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Minireview

The Ligand-Binding Domain of Rhodopsin and Other G Protein-Linked Receptors

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Rhodopsin is a member of the very large family of G protein-linked receptors. The members of this family show clear signs of evolutionary relatedness, primarily in amino acid sequence homology, topographical structure of the proteins in the membrane, and the fact that all of the receptors function through the intermediary action of a GTP-binding regulatory protein or G protein. Recently, it has become clear that the structural similarity of these receptors extends well beyond the rather crude comparison of membrane topography. Reviewed here are several studies in which site-directed mutagenesis and active-site-directed reagents were used to show that the ligand-binding pockets of these receptors are highly similar. They are similar despite the fact that the structures of their various ligands are very different.

KEY WORDS: Visual pigments; bacteriorhodopsin; retinal; agonist; antagonist.

INTRODUCTION

The visual pigment rhodopsin is a member of a very large family of hormone, neurotransmitter, and sensory receptors (Dohlman *et al.*, 1987). This family is defined by three criteria. First, all are cell surface receptors that enable a cell to communicate with its external environment. Second, all have a structural feature in common; they have seven highly hydrophobic transmembrane segments that serve to embed the proteins in the lipid bilayer. Third, all bring about their intracellular response through the intermediary action of a GTP-binding regulatory protein or G protein. Thus, by several criteria, it is reasonable to expect the structure and function of the different G protein-linked receptors to be highly similar.

But how far can this analogy be extended? Clearly, there are profound differences among these proteins. For example, the agonist ligands are quite divergent in structure. The sizes of the agonists range from the small-molecule biogenic amines, such as adrenaline and dopamine, to the large-protein hormones, such as thyrotropin and choriogonadotropin, which are several hundred amino acids in length. Even among the small-molecule agonists there is considerable diversity. For example, the visual pigments are distinguished as a group from all other receptors which bind small-molecule agonists because the visual pigments bind their ligands covalently whereas all of the other receptors bind their ligands noncovalently. This would seem to argue that the structure of the ligand-binding domain in the visual pigments is fundamentally different than the binding domain in the other receptors.

The purpose of this review is to summarize recent work which suggests that the binding domains of these various receptors are in fact highly similar and represent minor variations on an evolutionary theme. I will restrict the discussion for the most part to two subgroups, the visual pigments and the biogenic amine receptors, for the simple reason that much more is known about these receptors than is known about the others. However, the argument put forth in this review is that all of the G protein-linked receptors display similar characteristics in terms of ligand binding and mechanism of activation.

I have made no attempt here to provide a com-

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prehensive review of G protein-linked receptors. For this I refer the reader to the recent reviews by Dohlman *et al.*, (1991), Hargrave and McDowell (1991), and Strosberg (1991). Nor have I tried to be comprehensive in my selection of references. I have chosen a limited number of papers that I feel clearly demonstrate the thesis of this article.

BACTERIORHODOPSIN AS A MODEL FOR G PROTEIN-LINKED RECEPTORS

Bacteriorhodopsin is the photogenic proton pump of the halophilic archaebacterium *Halobacterium halobium* (Stoeckenius *et al.*, 1979; Khorana, 1988). In response to light, bacteriorhodopsin pumps protons out of the cell to establish a concentration gradient across the membrane enabling the synthesis of ATP. Bacteriorhodopsin is not a G protein-linked receptor, nor does it have any significant sequence homology with any of the G protein-linked receptors.

So why begin with bacteriorhodopsin? First, bacteriorhodopsin is an integral membrane protein composed of seven transmembrane α-helical segments (Henderson and Unwin, 1975). Second, bacteriorhodopsin has bound to it, a retinal chromophore which is covalently attached by means of a protonated Schiff base linkage to the ε-amino group of a Lys residue, Lys^{216}, present in the middle of the seventh transmembrane α-helical segment (Stockburger *et al.*, 1979; Bayley *et al.*, 1981). This is highly reminiscent of the chromophore-binding site in the visual pigments. The visual pigments have bound to them, an 11-*cis*-retinal chromophore which is covalently attached by means of a protonated Schiff base linkage to the ε-amino group of a Lys residue, Lys^{296}, present in the seventh transmembrane segment (Bownds, 1967; Oseroff and Callender, 1974: Ovchinnikov *et al.*, 1982; Dratz and Hargrave, 1983). The third reason to discuss bacteriorhodopsin is that a structural model is available for bacteriorhodopsin that was obtained by high-resolution electron cryo-microscopy of two-dimensional crystalline arrays of the protein (Henderson *et al.*, 1990). Nothing comparable to this structure is currently available for any of the G protein-linked receptors. Therefore, the structure of bacteriorhodopsin is useful as a model for the G protein-linked receptors.

