Molecular Model of the Cyclic GMP-Binding Domain of the Cyclic GMP-Gated Ion Channel

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Received December 20, 1991; Revised Manuscript Received March 11, 1992

ABSTRACT: The structure of the cyclic GMP-binding domain of the cyclic GMP-gated ion channel from bovine retinal rod photoreceptors has been modeled by analogy to the crystal structure of the homologous cyclic AMP-binding domain of catabolite gene activator protein (CAP). The modeled cyclic GMP-binding domain has a three-residue deletion and a five-residue insertion between β strands compared to CAP. The major interactions of the ion channel with cyclic GMP are similar to those observed for cyclic AMP bound to CAP and predicted for cGMP bound to the cGMP-dependent protein kinase: Gly 543 and Glu 544 make hydrogen-bond interactions with the ribose 2′-OH, Arg 559 forms an ion pair with the charged phosphate oxygen, and Thr 560 forms hydrogen-bond interactions with an exocyclic phosphate oxygen and with the 2-amino group of cGMP. Three additional potential interactions were predicted from the model structure. Ile 545 O and Ser 546 OH form hydrogen-bond interactions with an exocyclic phosphate oxygen, and Phe 533 may interact with the aromatic ring of cGMP. This model is in agreement with both the analogue binding experiments and the mutational analysis of Thr 560.

In vertebrates, rod and cone photoreceptors and olfactory cilia contain cation-selective ion channels that are directly and cooperatively gated by cyclic nucleotides (Fresenko et al., 1985). For instance, the cation channels which control the flow of ions across the surface membrane of vertebrate rod and cone photoreceptor cells are directly gated by guanosine 3′,5′-monophosphate (cGMP). The concentration of cGMP increases or decreases on the basis of the cell response to light and thus controls the channel opening or closing: cGMP serves as a intracellular excitatory messenger. A similar kind of channel gated by both cyclic adenosine 3′,5′-monophosphate and cyclic guanosine 3′,5′-monophosphate exists in vertebrate olfactory sensory neurons. Both of these ion channels regulate signal transduction. Cyclic nucleotides regulate many biological activities, so it is important to understand how cAMP and cGMP bind to their receptors and the mechanism of regulation.

The amino acid sequences of the cyclic GMP-gated ion channel (cGGC) from bovine rod photoreceptors (Kaupp et al., 1989) and the cyclic nucleotide-gated ion channel from olfactory neurons (Dhallan et al., 1990; Ludwig et al., 1990) have recently become available. The cGGC from bovine retina consists of a single polypeptide of 690 amino acid residues and relative molecular mass M_r of 63 000 (the cDNA has an open reading frame that encodes 690 amino acid residues with a calculated M_r of 79 601) (Kaupp et al., 1989). The hydropathicity profile (Kyte & Doolittle, 1982) of the rod channel suggested that there were four to six membrane-spanning regions (referred to as H1–H6), each comprising approximately 20 amino acid residues (Kaupp et al., 1989). A seventh hydrophobic region of approximately 80 amino acid residues (residues 498–577) located on the C-terminal side of segment H6 shows a significant sequence similarity with other cyclic nucleotide-binding sites, in particular to the cGMP-dependent protein kinase. This region of cGGC also shows similarities with other cyclic nucleotide-binding proteins such as the cyclic AMP-binding domain of the *Escherichia coli* catabolite gene activator protein (CAP) (Kaupp et al., 1989).

The crystal structure of catabolite gene activator protein (CAP) complexed with cAMP is the only experimentally determined structure that contains a cyclic nucleotide-binding site. This was previously used to model the cyclic nucleotide binding domains of mammalian cAMP- and cGMP-dependent protein kinases (cAPK and cGPK) (Weber et al., 1987; Weber et al., 1989), which are involved in the regulation of many biochemical pathways. These models were used to predict the amino acid residues that form the cAMP- and cGMP-binding sites. Subsequently, the positions of most of the important cAMP-binding residues have been confirmed by sequencing of mutations with altered cAMP-binding properties (Bubis et al., 1988; Ogreid et al., 1988; Woodford et al., 1989; Correll et al., 1989). The models of the cyclic nucleotide binding domains of the protein kinases have proved useful in analysis and design of mutations (Steinberg et al., 1991; Shabb et al., 1990). In the present study, we have used the information from sequence similarities among these cyclic nucleotide receptors to construct a molecular model of the cyclic nucleotide-binding domain of the ion channel by analogy to the crystal structure of CAP with cAMP.

Materials and Methods

The refined coordinates of wild-type CAP with bound cAMP provided the basic structure (Weber & Steitz, 1987). A preliminary sequence alignment was made for CAP, cGPK, and cGGC, in which deletions and insertions were positioned between elements of secondary structure in CAP. cGGC is predicted to contain a cyclic nucleotide-binding domain similar to those in CAP and cGPK. The amino acid sequence for this region of cGGC showed 17% identity with residues 10–110 of CAP and 21% and 24% identity with the corresponding first and second cGMP-binding domains of cGPK. Therefore, the previously built model of the second, or B, cGMP-binding domain of cGPK (Weber et al., 1989) was used as a starting point for modeling the cGGC. Earlier the cAMP-binding domain of cAPK had been built from the crystal structure of CAP refined at 2.5-Å resolution (Weber et al., 1987). The

