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Rapid report

Solution structure of the sixth transmembrane helix of the G-protein-coupled receptor, rhodopsin[1]

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Abstract

Low resolution electron density maps have revealed the general orientation of the transmembrane helices of rhodopsin. However, high resolution structural information for the transmembrane domain of the G-protein-coupled receptor, rhodopsin, is as yet unavailable. In this study, a high resolution solution structure is reported for a 15 residue portion of the sixth transmembrane helix of rhodopsin (rhovih) as a free peptide. Helix 6 is one of the transmembrane helices of rhodopsin that contains a proline (amino acid residue 267) and the influence of this proline on the structure of this transmembrane domain was unknown. The structure obtained shows an α-helix through most of the sequence. The proline apparently induces only a modest distortion in the helix. Previously, the structure of the intradiskal loop connected to helix 6 was solved. The sequence of this loop contained five residues in common (residues 268–272) with the peptide reported here from the rhovih. The five residues in common between these two structures were superimposed to connect these two structures. The superposition showed a root mean square deviation of 0.2 Å. Thus, this five residue sequence formed the same structure in both peptides, indicating that the structure of this region is governed primarily by short range interactions.

Keywords: Rhodopsin; Membrane protein structure; Nuclear magnetic resonance

Rhodopsin is the photosensitive pigment of the dim-light photoreceptor rod cell. Rhodopsin is a member of the super family of G-protein-coupled receptors that share the seven transmembrane helix structural motif. These receptors regulate a wide variety of sensory, hormonal and neural responses. The transmembrane domain of rhodopsin was suggested to consist of a bundle of seven helices from circular dichroism experiments [1], primary sequence [2] and recent low resolution structure determinations [3,4].

Rhodopsin is composed of the apoprotein, opsin, a single polypeptide chain of 348 amino acids, and 11-*cis* retinal linked to Lys-296 by a Schiff base. Upon absorption of a photon of light, the 11-*cis* retinal photoisomerizes to all-*trans* retinal, inducing a conformational change in the receptor protein, which allows the receptor to activate a G-protein, thereby initiating the signal transduction cascade.

To understand this process, detailed structural information is needed for this receptor. No high reso-

Abbreviations: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; rhovih, sixth transmembrane helix of rhodopsin

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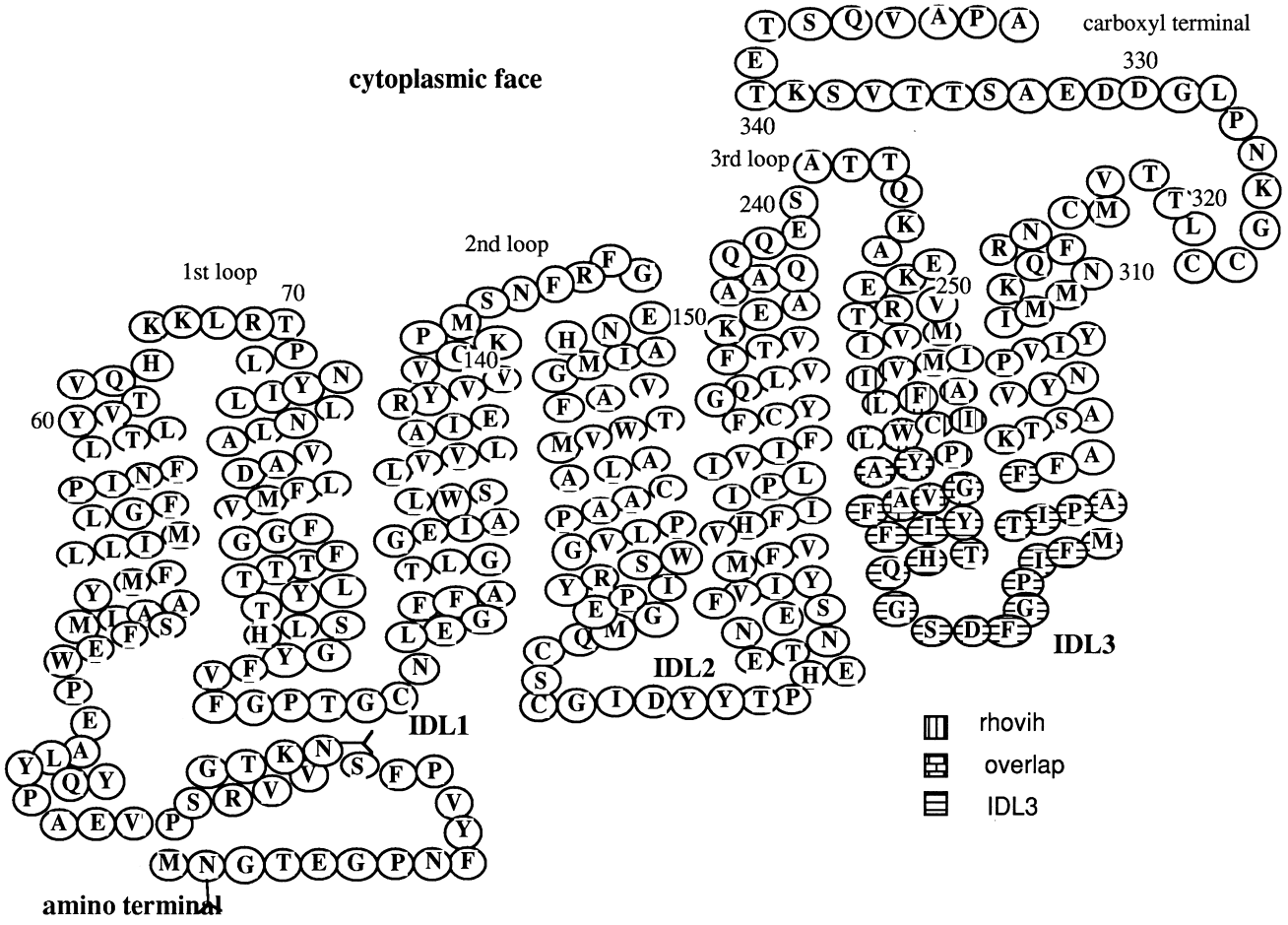

