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Hypothesis

A structural model for the α-subunit of transducin

Implications of its role as a molecular switch in the visual signal transduction mechanism

Vijay N. Hingorani and Yee-Kin Ho

Department of Biological Chemistry, University of Illinois at Chicago, Health Sciences Center, Chicago, IL. 60612, USA

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Transducin is a GTP-binding protein which mediates the light activation signal from photolyzed rhodopsin to cGMP phosphodiesterase and is pivotal in the visual excitation process. Biochemical studies suggest that the T_α subunit of transducin is composed of three functional domains, one for rhodopsin/$T_{\beta\gamma}$ interaction, another for guanine nucleotide binding, and a third for the activation of phosphodiesterase. The integration of the primary sequence of T_α along with secondary structure, hydropathy and folding topology predictions, and a comparison with homologous proteins have led to the construction of a three-dimensional model of the T_α subunit. A molecular mechanism which underlies the coupling action of T_α is suggested on the basis of this model.

Transducin; GTP-binding protein; Protein folding; Visual signal transduction; Retinal cyclic GMP cascade; Enzyme mechanism

1. INTRODUCTION

The primary event in visual excitation is the photolysis of rhodopsin. This triggers a series of biochemical events which lead to the transient hyperpolarization of the rod cells by reducing the inward Na^+ current across the plasma membrane (review [1]). This signal transduction process has been shown to involve the activation of a cGMP cascade. A GTP-binding protein called transducin is required for transmitting the light signal from photolyzed rhodopsin to the cGMP phosphodiesterase (PDE) [2]. Transducin contains three polypeptide chains which have been purified into two subunits (T_α, M_r 40000, and $T_{\beta\gamma}$, M_r 37000 and 8000) [3]. A photolyzed rhodopsin catalyzes the exchange of GTP for the bound GDP in hundreds of transducin molecules. The incorporation of GTP into T_α leads to the dissociation of transducin subunits from rhodopsin. The T_α-GTP complex then activates PDE which hydrolyzes thousands of cGMP to 5′-GMP. As a signal transducer, the T_α subunit must contain sites for interaction with rhodopsin, the $T_{\beta\gamma}$ subunit, guanine nucleotides, and the PDE. An understanding of the interactions between these functional sites is essential for the elucidation of the transduction mechanism.

Biochemical data on the structural and functional relationships of the T_α molecule are now

Correspondence address: V.N. Hingorani, Department of Biological Chemistry, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612, USA

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available and include the primary sequence elucidated via molecular cloning [4–7], peptide mapping [8], and functional domain analysis through chemical modification [9,10]. Moreover, the homology between the GTP-binding site of T_α and those of the G-proteins coupled to adenylate cyclase, elongation factor Tu (EF-Tu) and the *ras* p21 protein has been identified [4–7,11]. Recently, the tertiary structure of the GTP-binding domain of EF-Tu was solved to a resolution of 2.7 Å by X-ray crystallography [12,13] and has served as a model for the *ras* gene product [14] and for the partial folding of a consensus GTP-binding site [15]. We have extended these studies by integrating all the known biochemical characteristics of T_α and proposing a three-dimensional model. A molecular switching mechanism of T_α which controls the visual signal transduction process is also described.

2. THE MODEL

First, the secondary structure and hydropathy of the molecule were predicted from the primary sequence and served as a foundation for the folding efforts. Then, analysis of limited proteolysis and chemical modification studies have allowed us to assign all the functional sites of T_α on a linear tryptic peptide map. Next, the probable folding pattern for each of the domains was determined. The folding topology for T_α was established by comparison to other proteins which are either similar in secondary structure composition and arrangement or in their substrate binding sites, and whose tertiary structures have been solved by X-ray diffraction. This analysis has led to a general picture of the protein. Finally, all known biochemical data on T_α have been examined to verify the validity of the proposed structure.