A schematic representation of the structure published by Henderson *et al.* (1990) for bacteriorhodopsin is shown in Fig. 1. The perspective in this figure is from above the membrane looking down parallel to the axes of the transmembrane α-helical segments and perpendicular to the plane of the membrane. The helices are designated by Roman numerals in the order that they appear in the amino acid sequence from amino- to carboxyl-terminal ends. Embedded within the center of the helical bundle is a molecule of *all-trans*-retinal. The covalent bond to the lysine in helix VII and the positive charge on the nitrogen associated with the protonated state of the Schiff base are indicated. The retinal chromophore makes contact with amino acid residues from several helices within the bundle. Similarly, the binding pockets of G protein-linked receptors involve several transmembrane segments as judged by the results of mutagenesis studies and active-site-directed reagents (Dixon *et al.*, 1987; Kobilka *et al.*, 1988; Nakayama and Khorana, 1990). However, for the present purposes I will highlight interactions only with transmembrane segments III, V, and VII, since most of the experimental studies on the ligand-binding domains have focused on these three helices.

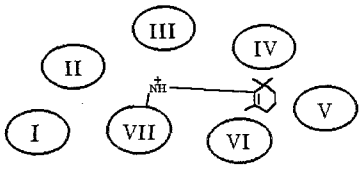

Fig. 1. Schematic representation of the structure for bacteriorhodopsin that was determined by Henderson *et al.* (1990). Helices are numbered sequentially with Roman numerals as they appear in the primary structure from amino terminus to carboxyl terminus. The helices are labeled with Roman numerals rather than letters so as to conform to the system more commonly used with G protein-linked receptors. The helices I–VII correspond to helices A–G, respectively, in the system commonly used with bacteriorhodopsin.

There is at least one amino acid residue in each of helices III, V, and VII that makes contact with the retinal chromophore. In helix III, Asp 96 is thought to be involved in the proton translocation mechanism, donating a proton to the Schiff base nitrogen (Otto *et al.*, 1989). Trp^{138} in helix V is seen in the electron density map of the protein to make contact with the β-ionone ring of the retinal (Henderson *et al.*, 1990). Lys^{216} in helix VII is covalently attached to the chromophore by means of a Schiff base linkage (Bayley *et al.*, 1981). Therefore, the orientation of the chromophore in the binding pocket of the protein is with positively charged nitrogen at one end, between helices III and VII, and β-ionone ring directed toward

helix V at the other end of the protein, as is shown in Fig. 1.

In the following discussion, the agonists and antagonists of the biogenic amine receptors are viewed as analogs of the retinal chromophore structure, with cationic ammonium group at one end of the molecule and catechol ring, or similar structure, at the other. From this viewpoint the structure of the G protein-linked receptors will be examined for similarities to the bacteriorhodopsin structure discussed above.

HELIX V: INTERACTION WITH THE RING

The aromatic ring of catecholamine ligands has two hydroxyl groups that are known to be important for binding of these ligands to their receptors. Strader *et al.* (1989) have suggested that these two hydroxyl groups are H-bonded to two Ser residues in helix V of the β-adrenergic receptors. The two residues, Ser^{204} and Ser^{207}, are separated by roughly one helical turn, and could H-bond to the catechol hydroxyls if the edge of the ring is directed toward helix V as is the β-ionone ring in bacteriorhodopsin. Strader *et al.* (1989) have shown that changing either of these residues to an Ala by site-directed mutagenesis results in a substantial decrease in affinity of the receptor for ligand. In an elegant extension of this initial observation, they analyzed the activation of the wild-type and mutant receptors with agonist analogs that were missing one or the other of the catechol hydroxyl moieties. The mutant S204A activated adenylyl cyclase with the agonist that was missing the *meta*-OH, but not with that missing the *para*-OH. In contrast, S207A activated adenylyl cyclase with the analog that was missing the *para*-OH, but not with that missing the *meta*-OH. They concluded from these results that Ser^{204} H-bonded to the meta-substituent, and Ser^{207} H-bonded to the *para*-substituent.