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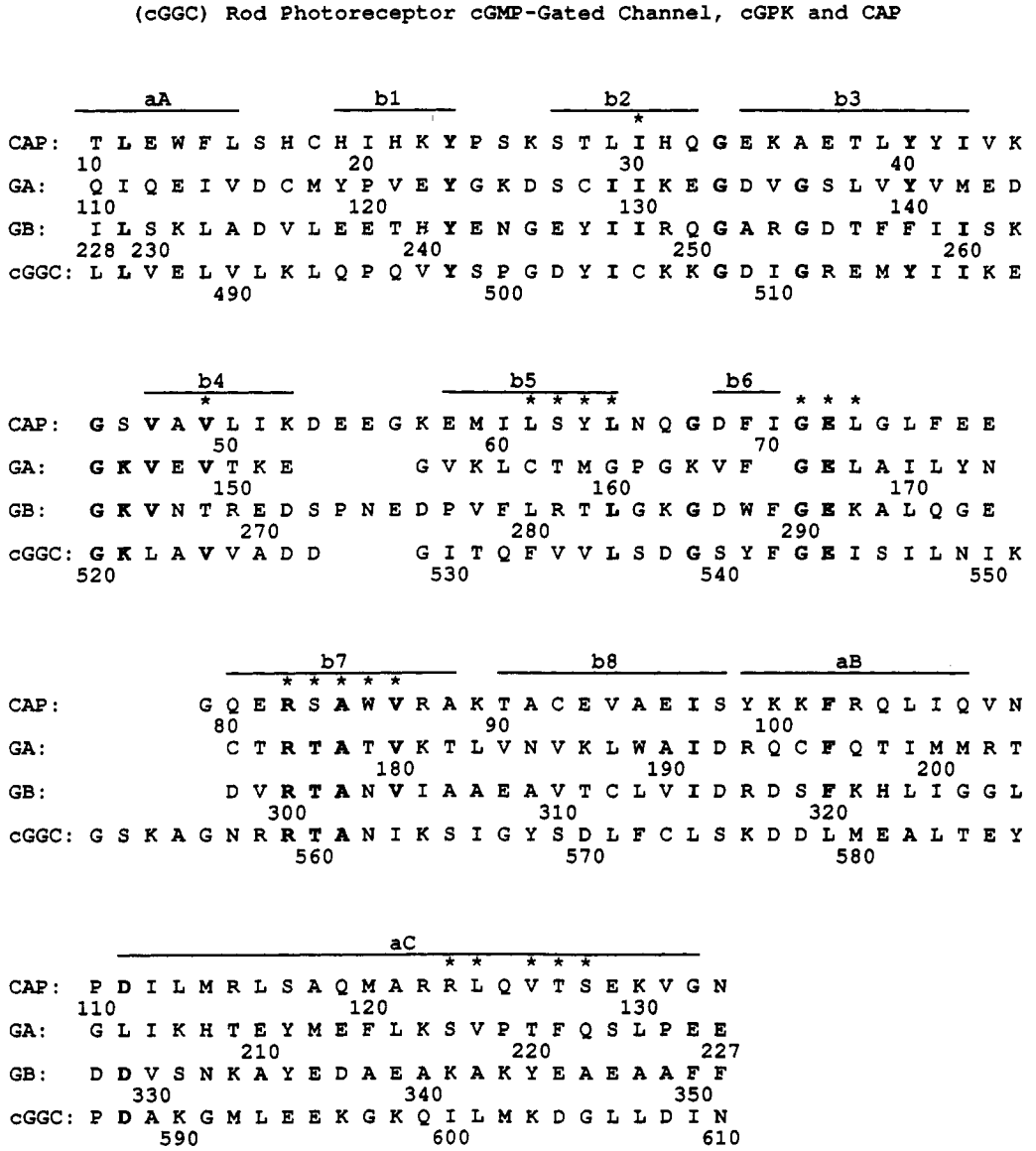

FIGURE 1: Amino acid sequences of the cyclic nucleotide-binding domains of CAP, cGPK, and cGGC in alignment based on the structure of CAP with cyclic AMP. The single-letter amino acid code is used, and alignments of three or more identical residues are indicated in bold letters. Residues forming the cAMP-binding site in CAP are indicated by asterisks. The elements of the secondary structure (aA–aC for α-helices, and b1–b8 for β-sheets) in CAP are indicated. GA and GB are the two cyclic nucleotide-binding domains of cGPK.

pattern of amino acid deletions or insertions also suggested that the cGMP-binding domain of cGGC was most similar to the B rather than the A domain of cGPK. In both the cAPK and cGPK, the B domains have more similarity in amino acid sequence with CAP than have the A domains. This suggested that the model three-dimensional structures for the B domains and for cGGC will be similar to the crystal structure of CAP. The molecular models, including amino acid substitutions, and side chain adjustments were examined with an Evans and Sutherland PS390 color graphics system using the program FRODO (Jones, 1978).

The model coordinates were energy minimized using the XPLOR package (Brunger, 1990) version 2.1 implemented on Cray-XMP computer at the Advanced Scientific Computing Laboratory at NCI–Frederick Cancer Research facility. The nonbonded terms of the empirical energy function consisted of a 6–12 van der Waals potential (approximated by the Lennard-Jones potential energy function) and a Coulombic potential with an 8-Å cut-off. No additional hydrogen-bond energy term was used. The effect of hydrogen bonds is taken into account by appropriate parameterization of the partial charges and van der Waals parameters (Brunger, 1990). The conformational terms of the empirical energy function consisted of terms involving dihedral angles, chirality or planarity, covalent bond, and bond angle energy. The hydrogen positions for the polar atoms are added and refined in XPLOR. The standard topologies and parameters for both the protein and DNA libraries were taken from CHARMM (Brooks et al., 1983) with slight modifications.