2.1. *Primary and secondary structure, hydropathy and functional domains*

The T_α molecule is composed of 350 amino acid residues with a calculated molecular mass of 39945 Da. Information concerning the secondary structure of the T_α peptide was obtained using the algorithms of Chou and Fasman [16] and Garnier et al. [17]. The algorithm of Kyte and Doolittle [18] was used to generate the hydropathy profile of the molecule (fig.1A). Fig.1B shows the peptide map of T_α. Trypsin treatment first cleaves off a 2 kDa peptide (Try-1 at Lys_{18}) from the amino-terminus. A second cleavage removes a 5 kDa peptide (Try-2 at Arg_{310}) from the carboxyl-terminus and generates a transient 33 kDa fragment which is finally cleaved to a 21 and 12 kDa fragment (Try-3 at Arg_{240}). The removal of the 2 kDa amino-terminal fragment disrupts the interaction of T_α with rhodopsin and $T_{\beta\gamma}$. The middle 33 kDa fragment containing the bound Gpp(NH)p remained capable of activating PDE [8]. Chemical modification has been used to identify a number of functional sites on the linear peptide map. Modification of a lysine residue on the 21 kDa fragment leads to the inhibition of PDE activation [10]. On the other hand, modification of a sulfhydryl group on the 12 kDa peptide affects the rhodopsin-transducin interaction [9]. Cholera toxin, which ADP-ribosylates the 21 kDa fragment (CT at Arg_{174}), blocks the hydrolysis of the bound GTP [19], whereas pertussis toxin, which ADP-ribosylates the 5 kDa carboxyl-terminal fragment (PT at Cys_{347}), inhibits the transducin-rhodopsin interaction thus preventing guanine nucleotide exchange [20]. This information indicates that the T_α molecule is composed of three functional domains (fig.1B). Domain 1 (D1) includes mainly the 12 kDa and part of the 21 kDa fragment and consists of the nucleotide-binding site; domain 2 (D2) which is contained entirely within the 21 kDa fragment is responsible for the interaction with and activation of PDE; and domain 3 (D3), consisting of the amino- and carboxyl-terminal (2 and 5 kDa) peptides, binds with $T_{\beta\gamma}$ and rhodopsin. In general, the link between functional domains is composed of extremely flexible regions which act as movable hinges and allow a conformational change in one domain to be conveyed to another domain. A careful inspection of the primary sequence of T_α reveals two such regions (Gly_{198}-Gly_{199} and Gly_{288}-Pro_{289}-Asn_{290}), both of which are located between the proposed functional domains of the T_α molecule (fig.1B, black triangles).

The primary sequence of T_α was compared to other GTP-binding proteins such as the elongation factor Tu (EF-Tu). T_α contains four regions that exhibit significant sequence homology at the nucleotide-binding site. The site is separated into two regions on the linear peptide map (the shaded