There is a good correlation among the biogenic amine receptors for the presence of a Ser or Thr residue at these conserved positions in helix V and the presence of an H-bond donor/acceptor in the ligand, as is illustrated in Fig. 2. The catecholamine receptors such as the α- and β-adrenergic receptors and the dopamine receptor have Ser residues at both positions, one for each OH moiety on the ligand. The serotonin receptor has only one Ser, and its ligand has only one OH group. The muscarinic acetylcholine receptor similarly has a single Thr in this region corresponding to the ester group on acetylcholine.

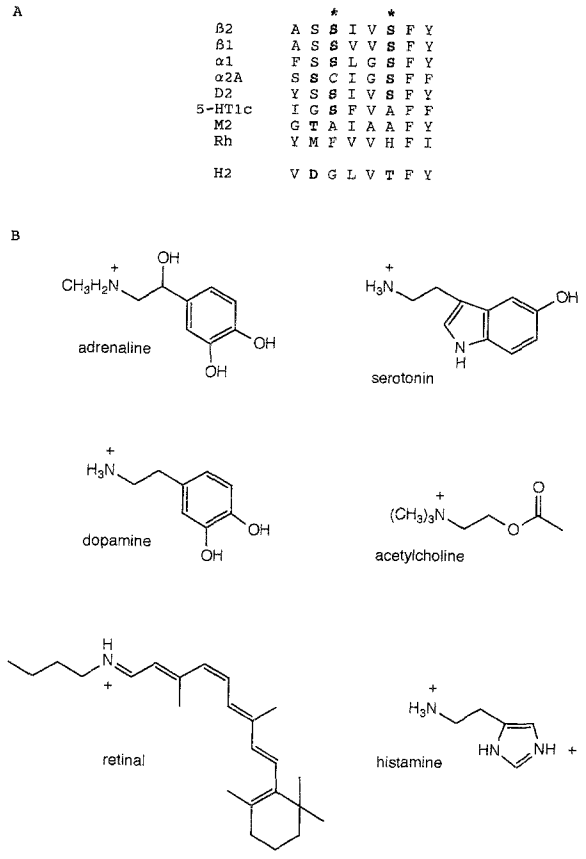

Fig. 2. (A) Sequences in helix V of several G protein-linked receptors. Asterisks identify Ser^{204} and Ser^{207} in the β_2-adrenergic receptor. Bold type are conserved residues. Note that conserved residues can be at position 203 instead of 204 in some receptors. Sequences of the receptors in this and other figures in this article are taken from the following references: β_2-adrenergic, Dixon *et al.*, (1986); β_1-adrenergic, Frielle *et al.* (1987); α_1-adrenergic, Cotecchia *et al.* (1988); α_2-adrenergic, Kobilka *et al.* (1987); D_2-dopamine, Bunzow *et al.* (1988); serotonin (5-HT1c), Julius *et al.* (1988); M_2-muscarinic acetylcholine, Kubo *et al.*, (1986); Rhodopsin (Rh), Ovchinnikov *et al.* (1982) and Dratz and Hargrave (1983); H_2-histamine, Gantz *et al.* (1991); substance k (SKR), Masu *et al.* (1987); thromboxane (TXR), Hirata *et al.* (1991). (B) Structure of ligands for the various receptors.

In contrast to these receptors, bovine rhodopsin, which has a ligand with no hydroxyl substituent, does not have a Ser or Thr residue in this region of the protein. Interestingly, Drosophila rhodopsins which use a 3-OH retinal chromophore (Tanimura *et al.*, 1986), where the hydroxyl group is a substituent on the β-ionone ring, have a Ser residue at one or the other of these conserved positions in helix V. Drosophila contains visual pigments that have absorption maxima in the ultraviolet region of the spectrum as well as pigments with maxima in the

visible region. The pigments with maxima in the visible region of the spectrum have a Ser corresponding to position 204 in the β-receptor numbering system (O'Tousa *et al.*, 1985; Zuker *et al.*, 1985; Cowman *et al.*, 1986), whereas pigments with maxima in the ultraviolet have a Ser at position 207 (Zuker *et al.*, 1987; Fryxell and Meyerowitz, 1987). This correlation raises the possibility that the two Ser residues stabilize different conformations of the retinal chromophore, which may account in part for the different absorption maxima of these pigments.