Five hundred energy minimization cycles were carried out with Powell-method conjugate gradient algorithm (Powell, 1977). The α-carbon atoms were restrained using harmonic restraints, and the minimization was carried out until the maximum gradient was less than 0.1 kcal/mol. The energies of the resulting model calculated from XPLOR are shown only for comparison between CAP and the ion channel model and are not absolute values. The accessible surface areas were calculated using the algorithm of Lee and Richards (1971) implemented in the XPLOR package.

RESULTS

Model Building. The alignment of the amino acid sequences of the cGMP-gated ion channel, cGPK, and CAP are shown for the cyclic nucleotide-binding domains (Figure 1). The

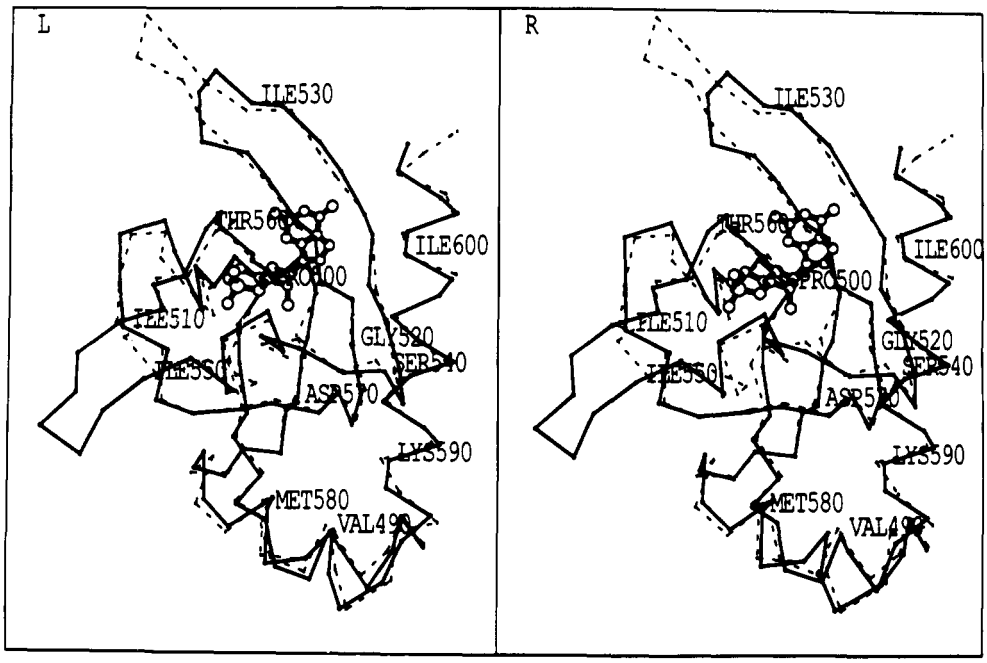

FIGURE 2: Stereoview of the Cα atoms of the modeled domain of cGGC (continuous lines) compared to the Cα atoms of the CAP cAMP-binding domain from the crystal structure (dashed lines). The cGMP is shown in a ball-and-stick representation in the binding site.

alignment is based on the crystal structure of CAP, and deletions and insertions were positioned between elements of secondary structure in CAP. The sequence numbering in CAP and cGGC is from 10 to 110 and 485 to 602, respectively. The numbering in CAP is used as a standard for comparison of the model throughout this section.

The amino acid side chains of the cGMP-binding domain of cGPK were replaced by those of cGGC in the positions were the sequences differed from residue Leu 485 to Met 602. During this procedure, the positions of the side-chain atoms of the substituted residues were checked on the computer graphics system for steric conflicts with neighboring atoms. Two non-hydrogen-bonding atoms at less than 2.6 Å apart was considered a steric collision. Occasionally an adjustment in the position of the amino acid side chain was required, and in such instances the torsion angles were adjusted to avoid such collisions. The model building and regularization routine REFINE (Hermans & McQueen, 1974) in FRODO was used during the substitutions to restore ideal geometry.

Deletions or insertions relative to CAP occur in only two places: between the antiparallel β strands 4 and 5 which form a surface turn; and near the start of β strand 7. The connection between β strands 4 and 5 has a variable length in the different CAP-like cyclic nucleotide-binding domains. It is four residues shorter than in CAP in the first cAMP-binding domain of type I regulatory subunit of cAPK and longer by seven residues in the second cAMP-binding domain of type II regulatory subunit (Weber et al., 1987). The ion channel is three residues shorter than CAP in this region. In CAP, the β4–5 turn lies near the DNA-binding domains. Since this region shows many variable residues, it may be involved in intersubunit or interdomain interactions in the other cyclic nucleotide-binding domains. The turn before β7 is one residue shorter in all the protein kinases; however, it is apparently extended by five residues relative to CAP in the ion channels. This extension was approximately modeled as an antiparallel β turn, since it is expected to form a loop on the protein surface. Residues from β7 in CAP form several important interactions with cAMP. These residues, in particular Arg 82, are conserved among all of these cyclic nucleotide-binding domains, so we expect that the structure will also be conserved, and no changes were made to the polypeptide backbone in β7.