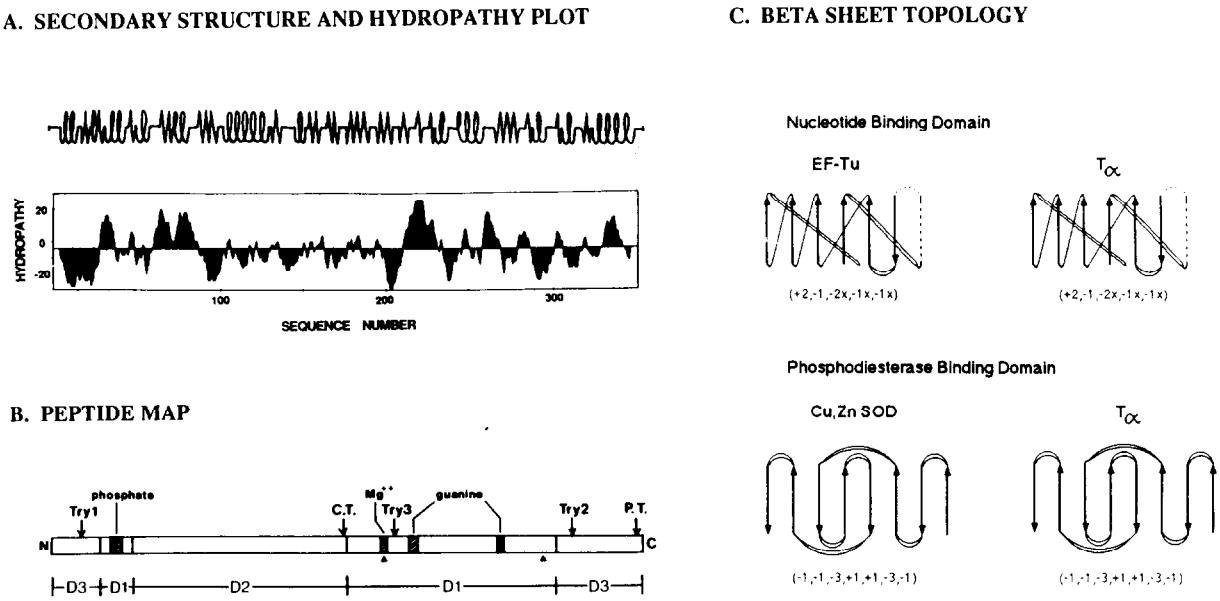

Fig.1. Secondary structure, hydropathy plot, peptide map, and β-sheet topology of T_α. (A) The predicted secondary structure of T_α is indicated in a schematic manner. Primary sequence information is from [4–7]. The coils, the zig-zags and the flat segments represent regions of α-helices, β-strands, and random coils and β-turns, respectively. The hydropathy plot of T_α is shown. The positive values indicate hydrophobic regions and the negative values indicate hydrophilic regions. (B) The linear peptide map of T_α. (C) Schematic diagram of the topology of the β-pleated sheets for the two major functional domains of T_α. The arrows correspond to individual β-strands. Comparison of the topology of the proposed nucleotide-binding domain of T_α with that of EF-Tu and the PDE site of T_α with Cu,Zn superoxide dismutase. In T_α the arrows, from left to right, represent β-strands N, M, L, A, K, J in the nucleotide-binding site and β-strands B, C, D, G, F, E, H, I in the PDE site as shown in fig.2.

area in fig.1B). The guanine ring-binding region is located towards the carboxyl-terminus and the regions responsible for phosphate binding and for hydrolytic activity are located close to the amino-terminus. Between these two segments is a variable region which represents the effector-binding site, i.e. the PDE-binding domain for T_α.

2.2. *A model for the tertiary structure*

The possible folding pattern for each domain of T_α was determined separately and then these structures were combined to build a complete three-dimensional model. The most reasonable folding topology for each domain was examined by comparing T_α with other homologous proteins that contain a similar arrangement in the primary or secondary structure and whose tertiary structures have been elucidated by X-ray crystallography. The similarity between T_α and EF-Tu suggests that the guanine nucleotide-binding domain of T_α consists of 157 residues and has the parallel α/β doubly wound structure. We propose that the GTP-binding site of T_α, like that of EF-Tu, has a β-sheet topology of $(+2, -1, -2x, -1x, -1x)$ according to the nomenclature of Richardson [21,22] and is shown in fig.1C.

An examination of the secondary structure within the PDE domain of T_α shows several β-strands in tandem, separated by turns and coils. Such an arrangement strongly suggests an anti-parallel β-sheet topology. We propose that this domain, which consists of eight β-strands, forms the 'Greek key' barrel structure as described by Richardson [21,22]. This is the most common subgroup of anti-parallel β-structures and is found in enzymes such as Cu,Zn superoxide dismutase and prealbumin. This domain consists of 127 residues and is contained entirely within the 21 kDa tryptic fragment (Leu_{19}–Arg_{204}). A $(-1, -1, -3, +1, +1, -3, -1)$ β-sheet topology similar to that of the Cu,Zn superoxide dismutase [23] is shown in fig.1C.