Perhaps the most compelling correlation is found in the sequence of the H2-histamine receptor. The H2-receptor has an Asp residue at position 203 in addition to the Thr at position 207 (Gantz *et al.*, 1991). This is the only receptor that has an acidic amino acid residue at this position in helix V. Presumably, the negatively charged Asp forms an ion pair with the positively charged imidazolium group on the ligand (Birdsall, 1991).

HELIX III: COUNTERION TO POSITIVELY CHARGED LIGANDS

Helix III of the G protein-linked receptors contains four highly conserved amino acid residues which define the boundaries of this transmembrane segment. Almost all of the G protein-linked receptors that have been sequenced contain a Cys residue on the amino-terminal side of helix III. This Cys is invariably followed by exactly 23 amino acids of nonconserved sequence, and then a triad of the following composition: an acidic amino acid followed by a basic amino acid followed by an aromatic amino acid. Using the numbering system of bovine rhodopsin, these amino acids are Cys^{110}, Glu^{134}, Arg^{135}, and Tyr^{136} (Dratz and Hargrave, 1983).

To complement the observation of evolutionary conservation in this region of the protein, mutagenesis studies have shown the amino acids in helix III to be important to both the structure and mechanism of action of these receptors. Cys^{110} in rhodopsin when changed to Ser disrupts the structure and post-translational processing of the protein (Karnik *et al.*, 1988). Karnik and Khorana (1990) have shown convincingly that Cys^{110} forms an essential disulfide bond with Cys^{187}. Cys^{187} is also a highly conserved residue, located in the extracellular loop between helical segments IV and V. Site-directed mutagenesis of these Cys residues in β-receptor has been shown to disrupt

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          *     *     *
  ß2      C E F W T S I D V L C V T A S
  M2      C D L W L A L D Y V V S N A S
  D2      C D I F V T L D V M M C T A S
5-HT1c    C P V W I S L D V L F S T A S
  H2      C N I Y T S L D V M L C T A S

  SKR     C Y F Q N L F P I T A M F V S

  Rh      C N L E G F F A T L G G E I A
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Fig. 3. Sequence of a portion of helix III for several G protein-linked receptors. Asterisks are placed above positions 110, 113, and 117 in the rhodopsin numbering systems. Position 117 in the rhodopsin numbering system corresponds to position 113 in the β_2-adrenergic receptor numbering systems.

ligand binding to the protein (Dixon *et al.*, 1987; Dohlman *et al.*, 1990), and it is generally thought that a disulfide bond between these cysteines is a common feature of the G protein-linked receptors.

The Arg at position 135 can be mutated without effect on the structure of rhodopsin as judged by the ability of the mutant R135Q to bind 11-*cis*-retinal and form a wild-type pigment (Sakmar *et al.*, 1989). However, Arg^{135} is clearly implicated as a crucial residue for the ability of rhodopsin to activate the G-protein transducin. R135Q is unable to activate transducin (Sakmar *et al.*, 1989). Franke *et al.* (1990) have shown that a mutant in which Glu^{134} and Arg^{135} are interchanged binds transducin, but does not catalyze exchange of guanine nucleotides. Clearly, residues at the boundary of helix III are of obvious importance for both the structure and function of these receptors.

Within helix III, the biogenic amine receptors contain a highly conserved Asp residue that is absent in all of the other receptors (Fig. 3). This residue, Asp^{113} in β_2-adrenergic receptor, has been identified by Strader *et al.* (1988) as the counterion to the positively charged ammonium group of the ligands. They have shown that changing this residue to an Asn decreases the affinity of the β-receptor for agonist by four orders of magnitude.