Table I: Energy Minimization of the Cyclic Nucleotide-Binding Domains of CAP and cGGC Ion Channel

	CAP	cGGC
RMS movements, main chain (Å)	0.637	0.770
RMS movements, side chain (Å)	1.039	1.208
av conformational energy per residue (kcal/mol)	5.219	5.228
accessible surface (Å²)[a]	6929.37	6963.49
hydrophobic accessible surface (Å²)	74.0%	74.2%
interaction energy (kcal/mol)[b]		
total	−22.514	−21.950
vdw	−8.811	−8.287
elec	−13.703	−13.663

[a] The hydrophobic accessible surface areas were the sums of exposed surface areas of carbon atoms. [b] Interaction energy was calculated as the sum of nonbonded energies between atoms of the cyclic nucleotide and atoms of the protein within a distance of 8.0 Å.

There are six residues that are identical in all of these CAP-like cyclic nucleotide binding domains. The glycines between β2 and -3 and between β3 and -4 are conserved and have a positive φ torsion angle in CAP. This conformation is energetically favorably only for glycine, so the conservation of Gly 33 and 45 indicates that the three-dimensional folding is also conserved. Gly 71, Glu 72, and Arg 82 are identical in all these domains. These residues form hydrogen-bond and ionic interactions with cAMP in CAP. Mutations of these residues reduce or eliminate cAMP binding to the altered site in cAPK (Steinberg et al., 1991; Shabb et al., 1990). Ala 84 is also conserved and forms part of the cyclic nucleotide binding pocket.

Model Structure. The model structure of the cGMP-binding domain of cGGC after energy minimization is compared with the cAMP-binding domain of CAP in Figure 2. The root mean square (rms) deviations of the protein atoms were higher for the cGGC model than after the same minimization procedure with the cAMP-binding domain of the CAP crystal structure. The rms difference between the cGGC model before and after energy minimization was 0.77 Å for the main-chain atoms and 1.21 Å for side chain atoms (Table I) compared to values 0.64 and 1.04 Å observed for CAP. The largest differences were centered around the insertion between

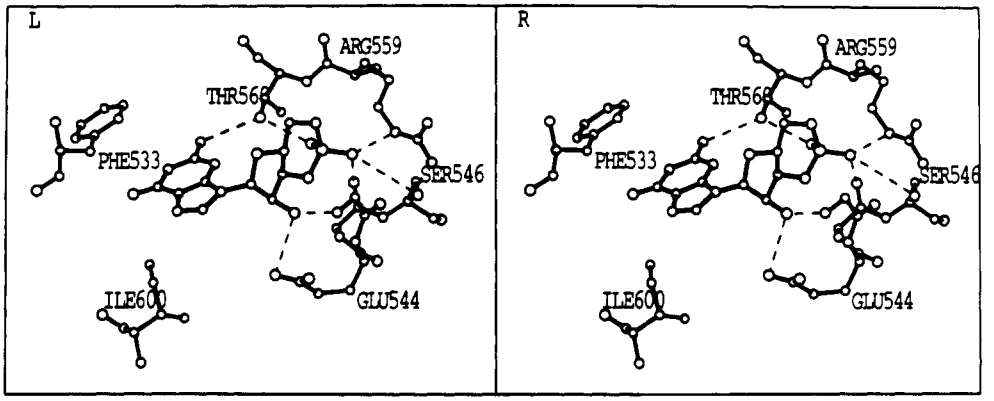

FIGURE 3: Stereoview of cGMP in the modeled binding site of cGGC. Only the amino acids that interact with cGMP are shown, and hydrogen-bond interactions are indicated by dashed lines.

β6 and β7 from residue 78 to 86 and deletion between β4 and β5 from residue 52 to 54. Comparison of the energy-minimized model of cGGC with that of CAP gave an rms difference of 0.53 Å for the main-chain atoms, less than the 0.82 Å observed between the two cAMP-binding domains in the CAP dimer crystal structure (Weber & Steitz, 1987). Most of the atomic differences were localized in the variable surface loops. It was also interesting to note that despite sequence divergence between the models there was no major rearrangement in the α-helices or the loops connecting them. In addition, despite an insertion and deletion in these loops, the β strands also appear to be well conserved in the cGGC model.

The conserved structure of the cyclic nucleotide-binding domains, as deduced from the CAP crystal structure, consists of a helix on either end of an eight-stranded antiparallel β roll, and the cyclic nucleotide is bound between the β roll and a third helix. This domain is connected to a DNA-binding domain in CAP and to several other domains in the protein kinases and the cGGC. The core structure contains an antiparallel β hairpin surface loop between strands β4 and β5 in the center of the β roll. The model structure of cGGC shows an additional extended surface loop located between β strands 6 and 7. The exact conformation of this surface loop is expected to be difficult to predict, as discussed by Greer (1990). Both of these surface turns are close to the residues forming the cyclic nucleotide-binding site and therefore may be involved in allosteric interactions with other domains in the intact protein. The A and B helices have the expected pattern of hydrophobic and polar residues for these helices. In the A helix, cGGC residues Leu 486, Leu 489, and Val 490 are conserved hydrophobic residues in CAP and cGPK. Helix B has conserved hydrophobic residues Leu 579, Ala 582, and Leu 583, and polar residues Asp 577, Asp 578, and Glu 581 at equivalent positions as in CAP and cGPK. The C helix has less sequence similarity and may be shorter than in CAP or in a different position as discussed for the cAMP-dependent protein kinase (Weber et al., 1987; Steinberg et al., 1991).