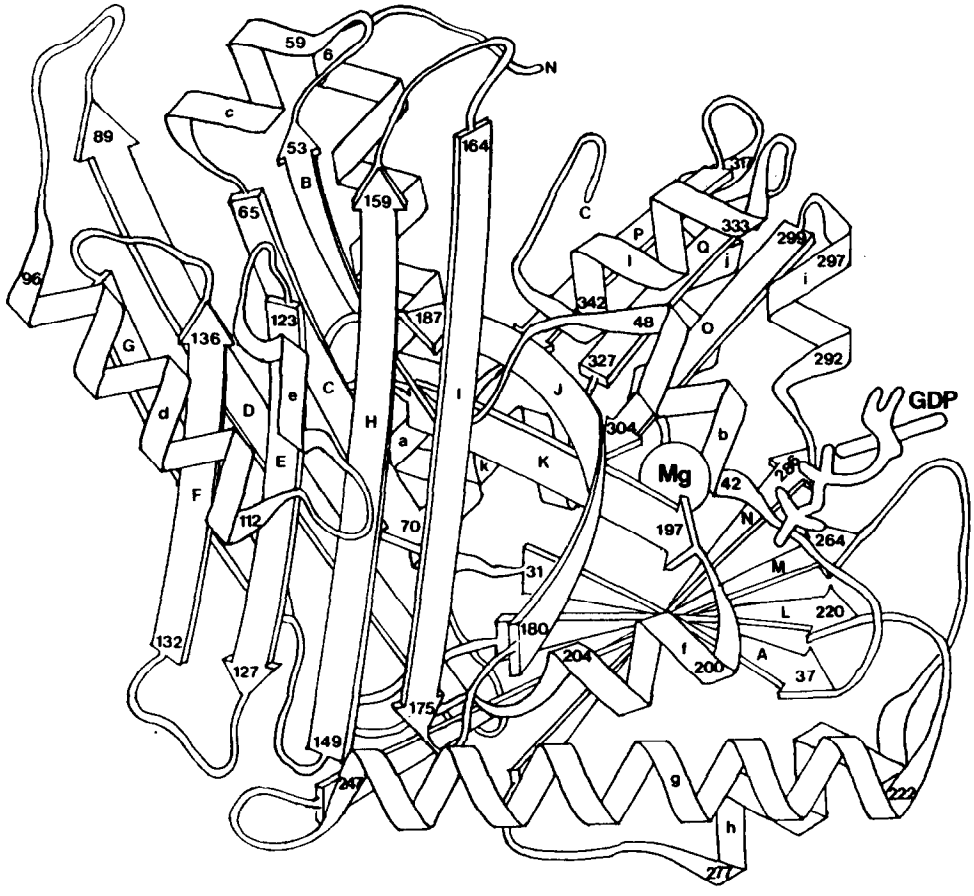

Fig.2. Proposed tertiary structure of the T_α molecule.

The proposed topology has allowed us to translate the primary and secondary structure of each functional domain of T_α into a three-dimensional structure. This was accomplished by placing the homologous sequences and corresponding secondary structures of T_α in positions identical to those found in the crystal structures of EF-Tu and Cu,Zn superoxide dismutase. A schematic model of the predicted tertiary structure of T_α is shown in fig.2. The α-helices and the β-strands of the molecule are designated as helices a–i and strands A–Q, respectively, starting from the amino-terminus. An essential rule of protein folding is to restrict the hydrophobic regions to the inside of the folded structure and to expose the hydrophilic segments to the solvent in order to maximize the stability of the system [24]. The hydropathy data shown in fig.1A suggest that strands B, C, E, F, J–N, P and Q are all relatively hydrophobic. According to the model these are all buried within the molecule and are protected from the solvent. On the other hand, strands H, I, and G along with helices a, b and d–k are hydrophilic and are somewhat exposed in the model. Other features such as the protection of the β-sheet by α-helices and the hydrogen bonding between strands of anti-parallel β-sheets are all accounted for in the proposed model.