Rhodopsin does not have an Asp at this position in helix III, which would correspond to position 117 in the rhodopsin numbering system. However, rhodopsin and all of the vertebrate visual pigments that have been sequenced to date, from human (Nathans *et al.*, 1986) to lamprey (Hisatomi *et al.*, 1991), have a Glu residue at position 113, four amino acid residues closer to the conserved Cys in helix III. Glu^{113} in rhodopsin has been shown to be the counterion to the positively charged Schiff base nitrogen of the 11-*cis*-retinal chromophore (Zhukovsky and Oprian, 1989; Sakmar

et al., 1989; Nathans, 1990). Changing Glu^{113} to Gln results in deprotonation of the Schiff base nitrogen and has a dramatic effect on the spectrum of rhodopsin, shifting the maximum 120 nm to the blue, from 500 to 380 nm. This has been shown to be a consequence of a large change in the pK_a of the Schiff base nitrogen in the mutant (Zhukovsky and Oprian, 1989; Sakmar *et al.*, 1989). The pK_a in the mutant is about 6, whereas the pK_a in the wild-type protein is estimated to be at least 11 since the protein denatures above this pH without reversibly losing the Schiff base proton (Radding and Wald, 1956). Substitution of Asp at position 113 restores wild-type spectral properties, demonstrating the requirement for a charged residue at this position.

Interestingly, invertebrate rhodopsins do not have an acidic amino acid at position 113. Nonetheless, conservation of amino acid is still observed at this position with the following correlation. All of the pigments that have absorption maxima in the visible region of the spectrum contain a Tyr at position 113 (Zuker *et al.*, 1985; O'Tousa *et al.*, 1985; Cowman *et al.*, 1986; Ovchinnikov *et al.*, 1988; Hall *et al.*, 1991), whereas all of the pigments with maxima in the ultraviolet region contain Phe (Zuker *et al.*, 1987; Fryxell and Meyerowitz, 1987). The Schiff base of the UV-absorbing pigments is probably not protonated, a conclusion based on the short-wavelength absorption maximum in these pigments. In contrast, the pigments with maxima in the visible range would be expected to contain a protonated form of the Schiff base. Octopus rhodopsin, which contains a Tyr at this position and has an absorption maximum in the visible range, has been shown by resonance Raman spectroscopy also to have a protonated Schiff base (Kitagawa and Tsuda, 1980; Pande *et al.*, 1987). The tyrosine hydroxyl group must fulfill a similar function to Glu^{113} in the vertebrate pigments: stabilizing the protonated state of the chromophore (see discussion in Zhukovsky and Oprian, 1989).

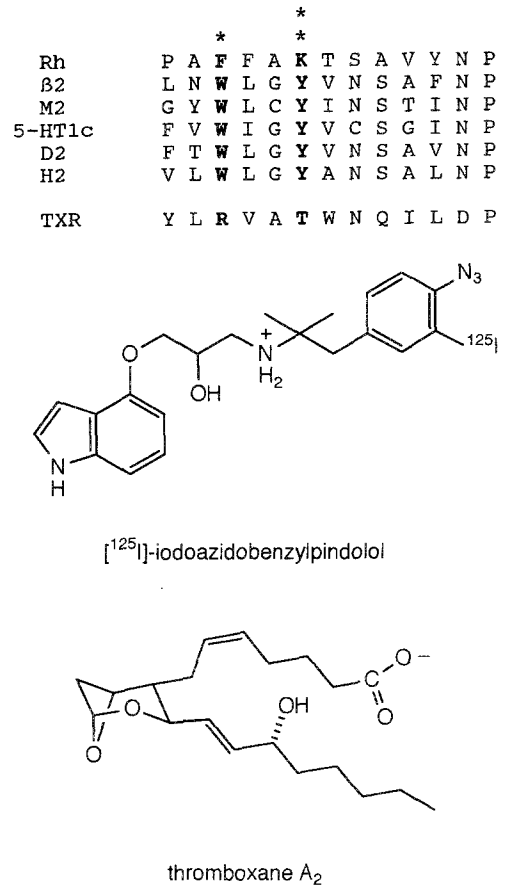

Fig. 4. Sequences of helix VII of several G protein-linked receptors. Single asterisk is placed above Trp^{330} of β_2-adrenergic receptor; double asterisk is placed above Lys^{296} of rhodopsin. The figure also shows the structure of the photochemical crosslinking agent used by Wong *et al.* (1988) to label Trp^{330}, iodoazidobenzylpindolol, and the structure of thromboxane A_2. The photochemical crosslinking experiment was performed with the turkey erythrocyte β-receptor, but for consistency with other figures in this article I have shown here the β_2-receptor sequence which is very similar. Strictly, the numbering system used for Trp^{330} is that of the avian receptor, not the β_2-receptor.