Internal Hydrophobic Packing. Two regions in the interior of the protein show similar hydrophobic interactions to those seen in CAP crystal structure. (1) The three helices, A, B, and C, are connected by the hydrophobic contacts of residues Phe 14, Leu 39, Tyr 41, Phe 69, Ala 95, and Phe 102 in CAP. The changes are (CAP/cGGC) Phe 14/Leu 489, Leu 39/Met 514, Tyr 41/Ile 516, Phe 69/Tyr 541, Ala 95/Phe 572, and Phe 102/Leu 579 (Figure 1). Three of the residues are smaller in cGGC than in CAP, while three are larger. The changes are complementary, forming good packing contacts in both cases. Similar complementary substitutions of residues in the hydrophobic core were observed for HIV-1 and HIV-2 proteases (Gustchina & Weber, 1991). A similar set of hydrophobic residues occur in cGPK and cAPK. (2) The second internal hydrophobic region lies at one side of the β roll and involves residues Tyr 23, Tyr 40, Val 47, Ile 70, and Val 94. The changes are (CAP/cGGC) Tyr 23/Tyr 498, Tyr 40/Phe 515, Val 47/Leu 522, Ile 70/Phe 542, and Val 94/Leu 571. These residues all are larger in cGGC than in CAP, and such larger residues are also seen in the protein kinase sequences. The major difference in this region is between the A and B domains of cAPK and cGPK. The A domains have a single amino acid deletion of Ile 70 in CAP, whereas the B domains all have Phe 70, which lies near the ribose of the cyclic nucleotide.

External Hydrophobic Regions. The protein surface was examined for large hydrophobic regions that might be indicative of interdomain contacts. There were no large surface patches of hydrophobic residues. In fact, the modeled domain of cGGC has 19 negative and 16 positive charges, compared with 15 negative and 19 positive in CAP. Therefore this domain of cGGC probably does not lie within the membrane. The percentages of accessible hydrophobic surface areas are very similar in the cGGC model and CAP structure (Table I), also suggesting the cGMP-binding domain, like that of the CAP, is not membrane bound. One part of the cGGC surface has an extended region of hydrophobic residues including Val 487, Val 490, Leu 491, Pro 495, Val 497, Pro 500, Ile 566, and Tyr 568, from helix A, β strands 1 and 8. In CAP, these residues are Glu 12, Leu 15, Ser 16, Ile 20, Lys 22, Ser 25, Lys 89, and Ala 91, which are obviously not all hydrophobic, and polar residues occur here in cGPK also. We predict that this region may be involved in hydrophobic contacts with another domain of cGGC or possibly may lie near the membrane.

Predicted Interactions with cGMP. The modeling indicated that the ion channel may assume a three-dimensional structure similar to that observed for the cAMP-binding domain of CAP. The major interactions between the protein and cyclic nucleotide are conserved and identical to those observed in CAP and deduced for the cyclic nucleotide-dependent protein kinases (Figure 3). Arg 82 (559) forms an ionic interaction with the phosphate of the cyclic nucleotide, Gly 71 (543) and Glu 72 (544) (cGGC numbering is in parentheses) form hydrogen-bond interactions with the ribose 2'-OH. In cGPK and the ion channels, Thr 83 (560) OH may form hydrogen-bond interactions with the 2-amino group of cGMP and with the exocyclic phosphate oxygen as observed for Ser 83 in CAP. This interaction was tested by mutation of Thr to Ala, which lowered the affinity for cGMP (Altenhofen et al., 1991). The model for the cyclic nucleotide-binding domain of the ion

channels predicts three additional interactions between the protein and the cyclic nucleotide. Ile 73 (545) C=O and Ser 74 (546) OH are both positioned to permit formation of hydrogen bonds with one of the exocyclic phosphate oxygens. Phe 61 (533) lies next to the adenine or guanine ring and probably forms favorable aromatic–aromatic interactions.

This model for the binding site can be examined in the light of experimental measurements of the binding constants of analogues of cGMP. Tanaka et al. (1989) have shown that the six-membered cyclic phosphate ring is essential for binding; GMP and 2'-deoxy-cGMP have extremely weak binding and do not activate the channel current. Zimmerman et al. (1985) showed that both phosphate exocyclic oxygens are required for channel opening. These results are very similar to those obtained for CAP and for the cyclic nucleotide-dependent protein kinases and indicate the importance of the ionic and hydrogen-bond interactions between the proteins and the ribose phosphate oxygens. The effects of substitutions of the purine ring differ in the various cyclic nucleotide-activated proteins. CAP is very specifically activated by cAMP, and hydrogen-bond interactions are observed with 6-NH_2 of adenine. The cAMP-dependent protein kinase experiments indicated that there were no hydrogen-bond interactions with the adenine ring (de Wit et al., 1984; Doskeland et al., 1983; Jastorff et al., 1979). The cGMP-dependent protein kinase results indicated the presence of a hydrogen-bond interaction with the 2-NH_2 group of cGMP (Weber et al., 1989), as shown in Figure 4. For the photoreceptor channel, both the 6-oxy and 2-amino groups of the purine were shown to be important for ligand binding (Tanaka et al., 1989). Cyclic IMP and cAMP have binding affinities of 50–60-fold less than cGMP. On the other hand, substitutions at the 8-position, such as bromine or fluorescein, bind more tightly (16–28-fold) than cGMP. The Thr 560 OH is predicted to interact with the 2-NH_2 group, as in the cGMP-dependent protein kinase. Interactions with the 8- and 6-positions are more difficult to predict since these are close to the region corresponding to the C-helix in CAP, which may differ in the protein kinases as suggested by analysis of mutations (Steinberg et al., 1991). The C-helix region has little sequence similarity among the different proteins: CAP, kinases, and the ion channels. In the cyclic nucleotide-dependent protein kinases, the C-helix may be shorter than in CAP due to the presence of a Pro. A further complication is that the C-helix in CAP makes many intersubunit contacts with the C-helix in the other subunit of the CAP dimer. This region may be involved in intersubunit or interdomain contacts in the photoreceptor channel which may alter the conformation of the protein. The large hydrophobic bromine or fluorescein at the 8-position is predicted to bind in a hydrophobic pocket, possibly between the C-helix and the β roll. Phe 61 may provide additional favorable hydrophobic interactions with these analogues (Figures 3 and 4). Site-directed mutagenesis may be required to identify the amino acid interactions with the 6-oxy of cGMP.

