial minimisation, molecular dynamics and final minimization was repeated three times to produce independent models with 12 different bundles as a starting structures (the stages 2–15). Those 12×3 models were filtered further using hydrophobic moments orientation, lipid facing sides and interaction energy in the TM helix bundles. A bundle, with low energy, hydrophobic moment orientation and lipid facing surfaces criteria fulfilled, was chosen for further model building.

The addition of the whole cytoplasmic tails was carried out only on the one chosen bundle. In the absence of the detailed experimental results, the structures of the both tails were set to those predicted by the secondary structure algorithms. The dielectric constant was set to = 80 Db, to emulate the polar environment in water. The stages 15 to 23 were performed only on the selected bundle.

2.3. Molecular Modelling of the β Subunit. Model II – Use of the Four Helix Bundle and the Addition of the Loops and Tails in One Stage

All calculation described in this section were also performed on the SGI O2 workstation with 64 MB RAM and 6 GB hard disk. The TM helices were constructed in the same manner as described in the previous section. In this work the version 6.3 and 6.4 of SYBYL [Tripos Association, 1994–1997] were used.

TABLE I Model building and MM/MD protocol for the β subunit – model I. This protocol is based on the SA/MD protocol for building ion channels (Kerr *et al.*, 1994). Stages 1 to 13 were performed on all twelve four-helix-bundles, while the rest of the protocol was applied only to one selected model

Stage	*Notes*	*Temperature* (*K*)	*Constraints*	*Time* (*ps*)	*Steps*
1	Helix building	–	$=-57°$; $=-47°$	–	–
2	Four helix bundle building	–	Docking procedure	–	–
3	Loops 1–2 and 2–3 building	–	NMR based models	–	–
4	Loop 3–4 and truncated tails building	–	Secondary structure prediction	–	–
5	Manual docking of loops and truncated tails into bundle	–	All structures fixed	–	–
6	Energy minimization	–	–	–	5
7	Molecular dynamics	1000	Fixed C-α of bundle + NOE constraints	3.7	–
8	Molecular dynamics	1000	Fixed C-α of bundle + NOE constraints	32.0	–
9	Molecular dynamics Cooling	from 1000 to 300	Fixed C-α of bundle + NOE constraints	8.4	
10	Energy minimization	–	–	–	2000
11	Molecular dynamics Cooling	from 500 to 300	Gradually releasing bundle constraints	4.0	–
12	Molecular dynamics	300	–	5.0	–
13	Energy minimization	–	–	–	1000
14	Building whole subunit	–	Secondary structure prediction of tails	–	–
15	Energy minimization	–	–	–	1000
16	Molecular dynamics Cooling	from 800 to 300	NOE constraints + fixed C-α of bundle	10.0	–
17	Energy minimization	–	–	–	1000
18	Molecular dynamics Cooling	from 350 to 300	Fixed C-α of bundle	10.0	–
19	Molecular dynamics	300	Gradually releasing bundle constraints	25.0	–
20	Energy minimization	–	–	–	1000
21	Molecular dynamics	300	–	100.0	–
22	Energy minimization	–	–	–	1000

The outline of the β subunit building from the lowest energy docked bundle is presented in Table II. The loops between helices and parts of N- and C-terminal tails were constructed, based on the available structural data (NMR based structures for loop 1–2 and loop 2–3 as described in previously; secondary structure prediction data for loop 3–4) using

TABLE II Model building and MM/MD protocol for the β subunit–model II. Protocol is based on the SA/MD protocol for building ion channels (Kerr *et al.*, 1994)

Stage	*Notes*	*Temperature* (*K*)	*Constraints*	*Time* (*ps*)	*Steps*
1	Helix building	–	$= -57°$; $= -47°$	–	–
2	Four helix bundle building	–	Docking procedure	–	–
3	Loops 1–2 and 2–3 building	–	NMR based models	–	–
4	Loop 3–4 and cyto-plasmic tails building	–	Secondary structure prediction	–	–
5	Manual docking of loops and tails into bundle	–	All structures fixed	–	–
6	Energy minimization	–	–	–	1000
7	Molecular dynamics Cooling	from 800 to 300	NOE constraints + fixed C-α of bundle	10.0	–
8	Energy minimization	–	–	–	1000
9	Molecular dynamics Cooling	from 350 to 300	Fixed C-α of bundle	10.0	–
10	Molecular dynamics	300	Gradually releasing bundle constraints	25.0	–
11	Energy minimization	–	–	–	1000
12	Molecular dynamics	300	–	100.0	–
13	Energy minimization	–	–	–	1000

the biopolymer module of SYBYL. The structures were converted into X-PLOR PDB and PSF files. The starting model of the β subunit was constructed by manual rotation and translation of the loops and tails and docking them into lowest energy four helix bundle helix bundle obtained by docking procedure of TM helices (as described in the previous section).

The molecular dynamics protocol was applied in order to sample the conformational space of the constructed model. The first stage of the 1000 steps of Powell minimization was performed in order to remove initial bad contacts. The rest of the protocol is same as the protocol steps 15 to 22 described in the previous section.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the Previously Published Model

The previously published model of the β subunit (Zloh *et al.*, 1995) was preliminary since only a small number of experimental and theoretical constraints were available to be incorporated in the short molecular

dynamics simulations. For example, the helix–helix arrangement was based only on the intuitive orientation of the hydrophobic moments and vertical docking of the TM helices was not allowed. The secondary structures of the connecting loops were based only on the CD spectra with some information from the secondary structure predictions. Therefore, further refinement in the model building was carried out and presented in this work.

3.2. Molecular Modelling of the β Subunit. Model I – Use of the Docked Four-helix Bundle and the Addition of the Loops and Tails in Two Stages

The uncertainties found in the previous model, had led to expanding the work in the several directions. The secondary structures of the loops (1–2 and 2–3) were examined further by NMR and NMR based molecular modelling (Zloh *et al.*, 1995; Zloh *et al.*, 1998). Also, the initial arrangement of the TM helices in the 7 helix bundle was unknown and some possible arrangements are presented in Figure 1. The absence of the experimental knowledge in the transmembrane part of the molecule has channelled this work towards helix–helix and helix–lipid interaction studies (Zloh and Gibbons, 1996). These experimental and calculated data were therefore used in new calculations of the β subunit.