2.3. *The GTP-binding domain*

The central core of the guanine nucleotide-binding site consists of a hydrophobic twisted β-sheet made up of five parallel β-strands (strands K, A, L, M and N) and one anti-parallel β-strand (strand J). These strands are connected by five hydrophilic α-helices which are partially exposed to the solvent. The nucleotide-binding site is situated at the carboxyl end of the β-sheet similar

to other nucleotide-binding proteins [25], and the points of interaction of the nucleotide with the protein are located in four loops connecting β-strands with α-helices. The phosphate-binding site is in the Rossmann fold containing residues Gly_{36}-Ala-Gly-Glu-Ser-Gly_{41}. Magnesium ion binding occurs in a loop between strand K and helix f situated diametrically opposite the β-phosphate of GDP and consists of the residues Asp_{196}-Val-Gly-Gly-Gln_{200}. A salt bridge may be formed between the Mg^{2+} and the carboxyl group of Asp_{196}. The GDP-binding pocket does not seem large enough to accommodate the additional γ-phosphate when GTP is bound to the site, unless the loop is moved to widen it. This movement could easily occur at the hinge region formed by Gly_{198}-Gly_{199} and influence the position of strand K and helix f. It is conceivable that the movement of strand K and helix f could directly control the activation of PDE and dissociation of the transducin subunits from rhodopsin.

2.4. *The PDE activation domain*

This region is composed of a segment between the γ-phosphate binding and the Mg^{2+} binding regions from Met_{49} to Val_{175} and is contained entirely within the 21 kDa tryptic fragment (Leu_{19}–Arg_{204}). The proposed Greek key barrel structure contains eight antiparallel β-strands (strands B, C, D, G, F, E, H and I) as shown in fig.2. The exact location of the site of interaction with PDE is still unclear. But since it involves protein-protein interactions, it is likely that it is located on the surface of the molecule.

2.5. *The receptor-binding domain*

This region is responsible for receiving the light activation signal from photolyzed rhodopsin which catalyzes the GTP exchange reaction. The $T_{\beta\gamma}$ subunit is absolutely required for the binding of T_α to rhodopsin [3]. Biochemical studies indicate that this domain involves the amino- and carboxyl-terminal peptides of T_α. However, the exact locations of the rhodopsin and $T_{\beta\gamma}$ interacting sites are still not clear. We presume that in the tertiary structure the two terminal regions are in close proximity, in a manner that provides the interacting sites for both rhodopsin and $T_{\beta\gamma}$ binding. The amino-terminal 2 kDa peptide, helix a, is an extremely hydrophilic α-helical structure. We suggest that the conformation of helix a may play an important role in regulating the interaction of transducin with rhodopsin. A careful examination of the organization of the charged and the hydrophobic residues on helix a (fig.3) reveals an interesting feature which may be functionally significant. A distinct hydrophobic groove composed of Ala_7, His_{11}, Leu_{15}, Leu_{19}, Ala_{23} and Ala_{27} can be identified on one side of the helical structure. Surrounding this groove are charged residues of lysine, arginine, aspartate, and glutamate which are arranged in an alternating charge-pair fashion. Such an arrangement can allow a small rotation about the helical axis to turn the hydrophobic groove away from the protein surface and replace it with an extremely hydrophilic surface. An alternation of hydrophobic and charged residues on the interacting surface could be the molecular basis for