Evaluation of the Model. In this work, we have predicted potential specific contacts between the protein and the cyclic nucleotide, on the basis of the sequence and functional homology between CAP, cGPK, and cGGC structures. However, it is clear that this form of analysis suffers from some limitations inherent to model building. The first limitation concerns the loops in the β structure in which insertions and deletions have been placed. The poor sequence conservation in these parts means that the model may be prone to large errors in these loops. The position of surface loops in particular may depend on interdomain contacts or crystal packing interactions even within the same protein. Also, surface loops frequently have more variation in sequence and the exact conformation may be difficult to model (Greer, 1990; Weber, 1990). The second limitation is that the real structures of CAP and cGGC are expected to be more divergent than the models are. The cyclic nucleotide-binding domains of cGGC and CAP show 17% identical amino acids, so that the expected rms deviation for similar pairs of structures, according to Chothia and Lesk (1986), would be about 1.7 Å. However, the modeled main-chain atoms of cGGC domain have an rms deviation of 0.53 Å compared to CAP. The closer than expected rms value to the real structure is primarily because the main-chain atoms of the cGGC model were restrained during the energy minimization procedure to be nearly identical to the CAP structure, since this was the only available model. In the real structures, the exact position of the helices (especially the C-helix) may vary and therefore be difficult to predict (Read et al., 1984). Another limitation of this model is that it predicts the structure of one domain in a multidomain oligomeric protein. In the absence of other information, we cannot easily predict the relative positions of other domains or subunits in the holoprotein. However, the structure of functionally conserved binding sites may be predicted most accurately (Weber, 1990). Despite these limitations, there is strong evidence supporting the correctness of this model: (a) conserved Gly residues between β strands and conserved residues at the cGMP binding site, (b) conserved hydrophobic packing between the three helices and within the β roll, and (c) correlation of the cGMP-binding site with results from analogue experiments and site-directed mutagenesis. We conclude that the proposed model of cGGC can be considered to be reasonable starting point for evaluation of the cGMP-binding domain of cGGC.

Discussion

The model presented here for the cGMP-binding domain of cGGC may not be correct in all details; however, it suggests several specific interactions between the protein and cGMP which may be tested by site-directed mutagenesis or analogue binding studies. It was proposed previously that the threonine to alanine difference in the cyclic nucleotide-binding pockets of cGPK and cAPK was responsible for specific recognition of cGMP or cAMP (Weber et al., 1989). However, the amino acid sequences of the cGMP- and cAMP-gated ion channels both have threonine at the equivalent position. The photoreceptor channel is primarily activated by cGMP (Tanaka et al., 1989; Kaupp et al., 1989), whereas the olfactory channel is activated by both cAMP and cGMP at similar concentrations (Nakamura & Gold, 1987) or at lower concentrations of cGMP compared with cAMP (Dhallan et al., 1990). Therefore, the presence of threonine in the binding site may be required for more effective binding of cGMP but evidently does not prevent the binding of cAMP. The results of mutating Ala to Thr in cAPK produced a protein with increased affinity for cGMP compared to wild type, but similar affinity for cAMP, so other factors must contribute to the recognition of specific cyclic nucleotide (Shabb et al., 1990). Altenhofen et al. (1991) have tested these interactions by site-directed mutagenesis in cGGC with similar results, as summarized in Table II. Substitution of the respective threonine with alanine in both cGGC and olfactory channels resulted in a 30-fold decrease in cGMP sensitivity of channel activation but had little effect on activation by cAMP.

In addition to the residues predicted from sequence alignment to interact with cGMP (Gly 543, Glu 544, Arg 559, and Thr 560), Ile 545, Ser 546, and Phe 533 are predicted to