3.2.1. Partially Truncated Models of the β Subunit

The twelve helix bundles (see Fig. 2) formed by docking TM helices [unpublished results] were chosen for further modelling. The loop peptides and 10 residues of each cytoplasmic tails were added to those initial four helix bundles. These were subjected to the SA/MD protocol described in the methods (stages 1–15). Three independent models for each bundle were obtained as a result of the SA/MD protocol. The interaction energies between TM helices within bundles were calculated for all resulting structures. The greatest interaction energies of the lowest energy models for each bundle are shown in Table III.

The adding of the loops and truncated 10 residue tails was therefore utilized to constrain the four helix bundle, since the distance between N- and C-terminals of two consecutive TM helices could not be larger than the distance between the two terminal ends of the loop that connects those helices. In such a way, the structure of the loop could aid the TM bundle folding. Also, new interactions were introduced which could lead

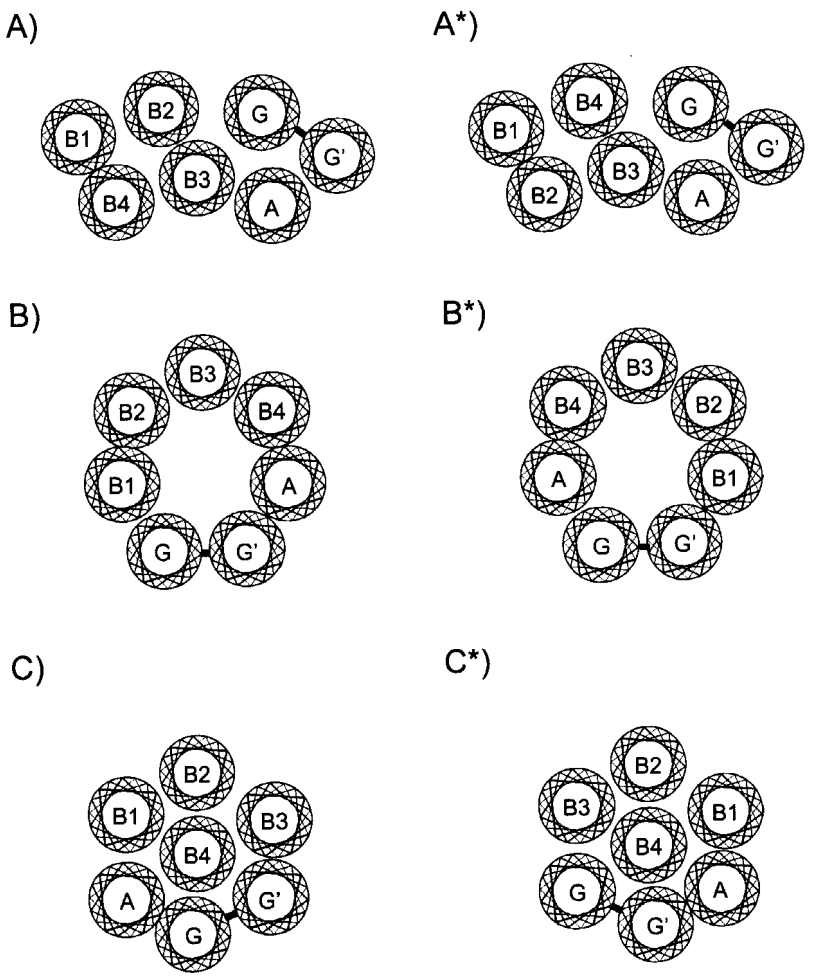

FIGURE 1 Some examples of the possible clockwise and anticlockwise arrangements of the TM helices of the high affinity IgE receptor: (A) *Bacteriorhodopsin* type, (B) channel type and (C) circular type arrangements (*denotes the anticlockwise arrangement).

to construction of a more detailed model, hopefully closer to reality. In the *Bacteriorhodopsin*, the flexibility of the connecting loops prevented the experimental elucidation of their conformation. Therefore, the modelling of some receptors homologous to the BR (human opoid receptors) was restricted to the modelling of the helix bundle alone (Habibi-Nezhad *et al.*, 1996). In the case of the 5-HT_2 receptor, the ligand interaction site was identified within the extracellular region. In the absence of the experimental data, the secondary structure prediction results were used as a starting