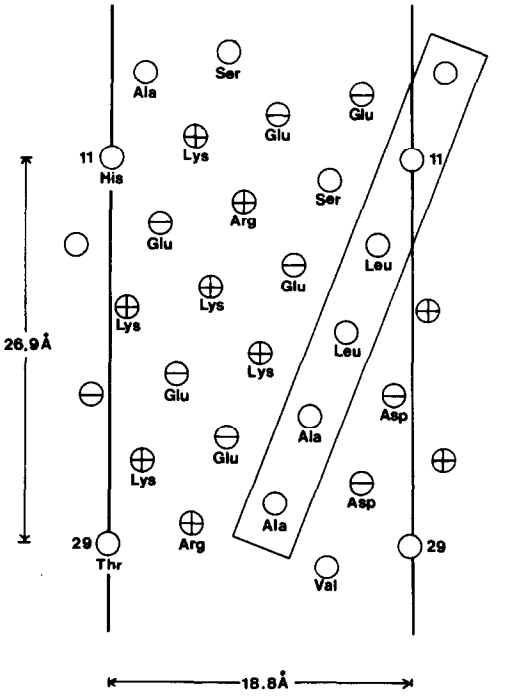

Fig.3. Helical net representation of helix a of T_α. The amino-terminal helix a of T_α is presented as an α-helical net. Uncharged residues are represented by open circles, basic and acidic residues are represented by circles containing pluses and minuses, respectively. Hydrophobic residues, Ala_7, His_{11}, Leu_{15}, Leu_{19}, Ala_{23} and Ala_{27}, forming a hydrophobic groove on the helical structure are boxed.

the regulation of the association and dissociation properties of T_α to rod outer segment membrane.

Another region in this domain is composed of the carboxyl-terminal 5 kDa tryptic fragment consisting of alternating α–β-structural sequences. It is likely that the three β-strands are arranged as a small anti-parallel β-sheet (strands O, P and Q) protected on both sides by α-helices as depicted in fig.2. The pertussis toxin-catalyzed ADP-ribosylation of the Cys_{347} is greatly enhanced in the presence of the $T_{\beta\gamma}$ subunit and the cleavage of the amino-terminal peptide of T_α reduces the $T_{\beta\gamma}$ enhancement on ADP-ribosylation [26]. This suggests that the conformation in this region is sensitive to the binding of $T_{\beta\gamma}$. Helix i connects directly to the carboxyl-end of the β-sheet in the nucleotide-binding domain with a Gly_{288}-Pro_{289}-Asn_{290} hinge region which can easily serve to transmit information between these two domains. This proximity can explain the influence of photolyzed rhodopsin and GTP exchange on each other and on the association and dissocation of the transducin-rhodopsin complex.

3. MECHANISM OF ACTION

The primary motivation for constructing a structural model for T_α was to provide a molecular mechanism for its coupling action. From the proposed model one can envision the rhodopsin-binding site of transducin as being composed of regions from both the T_α and $T_{\beta\gamma}$ subunits. Helix a, at the amino-terminal of T_α has been suggested to be essential for the interaction with rhodopsin. As $T_{\beta\gamma}$ associates with the carboxyl-terminal region of T_α, helix a may be anchored with the $T_{\beta\gamma}$ subunit to form the rhodopsin-binding site. Under these conditions, the transducin molecule is associated with the rhodopsin membrane and is available for activation by photolyzed rhodopsin. In the absence of photolyzed rhodopsin, the guanine nucleotide-binding site is in a closed conformation. Interaction with photolyzed rhodopsin opens up the nucleotide-binding site and allows rapid GTP/GDP exchange. Such an interaction must involve the transfer of information between the receptor-binding domain and the nucleotide-binding domain. In the closed conformation, helix i, located near the opening of the guanine nucleotide-binding pocket, is in a position to hinder sterically the exchange of the bound nucleotide. Hence, it provides tight binding for the bound nucleotide with a dissociation constant smaller than 10^{-7} to 10^{-8} M [27]. The light-activated signal from the binding of photolyzed rhodopsin can be propagated to the nucleotide-binding site through the carboxyl-terminus which is complexed with rhodopsin/$T_{\beta\gamma}$. A conformational change induced by the photolyzed rhodopsin is transmitted to helix i. A slight tilting of helix i results in opening of the nucleotide-binding pocket through a flexible hinge region (Gly_{288}-Pro_{289}-Asn_{290}). This open conformation enables nucleotide exchange to occur.