Fig. 1. Schematic representation of the amino acid sequence of rhodopsin. The darkly shaded region indicates the sequence from the putative rhovih that was used in the peptide in this structural study. The lightly shaded region represents the intradiskal loop connected to the sixth transmembrane helix discussed in the text.

lution structure of rhodopsin has yet been reported. We have been pursuing an alternative approach to rhodopsin structure. Previously, we reported that peptides corresponding to the cytoplasmic loops and carboxyl-terminus of rhodopsin formed stable structures in solution [5–8]. These experiments suggested that secondary structures such as α-helix and β-turn in rhodopsin were stabilized by short range interactions coded by the primary structure. In this report, the structure of a peptide encompassing much of the sequence of the sixth transmembrane helix was determined by two-dimensional nuclear magnetic resonance (NMR).

The following peptide representing the sequence of the sixth transmembrane helix of rhodopsin (rhodopsin amino acid residues 258–271, rhovih) was synthesized through solid phase synthesis in the Biotechnology Center at the University of Connecticut: VIAFLICWLPYAGVA.

Fig. 1 shows this sequence in the primary structure of rhodopsin [2].

All NMR spectra were recorded on a Bruker AMX-600 spectrometer at 30°C in DMSO because this peptide was not stable in aqueous solution (the peptide aggregated soon after being introduced into solution) or in trifluoroethanol. This is in agreement with the results on the solubility of the seventh transmembrane helix of both the tachykinin receptor and rhodopsin [9]. Standard pulse sequences and phase cycling were employed to record double quantum-filtered COSY spectra and NOESY spectra (400 ms mixing time) [10]. All spectra were accumulated in a

Table 1
Chemical shifts for rhovih

Residue	HA	NH	HB	Others
Val-1	4.23		2.02	0.79
Ile-2	4.26	7.89	1.72	HG 1.44, 1.05; HD 0.77
Ala-3	4.26	8.04	1.13	
Phe-4	4.54	7.9	2.78, 300	
Leu-5	4.36	8.055	1.44, 1.45	HG 1.44, 0.78
Ile-6	4.18	7.83	1.67	HG 1.43, 1.21
Cys-7	4.31	7.84	2.71, 2.91	
Trp-8	4.59	7.97	2.90, 3.11	7.50, 7.48
Leu-9	4.4	8.09	1.43, 1.56	0.8
Pro-10	4.51		1.74	HD1 3.47, 3.38
Tyr-11	4.72	7.68	2.69, 2.91	2,6H/6.97
Ala-12	4.46	7.53	1.36	
Gly-13	3.73	7.95		
Val-14	4.18	7.7	1.74	QG1 0.86; QQG 0.78
Ala-15	4.47	7.576	1.35	