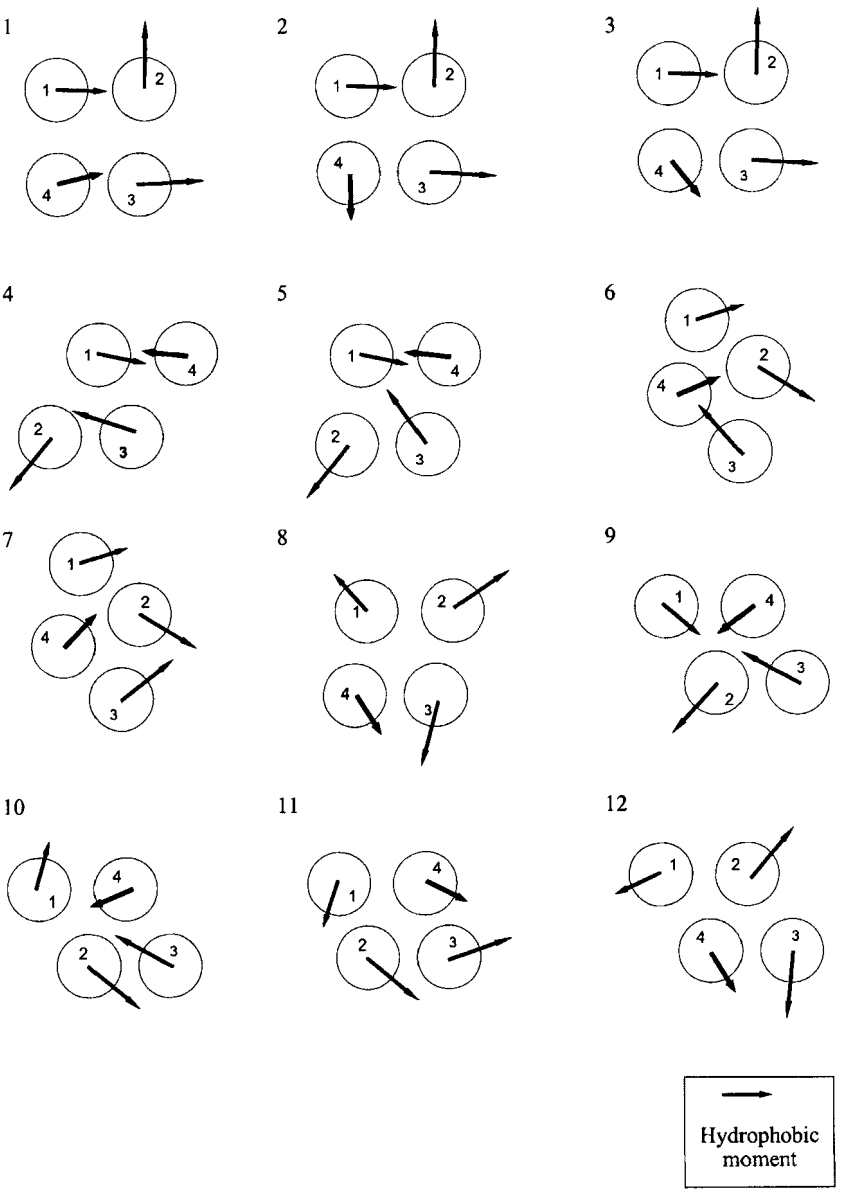

FIGURE 2 The orientation of the helix hydrophobic moments in the anticlockwise and clockwise four helix bundles formed by helix–helix docking (perpendicular view to the plane of the membrane). Arrows depicted the hydrophobic moments.

conformation for the loop and tail regions in the building 3-D models and to explain ligand–receptor interactions (Kristiansen *et al.*, 1993; Kristiansen and Dahl, 1996; Sytle *et al.*, 1993; Sytle *et al.*, 1996).

TABLE III The interaction energies between the TM helices of the lowest energy four helix bundles of the partially truncated β subunit of high affinity IgE receptor

Bundle	E_{VDW}(*kcal/mol*)
1	−99.9
2	−87.2
3	−123.6
4	−72.2
5	−95.2
6	−103.2
7	−107.6
8	−93.6
9	−81.6
10	−86.5
11	−90.1
12	−89.7

In this work, the experimental NMR and CD data of connecting loops were combined with molecular mechanics calculations of lipid–helix interaction sites (Zloh and Gibbons, 1996) together with secondary structure prediction of the tails to build the subunit model. These experimental and empirical constraints should therefore be used as criteria for choosing the correct model(s) out of the twelve partially truncated models of the β subunit. In this case, the TM helix–TM helix packing, expressed through interaction energy between helices within the transmembrane bundle, was also an important determinant. The orientation of the hydrophobic moments and lipid binding sites were additional and influential factors in making the decision of which model should be subjected to further modelling.

Bundle 3 had greatest individual interaction energy (Tab. III, $E = -123.6$ kcal/mol), but it was not chosen as a suitable model for further modelling. The lipid facing side of TM helix 4, as predicted by dodecane and PFA molecular mechanics calculations, was involved in TM helix–TM helix contact surface. However, this bundle could be considered in the future work as a low energy structure.

Bundle 7 had the second greatest interaction energy ($E = -107.6$ kcal/mol). This bundle had the hydrophobic moment of TM 4 facing inside the bundle and not to the lipid exterior. This could be explained by the small value of the hydrophobic moment for this helix, and in this model of the β subunit there is very good agreement between lipid facing sides determined by molecular mechanic calculations and helix–helix docking. Therefore, this model was considered to be a good basis for further modelling of the 7 helix bundle of the high affinity IgE receptor.

The overlap of three models for the partially truncated β subunit based on this bundle is shown in Figure 3. The RMSD for the C-α of the bundle was 4.5 Å, indicating a substantial difference in the three models – even the starting point was same. The sampling of the conformational space using this approach could be considered substantial. The important feature of these models, was that the helical structure of the TM helices was conserved during SA/MD protocol.

Bundle 7 had an excellent agreement between prediction of lipid facing surface on the TM helices by two the different approaches used independently in this work. The lipid favourable surfaces calculated by molecular mechanics methods were not involved in the helix–helix contact interface determined by docking procedure (Fig. 2).

3.2.2. Complete Model of the β Subunit

This model of the truncated β subunit, based on Bundle 7, was completed by adding the cytoplasmic tails whose conformation were based principally on secondary structure prediction. The initial minimization, followed by several different runs of molecular dynamics (stages 15 to 22 in Tab. I)

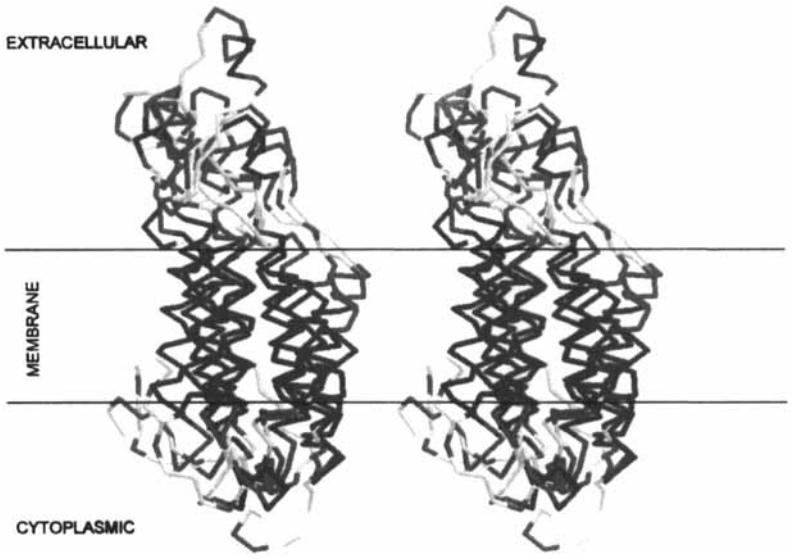

FIGURE 3 Stereoview of the three truncated models overlap of the β subunit of high affinity IgE receptor built starting from bundle 7. Overlapping was done on the C-α of the four helix bundle using Profit software. (See Color Plate XXI).