Upon binding of GTP two major changes occur in the T_α-GTP complex. First, it dissociates from photolyzed rhodopsin and the $T_{\beta\gamma}$ subunit. Second, the PDE activation site of the Tα-GTP complex is exposed for interaction with the latent PDE. Based on the proposed model, when GTP binds to the guanine nucleotide-binding pocket additional space is needed to accommodate the γ-phosphate of GTP. Strand K and helix f are pushed away from strand A and as a result, the groove between strand K and the nucleotide-binding domain is widened. The shift of the position of strand K also includes similar movement on the adjacent strand J. Such spatial rearrangement of strands K and J provides the molecular basis for the T_α coupling function. As can be seen in fig.2, one end of strand K is linked directly to a flexible hinge region (Gly_{198}-Gly_{199}) which is directly attached to helix f. The movement of strand K mechanically triggers the movement of helix f toward the PDE-binding domain. Hence, the conformational changes originating at the guanine nucleotide-binding domain are now transmitted to the PDE-binding site and cause it to be exposed or assembled for PDE activation. The other ends of strands K and J are directed toward the rhodopsin/$T_{\beta\gamma}$-binding domain. The GTP-induced movement could disrupt the rhodopsin/$T_{\beta\gamma}$-binding site that is formed by the amino-terminal helix a and the carboxyl-terminal peptide which may lead to the dissociation of both the rhodopsin and the $T_{\beta\gamma}$ subunit from the T_α-GTP complex. The flow of information between the three functional domains of T_α can be accomplished by shifting the spatial arrangement of a few β-strands and α-helices located in the interface of the three domains.

4. BIOCHEMICAL EVIDENCE

4.1. *Limited proteolysis*

It has been shown that tryptic cleavage at Arg_{204} is protected by the binding of the non-hydrolyzable Gpp(NH)p. This site is located on the movable region of helix f. The movement of helix f induced by the binding of GTP to T_α pushes helix f toward the PDE domain and as a result, Arg_{204} is buried inside the T_α molecule and is not susceptible to tryptic digestion [8].

4.2. *Chemical modification of T_α*

Modification of a single sulfhydryl group has the opposite effect to modification of the lysine residues. The former only blocks the interaction with rhodopsin and $T_{\beta\gamma}$, whereas the latter inhibits the PDE activation and the GTP hydrolytic activity [9,10]. These results can be interpreted in the light of the model. Since the coupling function of T_α relies on the communication between the three domains, site-specific modification may block the interaction between only two of the three domains, hence inhibiting only part of the T_α catalytic functions. Photoaffinity labeling of the GTP-binding sites with 8-azido-[α-^{32}P]GTP showed that the guanine ring-binding site is linked to the 12 kDa tryptic fragment. However, using the [γ-^{32}P]-P_γ(4-azidoanilido)-P_α-5′-GTP as an affinity probe for the γ-phosphate-binding site results in the formation of a covalent adduct with the 21 kDa tryptic fragment (Hingorani and Ho, unpublished). These results are in complete agreement with the proposed structure of the GTP-binding site of the T_α molecule shown in fig.2.

4.3. *Comparison of the T_α molecules of the rod and cone cells*

It is known that a similar but not identical transduction system exists in the cone photoreceptor cells for color perception [28]. A distinct PDE which interacts with the cone T_α has been suggested. The primary sequence homology between these two T_α molecules is more than 85% [29] and the predicted secondary structures are the same, indicating that they may share a similar folding pattern. The guanine nucleotide-binding domain is essentially identical except for a variation on the suggested hinge region for the rod T_α (Gly_{288}-Pro_{289}-Asn_{290}). The change in cone T_α to Gly-Asn-Asn may have functional significance, such as differences in the affinity and exchange rates for guanine nucleotides. There are several variations in the proposed PDE activation domain, especially on helix d and the turn between strands H and I which may represent part of the PDE-interacting site. Indeed, the two variable locations are on the surface of the molecule and could easily interact with the PDE complex. The receptor-binding domain shows interesting variations. There are four additional residues (Glu-Leu-Ala-Lys) on helix a of cone T_α. However, the hydrophobic groove as well as the charge-pair characteristic surrounding the groove remains unchanged. This observation implies that in spite of the added residues the nature of the interaction of cone T with color rhodopsin remains the same. It has been suggested that both T_α molecules use the same T_β subunit as their modulator indicating that the T_β interacting sites are similar. The carboxyl-ends of the two T_α molecules are identical. These comparisons provide additional support for the proposed model.

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