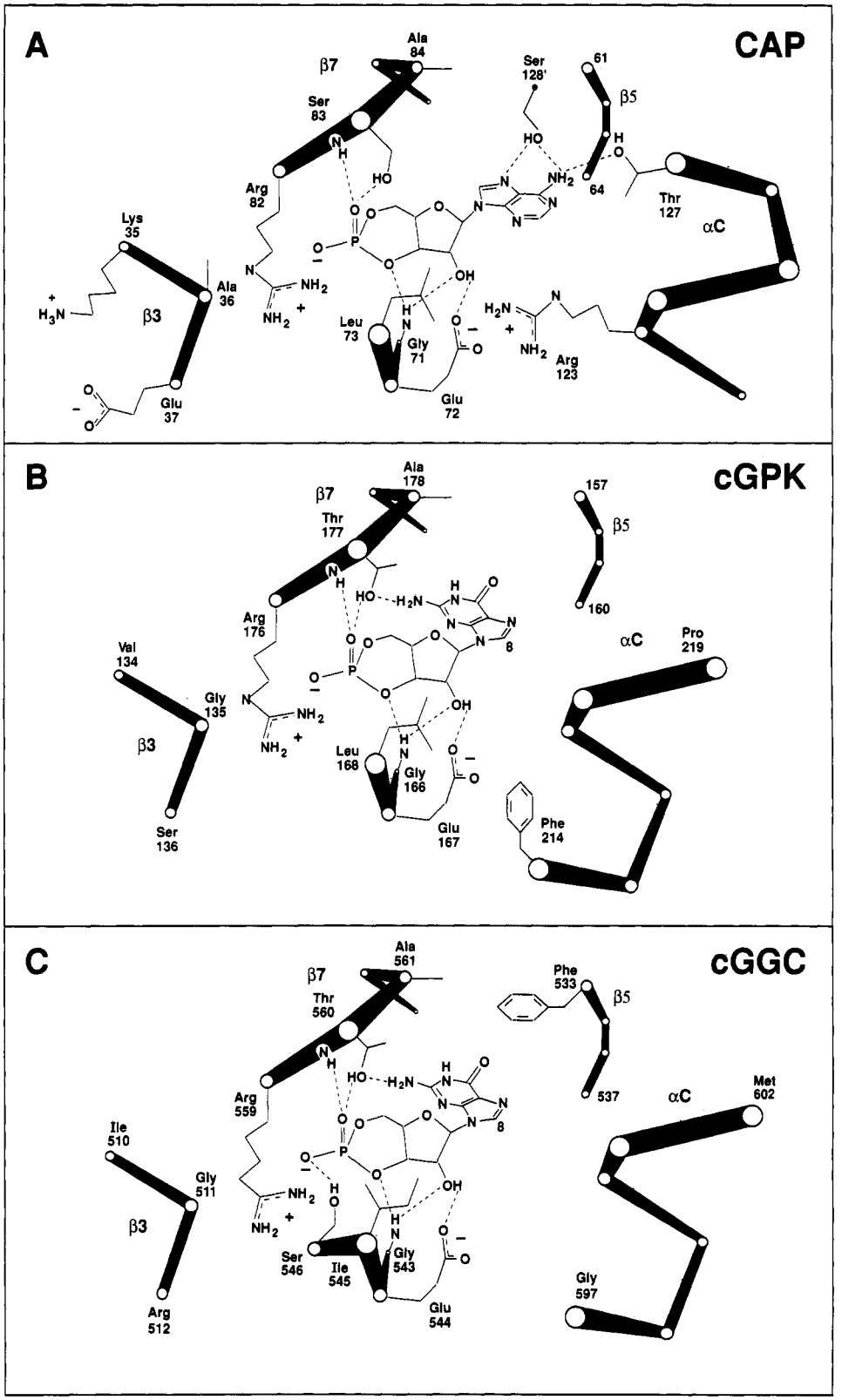

FIGURE 4: Cyclic nucleotide-binding sites in a schematic representation. (A) cAMP binding site of CAP (from crystal structure). Ser 128′ is from the other subunit in the dimer. (B) cGMP-binding site of cGPK (model) and (C) cGMP-binding site of cGGC (model). The side chains that are conserved or interact with cyclic nucleotide are shown, and hydrogen-bond and ionic interactions are indicated.

interact with cGMP. These additional interactions cannot easily be predicted from the sequence alignment but become obvious only when the three-dimensional structure is examined. Mutations of Ser 546 and Phe 533 in particular are expected to alter the binding affinity for cGMP.

The sequences of cGGC and olfactory cyclic nucleotide-gated ion channel were examined for differences within the cyclic nucleotide-binding sites. Differences occur only in residues 533–535 that are predicted to form part of $\beta 5$ and the binding pocket. Residue 533 is Phe in cGGC and Tyr in cAGC and is predicted to make aromatic interactions with the adenine or guanine ring of the cyclic nucleotide. Residue 534 is Val or Ala and is predicted to lie close to the guanine or adenine ring and provide hydrophobic interactions with the

Table II: Affinity of Cyclic Nucleotide-Binding Sites for cAMP and cGMP

protein	$K_{1/2}$ cAMP (μM)	$K_{1/2}$ cGMP (μM)	reference
cAPK wt	0.021	4.100	Shabb et al. (1990)
cAPK A334T	0.028	0.250	Shabb et al. (1990)
cGPK	29.700	0.110	Corbin et al. (1986)
rod cGGC	1500.000	24.000	Tanaka et al. (1989)
rod cGGC	1200.000	32.600	Altenhofen et al. (1991)
rod cGGC T560A	2750.000	940.000	Altenhofen et al. (1991)
olfact cGGC	2.400	1.600	Nakamura and Gold (1987)
olfact cGGC	39.000	1.200	Dhallan et al. (1990)
olfact cGGC T537A	16.300	53.030	Altenhofen et al. (1991)

cyclic nucleotide. Residue 535 is Val or Leu; however, the equivalent amino acid side chain in CAP is directed to the surface of the domain and not close to the cAMP. The differences in residues 533 and 534 are expected to alter the hydrophobicity of the binding site and may affect the selectivity for cyclic nucleotide.

Selected regions of the model can be targeted as potential contact sites for other domains of cGGC or other subunits in the intact ion channel. These are predicted by analogy to CAP and include the N-terminus of the cGMP-binding domain, residues 485–490, residues 521–537 between β4 and β5, residues 547–558 between β6 and β7, and the region of the C-helix, residues 588–610. Therefore, this model for the cGMP-binding domain of cGGC provides targets for mutational analysis of cyclic nucleotide binding and for interactions with other domains or subunits within the ion channel.