were applied to this model. Proteins, including integral membrane proteins in cells have constantly changing geometries, with movements occurring on femtosecond to millisecond (10^{-15} to 10^{-3} s) time scales. Molecular dynamics simulations, which combine molecular mechanics energy functions with Newton's equations of motions, have been used to study the internal movements in proteins, and also to refine 3D molecular structure. For example, a seven helix protein model can change from an initial circular arrangement (Fig. 1B) into a *Bacteriorhodopsin*-like shape (Fig. 1A) during 20–25 ps of molecular dynamics simulation in vacuo (Edvardsen *et al.*, 1992; Jahnig and Edholm, 1992). In this work, molecular dynamics calculations were used to study changes in molecular conformations, since upon addition of kinetic energy to the molecular system, a structure may move across conformational barriers and undergo substantial changes during such simulations.

The geometric changes during molecular dynamics were followed through displacement of the C-α atoms. The RMS deviation from the starting conformation were calculated and presented in different forms (Figs. 4 and 5). In these diagrams, there were initial molecular dynamics stages of cooling and minimizations (stages 15–18; Tab. I) after model building, and before simulation at 300 K presented as 0 ps in Figures 4 and 5. Also, there was an energy minimization at 25 ps (depicted as a vertical bar in

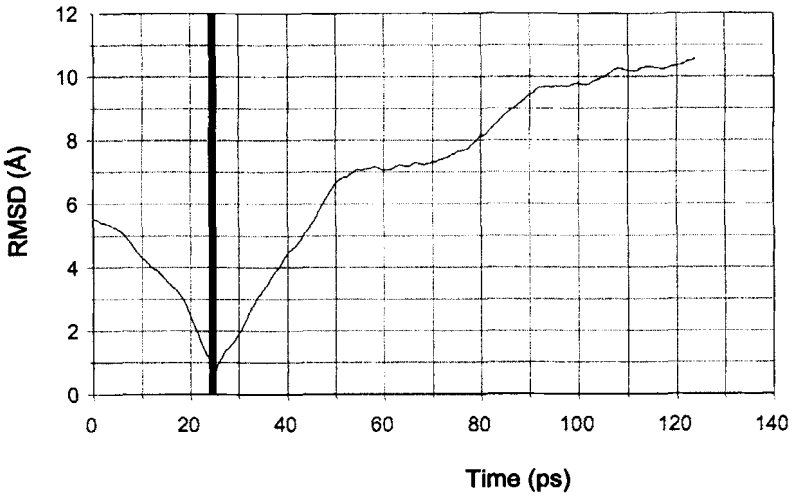

FIGURE 4 Geometric analysis of the β subunit based on bundle 7 at each 0.25 ps of the molecular dynamics run at 300 K. RMSD was calculated against starting conformation. C-α of the whole subunit were used for the calculations.