HELIX VII: LOCATION OF THE POSITIVELY CHARGED AMMONIUM ION

That helix VII is involved in ligand binding to the visual pigments has been known for some time from the fact that the chromophore is bound covalently to the protein through a Schiff base linkage to the ε-amino group of Lys^{296} in the seventh transmembrane segment (Bownds, 1967; Ovchinnikov *et al.*, 1982; Dratz and Hargrave, 1983). More recently, helix VII has been shown to be involved in ligand binding to other receptors. Wong *et al.* (1988) have shown that the photochemical cross-linking reagent [^{125}I]-iodoazidobenzylpindolol (Fig. 4), a β-receptor antagonist in which the relative nitrine is located on the ammonium-ion side of the molecule, covalently attaches to Trp^{330} in the seventh transmembrane segment of the avian β-receptor. As shown in Fig. 4, Trp^{330} is strictly conserved among the biogenic amine receptors. Trp^{330} in β-receptor corresponds to Phe^{293} in rhodopsin, located three amino acids away from

Lys^{296}, which orients the β-receptor ligand in the binding pocket with positively charged ammonium group near helix VII in a manner analogous to the positively charged Schiff base nitrogen of bacteriorhodopsin and the visual pigments.

Recently, the sequence for the thromboxane receptor has been determined by Hirata *et al.* (1991). Thromboxane has a carboxylic acid group on the side of the molecule opposite to that of the ring. This is analogous to the ammonium ion of biogenic amines and the Schiff base nitrogen of the visual pigment chromophores. To complement the carboxylate group on the ligand, the thromboxane receptor has an Arg residue instead of the conserved Trp at position 330 (β-receptor numbering system), as is shown in Fig. 4. This suggests that part of the ligand binding energy in the thromboxane receptor is derived from an electrostatic interaction between the negatively charged carboxylate on the ligand and the positively charged guanidinium group on the receptor.

CONCLUSION

It is clear from the preceding discussion that there is good reason to view the ligand binding pocket of visual pigments and biogenic amine receptors as evolutionary variations on a common theme. The ligands appear to be bound to the proteins with similar orientation, and make contacts with amino acid residues that are highly conserved among the different receptors. Even the fact that visual pigments bind their ligands covalently whereas other receptors do not is no longer an obstacle to this comparison since the covalent linkage in the visual pigments has recently been shown to be dispensable for the spectral properties of rhodopsin as well as its ability to activate transducin (Zhukovsky *et al.*, 1991).

One of the challenges remaining in this area is to reconcile the binding site structure and activation mechanism of the visual pigments and biogenic amine receptors with those of the large peptide hormone receptors such as thyrotropin receptor (Parmentier *et al.*, 1989) and lutropin-choriogonadotropin receptor (MacFarland *et al.*, 1989). These receptors contain a large amino terminal extension not seen with other G protein-linked receptors, and recent evidence suggests that it is to this extension that the hormones bind (Chazenbalk *et al.*, 1990; Wadsworth *et al.*, 1990; Nagayama *et al.*, 1991a, b; Moyle *et al.*, 1991). This is a surprising observation because it suggests that the ligand-binding site and activation mechanism in the large-peptide hormone receptors differs from those of the other G protein-linked receptors. In this regard, we note a recent study by Ji and Ji (1991) in which choriogonadotropin was shown to activate a mutant lutropin receptor missing almost all of the amino-terminal extension, suggesting that a unique mechanism does not exist for these receptors. It seems highly unlikely that a different activation mechanism would be employed by the lutropin and thyrotropin receptors, and with further study, the ligand binding and activation sites of these receptors will undoubtedly be shown to be similar to those of the other G protein-linked receptors. After all, it was not long ago that the ligands of the visual pigments and biogenic amine receptors were thought to have little in common.

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