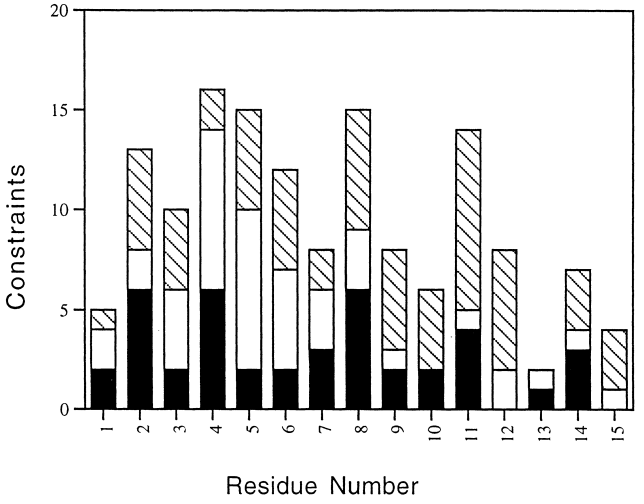

Fig. 2. Number of constraints per residue for the rhovih: intra-residue (filled bars), sequential (open bars), long range (hatched bars).

phase sensitive manner using time proportional phase incrementation for quadrature detection in F1. Chemical shifts were referenced to internal water in DMSO. Sequence-specific assignments were obtained using standard approaches and the results are listed in Table 1.

The sequence-specific assignment of the ^{1}H NMR spectrum for the peptide was carried out using standard methods employing FELIX (MSI). Assigned nuclear Overhauser effect (NOE) cross peaks were segmented using a statistical segmentation function and characterized as strong, medium and weak corresponding to upper bounds distance range constraints of 2.7, 3.5 and 5.0 Å, respectively. Lower bounds between non-bonded atoms were set to the sum of their van der Waals radii (approximately 1.8 Å). Pseudoatom corrections were added to interproton distance restraints where necessary [11]. Distance geometry calculations were carried out using the program DIANA [12] within the SYBYL 6.4 package (Tripos Software, St. Louis). First generation DIANA structures, 150 in total, were calculated. Energy refinement calculations (restrained minimizations/dynamics) were carried out on the best distance geometry structures using the SYBYL program implementing the Kollman all-atom force field. Statistics on structures were obtained from X-plor. These calculations were performed on a Silicon Graphics R10000 computer. Imaging and superposition of the resulting structures and construction of the transmembrane helices of rhodopsin were performed on a Power Mac with MacImdad (Molecular Applications Group, Palo Alto, CA, USA).

Fig. 3. Long range NOEs observed for rhovih.

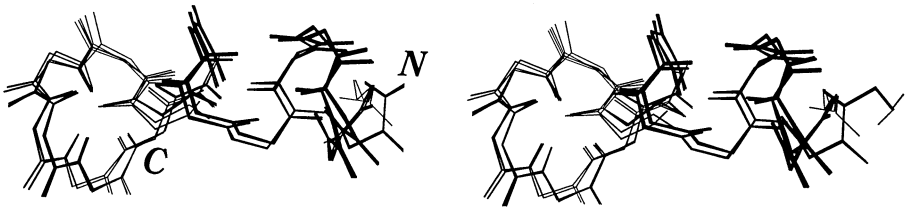

Fig. 4. Overlay of the six best DIANA structures for rhovih.

Residues 258–272 of bovine rhodopsin constitute a significant portion of the putative sixth transmembrane helix of the transmembrane helical bundle of this G-protein-coupled receptor (see Fig. 1). This peptide was synthesized and the solution structure was determined by homonuclear two-dimensional NMR as described above. NOESY and COSY data were assigned and used to generate 92 distance constraints: 42 intraresidue, 20 sequential and 11 long range. Only one set of NOEs was observed for each residue, consistent with a single dominant structure in solution. Fig. 2 shows the number of constraints per residue. Fig. 3 shows the pattern of long range constraints, including the i to i+3 and i to i+4 interactions characteristic of α-helix. DIANA structures were generated and only one family of structures was observed. Fig. 4 shows an overlay of the six best DIANA structures. Fig. 5 shows the root mean square deviation (rmsd) values for this family of structures. The peptide is predominantly helical in structure. This peptide includes a proline at rhodopsin position 267. This proline does not appear to cause a strong perturbation in the helical structure. It is interesting to contrast this to helix 7 in which the highly conserved Pro-303 induces a break in the helix (unpublished). Mutations in which Pro-267 is substituted for Leu or Arg lead to retinitis pigmentosa [13,14].