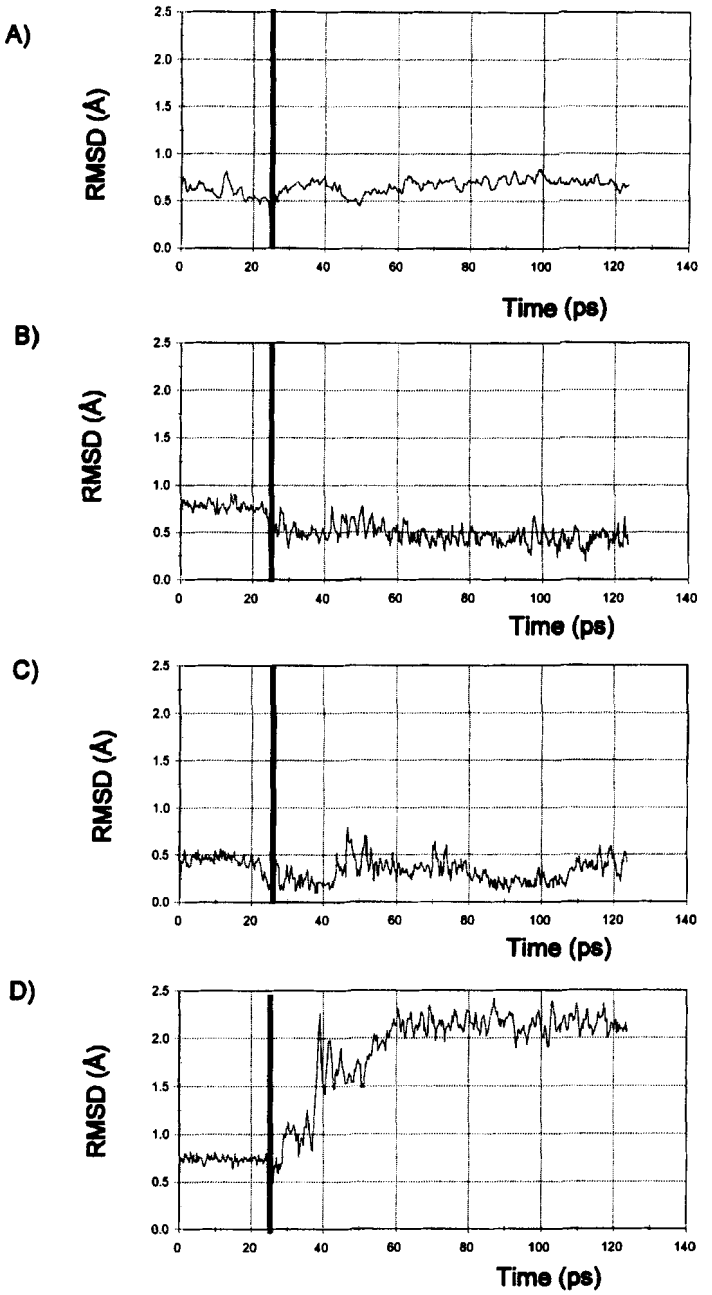

FIGURE 5 Geometric analysis of the β subunit based on bundle 7, at each 0.25 ps of the molecular dynamics run at 300 K. RMSD was calculated against the starting conformation, for the secondary structure elements set during building of the initial model. ((A) bundle - residues 60–80, 98–117, 130–150 and 180–200; (B) Loop 1–2 - residues 86–91; (C) Loop 2–3 - residues 121–126; (D) Loop 3–4 - 156–164). C-α atoms were used for the calculations.

the graphs), after all constraints were removed from the simulation process and dielectric constant was changed to 80 Db.

The RMSD calculations were performed against the initial model of the β subunit based on the Bundle 7. The conformation of the whole subunit changed considerably and the average structure would not be a good representative, since averaging of the model during simulation could lead to unrealistic geometries. The RMSD for the all atoms of the β subunit is presented in Figure 4. The initial displacement of the atoms during stages 15–18 was around 5.5 Å. This was followed by a decrease in the RMSD in the first 25 ps of the simulation (stage 19, Tab. I), while the dielectric constant was set to 1Db. The α helical hydrogen bonding constraints (called NOE constraints) were absent, and C-α atom constraints applied on the bundle were gradually relaxed. The simulation conditions directed changes towards the initial conformation before heating the structure to 500 K as seen in Figure 4. After the first 25 ps, the dielectric constant was changed, and the RMSD of all C-α atoms gradually increased to the 10.5 Å, indicating substantial change in the conformation.

The details of RMSD changes was followed both by behaviour of the bundle and of the helical motifs within connecting loop. The bundle and connecting loops parts did not change significantly during the first 25 ps (Figs. 5A–D), indicating that the cytoplasmic tails were principally responsible for the change. Between 25 and 125 ps of the simulation the bundle conformation did not change (RMSD fluctuated around 0.6 Å). The helical secondary structural elements of loops 1–2 and 2–3 (as determined by NMR based modelling) behaved similarly with an RMSD fluctuation between 0.4 and 0.5 Å. This implied that they possessed conformations similar to the initial model structures based on experimental data. Loop 3–4 exhibited a different behaviour, since the RMSD increased to 2.3 Å, and the structure changed significantly from the initial conformation (based on structure prediction). The structure of loop 3–4 was not significant for docking to Bundle 7. Specifically, the distance between the C-terminus of the TM helix 3 and N-terminus of the TM helix 4 was 20.1 Å in bundle 18, which could not be accommodated by the initial conformation of loop 3–4. Unfolding of loop 3–4 was thus observed. The presence of the two cysteine residues (C162 and C171) in the loop 3–4 further complicated the secondary structure prediction, since it has never been established whether or not they form a disulfide bridge.

The snapshot structures from the beginning and the end of the simulation are shown in Figure 6. In the agreement with the above arguments, the biggest change in the structure was observed for the cytoplasmic tails.

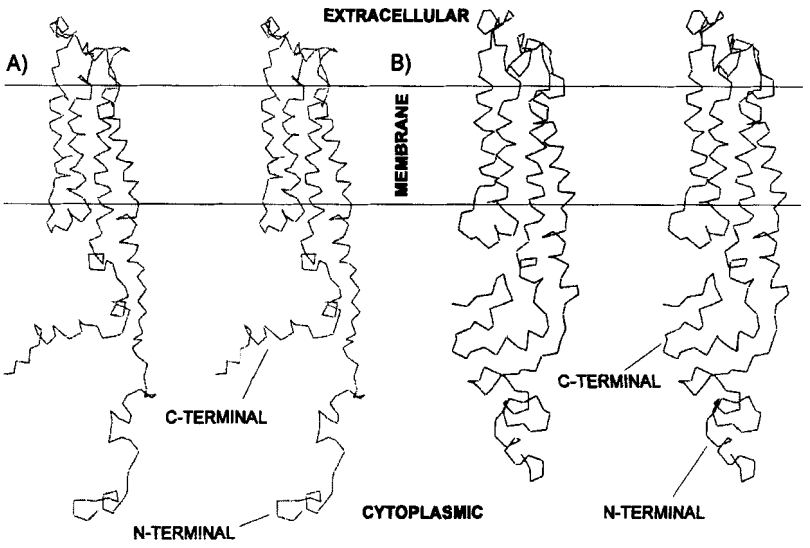

FIGURE 6 Stereoview of the C-α atoms representation of the β subunit models based on the bundle 7. Snapshot from the molecular dynamics simulation at (A) 0 ps and (B) 125 ps as defined for the Figure 4.

The relevant biological feature (cell signalling) of the β subunit detected experimentally was phosphorylation of the cytoplasmic C-terminal tail, which then interacted with Lyn SH2 domain of tyrosine kinase (Kihar and Siraganian, 1994).

The search of the PROMOTIF database detected two motifs – containing tyrosine phosphorylation sites in the C-terminal sequence. Both motifs had the sequence which appears twice in β subunit, cytoplasmic C-terminal tail:

$$[\mathrm{RK}] - X(2,3) - [\mathrm{DE}] - X(2,3) - \mathrm{Y}.$$

These were:

Site I – $\mathbf{K}^{211}\mathrm{VPD}^{214}\mathrm{DRLY}^{218}$;
Site II – $\mathbf{R}^{216}\mathrm{LYEE}^{220}\mathrm{LHVY}^{224}$.