ACKNOWLEDGMENTS

We are grateful to Dr. Jacqueline Tanaka for helpful discussions. We thank the Advanced Scientific Computing Laboratory at NCI-FCRDC, Frederick, MD, for the Cray time to run energy minimization calculations using XPLOR.

REFERENCES

Altenhofen, W., Ludwig, J., Eismann, E., Kraus, W., Bönigk, W., & Kaupp, U. W. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 9868–9872.

Beebe, S. J., & Corbin, J. D. (1986) *Enzymes (3rd Ed.) 17*, 43–111.

Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, J. S., Swaminathan, S., & Karplus, M. (1983) *J. Comput. Chem. 2*, 187–217.

Brunger, A. T. (1990) XPLOR software version 2.1, Yale University, New Haven, CT.

Bubis, J., Neitzel, J. J., Saraswat, L. D., & Taylor, S. S. (1988) *J. Biol. Chem. 263*, 9668–9673.

Chothia, C., & Lesk, A. M. (1986) *EMBO J. 5*, 823–826.

Corbin, J. D., Ogreid, D., Miller, J. P., Suva, R. H., Jastorff, B., & Doskeland, S. O. (1986) *J. Biol. Chem. 261*, 1208–1214.

Correll, L. A., Woodford, T. A., Corbin, J. D., Mellon, P. L., & McKnight, G. S. (1989) *J. Biol. Chem. 264*, 16672–16678.

de Wit, R. J. W., Hekstra, D., Jastorff, B., Stec, W. J., Baraniak, J., van Driel, R., & van Hasstert, P. J. M. (1984) *Eur. J. Biochem. 142*, 255–260.

Doskeland, S. O., Ogreid, D., Ekanger, R., Sturm, P. A., & Suva, R. H. (1983) *Biochemistry 22*, 1094–1101.

Dhallan, R. S., Yau, K.-W., Schrader, K. A., & Reed, R. R. (1990) *Nature 347*, 184–187.

Flockhart, D. A., & Corbin, J. D. (1982) *CRC Crit. Rev. Biochem. 13*, 133–186.

Fresenko, E. E., Kolesnikov, S. S., & Lyubarsky, A. L. (1985) *Nature 313*, 310–313.

Greer, J. (1990) *Proteins: Struct., Funct., Genet. 7*, 317–334.

Gustchina, A., & Weber, I. T. (1991) *Proteins: Struct., Funct., Genet. 10*, 325–339.

Hermans, J., Jr., & McQueen, J. E. (1974) *Acta Crystallogr. A30*, 730.

Jastorff, B., Hoppe, J., & Morr, M. (1979) *Eur. J. Biochem. 101*, 555–561.

Jones, A. T. (1978) *J. Appl. Crystallogr. 11*, 268–272.

Kabsch, W., & Sanders, C. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1075–1078.

Kaupp, U. B., Niidome, T., Tanabe, T., Terada, S., Bönigk, W., Stuhmer, W., Cook, N. J., Kangawa, K., Matsuo, H., Hirose, T., Miyata, T., & Numa, S. (1989) *Nature 342*, 762–766.

Krebs, E. G., & Beavo, J. A. (1979) *Annu. Rev. Biochem. 48*, 923–960.

Lee, B., & Richards, F. M. (1971) *J. Mol. Biol. 55*, 379–400.

Lincoln, T., & Corbin, J. D. (1983) *Adv. Cyclic Nucleotide Res. 15*, 139–192.

Ludwig, J., Margalit, T., Eismann, E., Lancet, D., & Kaupp, U. B. (1990) *FEBS Lett. 270*, 24–29.

Nakamura, T., & Gold, G. H. (1987) *Nature 325*, 442–444.

Ogreid, D., Doskeland, S. O., Gorman, K. B., & Steinberg, R. A. (1988) *J. Biol. Chem. 263*, 2664–2671.

Powell, M. J. D. (1977) *Math. Programming 12*, 241–254.

Read, R. J., Brayer, G. D., Jurasek, L., & James, M. N. G. (1984) *Biochemistry 23*, 6570–6575.

Shabb, J. B., Ng, L., & Corbin, J. D. (1990) *J. Biol. Chem. 265*, 16031–16034.

Steinberg, R. A., Gorman, K. B., Ogreid, D., Doskeland, S. O., & Weber, I. T. (1991) *J. Biol. Chem. 266*, 3547–3553.

Takio, K., Wade, R. D., Smith, S. B., Krebs, E. G., Walsh, K. A., & Titani, K. (1984) *Biochemistry 23*, 4207–4218.

Tanaka, J. C., Eccleston, J. F., & Furman, R. E. (1989) *Biochemistry 28*, 2776–2784.

Taylor, S. S. (1989) *J. Biol. Chem. 264*, 8443–8446.

Walsh, D. A., Perkins, J. P., & Krebs, E. G. (1968) *J. Biol. Chem. 243*, 3763–3765.

Weber, I. T. (1990) *Proteins: Struct., Funct., Genet. 7*, 172–184.

Weber, I. T., & Steitz, T. A. (1987) *J. Mol. Biol. 198*, 311–326.

Weber, I. T., Steitz, T. A., Bubis, J., & Taylor, S. S. (1987) *Biochemistry 26*, 343–351.

Weber, I. T., Shabb, J. B., & Corbin, J. D. (1989) *Biochemistry 28*, 6122–6127.

Woodford, T. A., Correll, L. A., McKnight, G. S., & Corbin, J. D. (1989) *J. Biol. Chem. 264*, 13321–13328.

Zimmerman, A. L., Yamanaka, G., Eckstein, F., Baylor, D. A., & Stryer, L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8813–8817.