The seven transmembrane helix motif of rhodopsin is now well accepted. As described above, several lines of evidence have suggested that the transmembrane domain of rhodopsin is a helical bundle. The residues that constitute the helices were assigned based largely on the hydrophobicity of the amino acid residues [2]. The Baldwin model has assigned residues to helical positions based upon extensive comparisons to other G-protein receptors [15]. The data presented here demonstrate experimentally that the peptide consisting of the amino acid sequence assigned to helix 6 readily assumes an α-helical conformation and is consistent with prior analysis suggesting a transmembrane helical bundle [1–4]. Furthermore, this secondary structure is not dependent upon the sequence being part of the intact protein.

The investigation of peptide structures as a means to study structural elements of the intact protein is valid if the conformation assumed by a sequence of amino acid residues is essentially the same whether it is part of a peptide or part of a protein. We have previously shown that the structural elements exhibited by the peptide consisting of carboxyl-terminal 19 amino acid residues of rhodopsin were substantially the same when the peptide was lengthened to include 33 then 43 amino acid residues [7,8,16]. We have also observed that while structures of the loops became better defined as the peptides representing these regions were lengthened, the basic loop motif remained (unpublished observations).

Helix 6 is connected to the third intradiskal loop, whose structure has been determined (manuscript

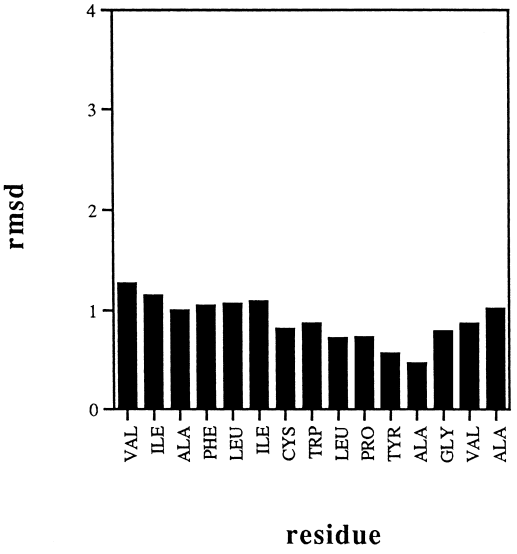

Fig. 5. rmsd values for the family of structures in Fig. 4.

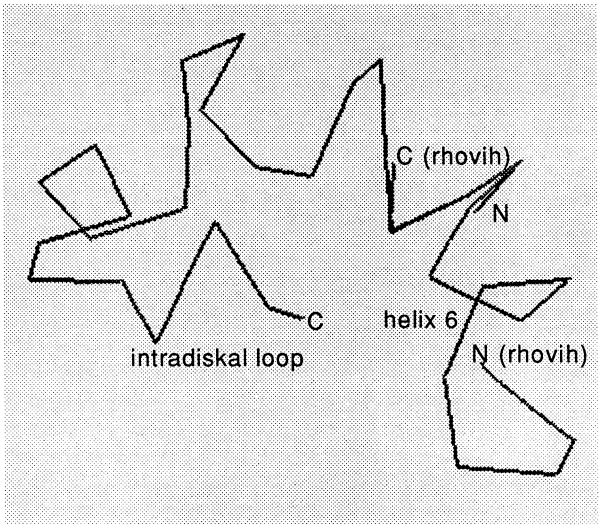

Fig. 6. Result when the five residues in common between rhovih and the structure of the connect intradiskal loop are superimposed.

submitted). That structure contains five residues in common with the peptide from the rhovih. Thus, the carboxyl-terminus of the peptide representing helix 6 is identical to the amino-terminus of the peptide representing the third intradiskal loop. If the conformations assumed by these peptides are governed by short range interactions, the conformations of the overlapping ends should be the same. Therefore, the five residues in common between these two structures (Tyr-268 → Ala-272) were superimposed and the result appears in Fig. 6. The five residue segment from both peptides superimposes well, with an rmsd of 0.2 Å for the backbone atoms.

The excellent agreement between the structures that the segment Tyr-268 → Ala-272 adopts in the peptide from transmembrane helix 6 and in the peptide from the third intradiskal loop of rhodopsin (see Fig. 1) indicates that this structure is stabilized by short range interactions coded by the amino acid sequence. This observation is consistent with a body of information that indicates that secondary structures such as α-helices and β-turns are stabilized by short range interactions. These observations suggest that such secondary structures may be stable both in the intact protein and in peptides derived from that protein, as was shown for myohemerythrin [17].

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