The analysis of the van der Waals surface of the cytoplasmic domains in this work found that the relevant residues belonging to tyrosine phosphorylation site I were exposed to the solvent (Fig. 7) and available for the interaction with signal transduction kinases. The other site was not fully exposed in this model. However, the model was refined in the absence of the water molecules and lipid bilayer, so the further refinement

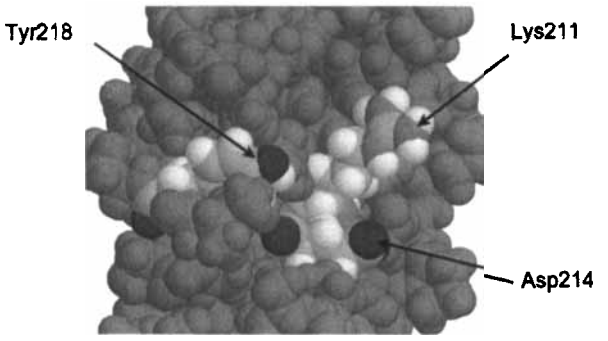

FIGURE 7 The van der Waals surface presentation of the cytoplasmic tails of the β subunit model I based on the bundle 7. All residues were coloured in green, except residues belonging to tyrosine phosphorylation sites, namely K211, D214 and Y218, which were coloured according to the CPK scheme. (See Color Plate XXII).

would be necessary, before studying phosphorylated subunit - SH2 protein interaction.

3.2.3. Summary of Model I

Few interesting features of this model could be pointed out:

- This model of the β subunit presented here was based on the docking of the TM helices and in every attempt the result was the four helix bundle, without introducing any constraints during docking procedure.
- The excellent agreement between calculated lipid-facing surfaces by helix–helix docking and molecular mechanics hydrophobic surface mapping.
- The NMR based structure of the connecting loops fit well into four helix bundle.
- The C-terminal tail of the β subunit in this model possessed the conformation adequate for the possible explanation of the signal transduction mechanism, but further work would be required in this direction.

3.3. Molecular Modelling of the β Subunit. Model II – Use of the Docked Four Helix Bundle and the Addition of the Loops and Tails in One Stage

The other approach cited in the literature has been applied to the dopamine receptor (Dahl and Edvardsen, 1994) and to the 5-HT_2 receptor (Kristiansen

et al., 1993; Kristiansen and Dahl, 1996; Sytle *et al.*, 1993; Sytle *et al.*, 1996). The loops and tails with predicted secondary structure were added to the 7-helix bundle, and resulting models were subjected to molecular dynamics simulation.

The work presented here was based on the similar approach. The use of the experimental data (NMR based structure of the loops 1–2 and 2–3) gives the extra strength to this work. According to the results of the TM helix docking (unpublished results), the bundle depicted as 8 could be most suitable subject for the modelling.

3.3.1. Molecular Dynamics Simulation of the Whole β Subunit

The geometric changes during molecular dynamics were again followed through displacement of the C-α atoms. The RMS deviations from the starting conformation were calculated and presented in different forms (Figs. 8 and 9). There were initial molecular dynamics stages of cooling and minimizations (stages 6–10; Tab. II) after model building, and before simulation at 300 K presented as 0 ps in the graphs 8 and 9. In addition, there was an energy minimization at 25 ps (depicted as a vertical bar in the graphs), after all constraints were removed from the simulation process and dielectric constant was changed to 80 Db.

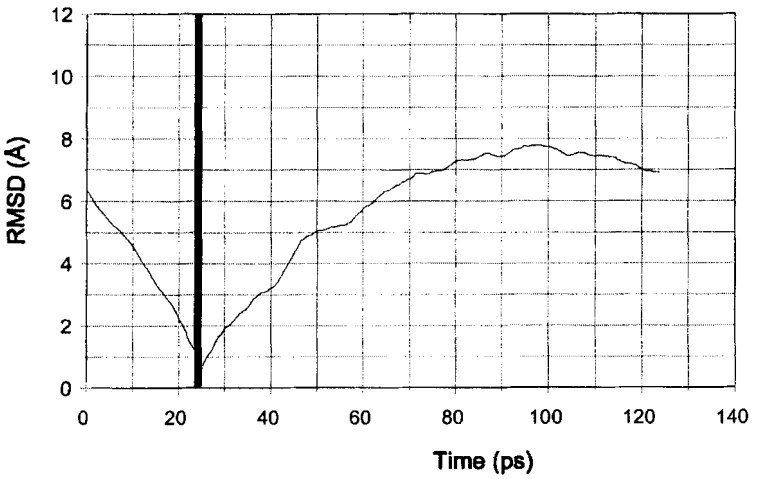

FIGURE 8 Geometric analysis of the β subunit based on the bundle 8, at each 0.25 ps of the molecular dynamics run at 300 K. RMSD was calculated against starting conformation. C-α atoms of the whole subunit were used for the calculations.

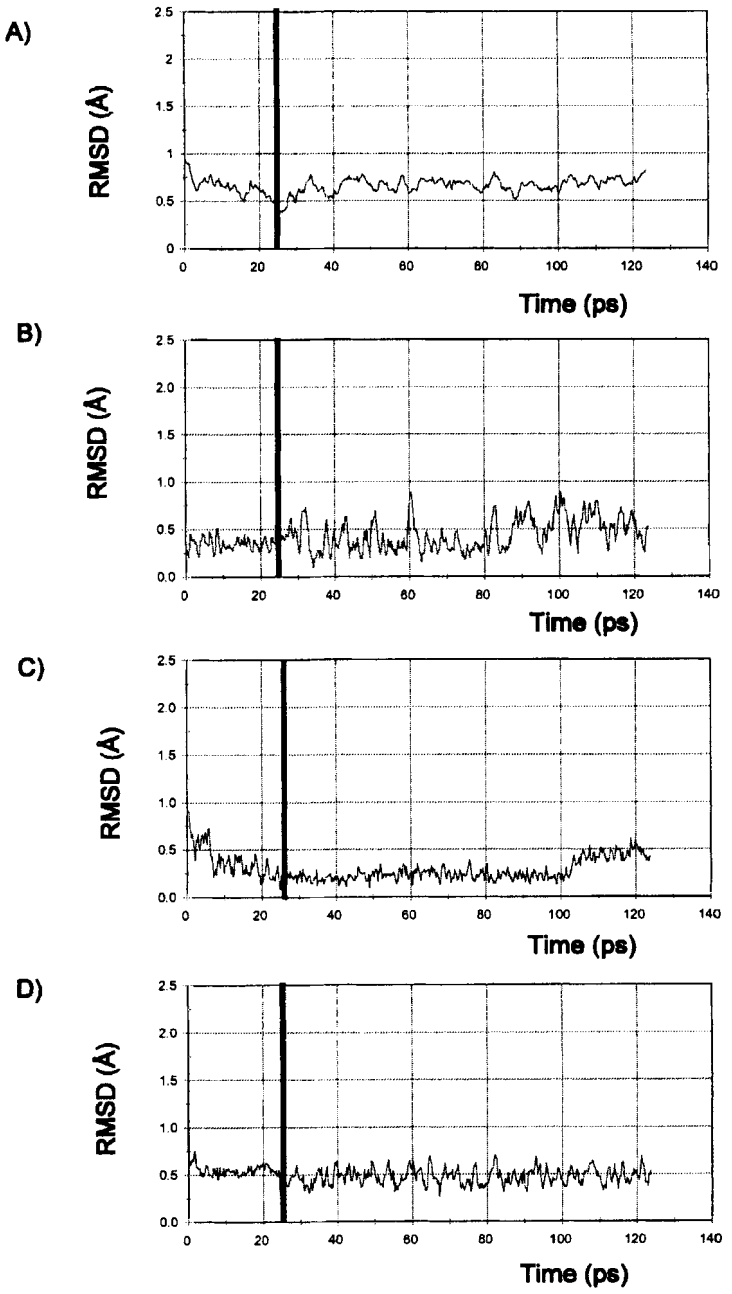

FIGURE 9 Geometric analysis of the β subunit based on the bundle 8, at each 0.25 ps of the molecular dynamics run at 300 K. RMSD was calculated against starting conformation, for the secondary structure elements set during building initial model. ((A) bundle - residues 60–80, 98–117, 130–150 and 180–200; (B) Loop 1–2 - residues 86–91; (C) Loop 2–3 - residues 121–126; (D) Loop 3–4 - 156–164). C-α atoms were used for the RMSD calculations.

The RMSD calculations were performed against the initial model of the β subunit. The RMSD for the all atoms of the β subunit is presented in Figure 8. The initial displacement of the atoms during stages 6 to 9 (Tab. II) was around 6.0 Å. This was followed by a decrease in the RMS in the first 25 ps of the simulation (stage 10), while the dielectric constant was set to 1 Db. The "NOE type constraints" were absent, and C-α atom constraints applied on the bundle were gradually relaxed. These simulation conditions directed changes towards the initial conformation. After the first 25 ps, the dielectric constant was changed to 80 Db as for the model I. The RMSD gradually increased to the 7.5 Å during this procedure, indicating changes in the conformation of the whole subunit. This RMSD change was smaller than the corresponding change of RMSD for the β subunit model I based on the Bundle 7.

The details of the RMSD changes were once again followed by monitoring both the behaviour of the bundle and the helical motifs of the connecting loops. The bundle and connecting loop helices did not change significantly during the first 25 ps (Figs. 9A–D), indicating that the cytoplasmic tails must have been changing. In the later stages of the simulation the bundle conformation did not change (RMSD fluctuated around 0.7 Å). The helical secondary structure elements of all three loops exhibited similar behaviour, with their RMSD fluctuating between 0.2 and 0.7 Å. This implied that they had similar conformations to the initial models based on the experimental and theoretical data. The results for loop 3–4, however, were different from the results in the model II section, where the RMSD increased to the 2.3 Å. In this case, structure did not deviate far from of the initial conformation (based on the structure prediction). The initial structure prediction of loop 3–4 was not suitable for the conformation Bundle 7, where the distance between the C-terminus of the TM helix 3 and the N-terminus of the TM helix 4 was 20.1 Å. The distance between C-terminus of the TM helix 3 and N-terminus of the TM helix 4 for the Bundle 8 was by comparison only 5.4 Å. The loop 3–4 initial conformation was therefore more suitable to Bundle 8 than Bundle 7. The unfolding of this loop was not observed when attached to Bundle 8.

A comparison of the snapshot structures from the beginning and the end of the simulation were shown in Figure 10. Again, the biggest change in the structure was observed at cytoplasmic tails.

The analysis of the van der Waals surface of the cytoplasmic domains revealed that residues belonging to tyrosine phosphorylation site II were exposed to the solvent (Fig. 11) and available for the interaction with signal transduction proteins. The other site was not fully exposed in this

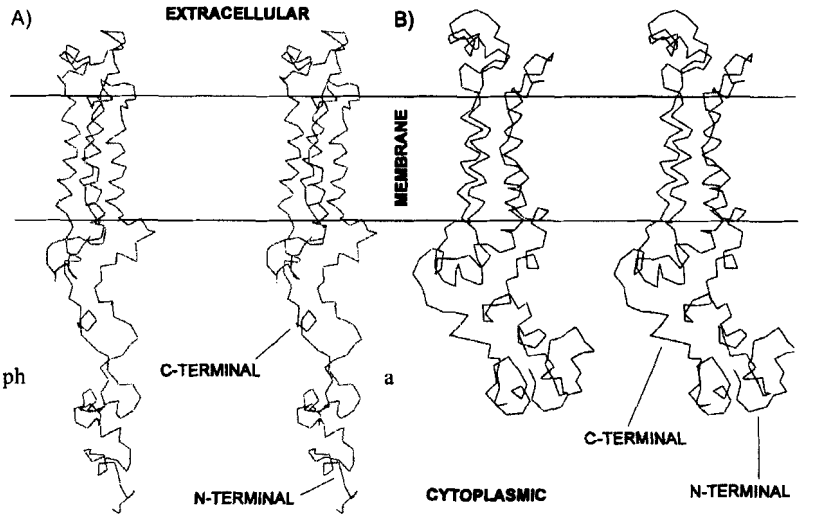

FIGURE 10 Stereoview of the C-α presentation of the β subunit models based on the bundle 8. Snapshots from the molecular dynamics simulation at (A) 0 ps and (B) 125 ps as defined for the Figure 8.

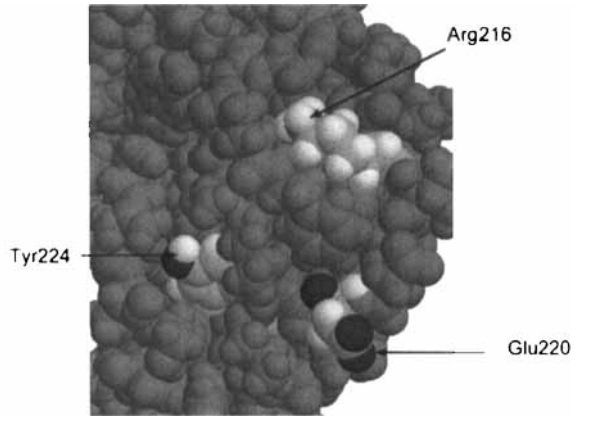

FIGURE 11 The van der Waals surface presentation of the cytoplasmic tails of the β subunit model based on the bundle 8. All residues were coloured in green, except residues belonging to tyrosine phosphorylation sites, namely R216, E220 and Y224, which were coloured according to the CPK scheme. (See Color Plate XXIII).

model as it was in model I. Again, the model was refined in the absence of the water molecules and lipid bilayer, and thus further refinement would be necessary in order to study phosphorylated subunit – SH2 protein interaction.

3.3.2. Summary of Model II

The conclusions that could be drawn for this model.

- The model of the β subunit presented here had the four helix bundle motif in the transmembrane domain.
- It had a clockwise arrangement of the four helix bundle as viewed from the extracellular side, but the question of the clockwise or anticlockwise arrangement is still not resolved.
- The very good agreement between calculated lipid-facing surfaces by helix–helix docking and molecular mechanics hydrophobic surface mapping.
- The NMR based structure of the connecting loops fit well into four helix bundle.
- The C-terminal tail of the β subunit in this model possessed the conformation adequate for the possible explanation of the signal transduction mechanism, but further work would be required in this direction.

This and the previous model have to be tested for correctness and accuracy through obtaining further experimental data and explaining the biological and biochemical facts described in the introduction.

4. CONCLUSION

Computer modelling of the 3D structure of the 243 residue β subunit if the Fc-ε-RI, high affinity IgE receptor, was carried out using some combination of molecular mechanics, molecular dynamics and spectrometry data (circular dichroism and NMR distances). It was assumed (based on the Blank *et al.*, 1989) that the β subunit consisted of four transmembrane α helices, three "loop peptides" that connected these helices, and two cytoplasmic domain peptides.

Through CD and NMR spectroscopy, the conformational behaviour of the three interconnecting loop peptides of the β subunit of the high affinity IgE receptor was studied. In all three cases some degree of helical content was found. NMR and CD were used to calculate the structure of two loop peptides (loop 1–2 and loop 2–3). Loop 3–4 peptide structure was calculated using only structure prediction and molecular mechanic calculation and the structure were demonstrably consistent with the CD data. The two cytoplasmic peptide structure were calculated in similar manner as for loop 3–4.

The calculation of the three-dimensional structure of the four membrane-spanning segments of the β subunit using rules of helix–helix packing arrangements and molecular dynamics yielded a four-helix bundle with specific van der Waals interactions between the TM helices. These four-helix bundles were used as a framework upon which to calculate the conformation(s) of the β subunit.

Although different starting points were used, essentially similar conformations were reached for the two models, namely:

- The β subunit has a 4 helix bundle structure.
- For each pair of consecutive transmembrane helices and their interconnecting loop peptide, a **TM helix - turn - loop helix - turn - TM helix** motif was found to be stable and within the range of possibilities of secondary structure elements shown by the CD and NMR spectroscopy of the peptide loops.
- Similar external (helix–lipid interacting) and internal (helix–helix interacting) surfaces were predicted for TM helices in both models.

In these calculations it was assumed that the β subunit existed as a single monomeric species. We are aware that the spatial arrangement of the transmembrane domains and disposition of helices of the β subunit could change when interacting with α and γ dimer structural subunits. The relationship to these other subunits, which probably occurs after the folding of the β subunit, is at present unknown and could be due to both intra and extramembraneous interactions, some of which can be hypothesized from the present data.

The other important feature of this work is the possible explanation of the signal transduction mechanism on the molecular level. The tyrosine phosphorylation motif, found twice in the sequence of the C-terminal tail of the β subunit, was on the exterior of the van der Waals surface of the cytoplasmic domains in molecular models. The solvent exposed residues of the motif would be available for the interactions with the signal transduction proteins. Thus, these models could be basis for the studying of the signal transduction pathways in allergy and inflammation.

The elucidation of domain conformations opens new possibilities for exploring *in vitro* a model system of high affinity IgE receptor for investigating the mechanisms of immediate hypersensitivity and the general mechanisms that mediate stimulus-secretion coupling. The next step in this set of calculations will be the addition of the α subunit and of the γ dimer TM helices and cytoplasmic and extracellular domains. The determination

of the mode of packing of the seven helices of the receptor should allow the modelling of all the extracellular and cytoplasmic loops and tails of the four subunits, as it was partially described in this work. A better understanding of the receptor conformation is crucial for improving drug design targets related to allergy and inflammations.

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