Structure determination of a retinal rod G protein peptide segment bound to rhodopsin by nuclear magnetic resonance spectroscopy

by Julie Ege Furstenau

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University
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Abstract:
The goal of this research project is to investigate the three-dimensional structure of a specific peptide segment of the retinal rod G protein, transducin, bound to light excited rhodopsin. This segment has been shown to exhibit biological activity in much the same fashion as the full G protein. The segment studied consists of a modified portion of the C-terminus of the alpha subunit of transducin that runs from amino acid numbers 340 to 350. The peptide chain has been modified to prolong its biological activity by the addition of N-terminal acylation, and the substitution of a lysine for an arginine in the 341 position.

This project studied this peptide segment using two-dimensional nuclear magnetic resonance and computer refinement methods. The goal was to determine if the peptide has significant structure when free in solution, in the presence of bovine rhodopsin in an unactivated state, and finally bound to rhodopsin in its light excited form.

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This project has yielded preliminary structures for the metarhodopsin 11-bound peptide segment. Work continues in this laboratory to better define the dark- and light-bound structures and yield further understanding of this protein interface.

STRUCTURE DETERMINATION OF A RETINAL ROD G PROTEIN PEPTIDE SEGMENT BOUND TO RHODOPSIN BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

by

Julie Ege Furstenau

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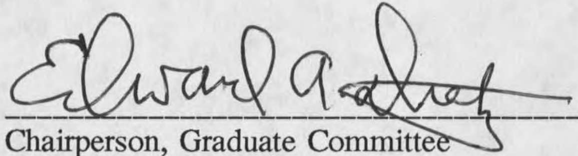
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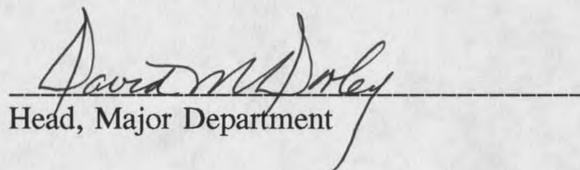
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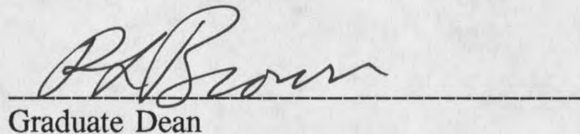
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ABSTRACT

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This project has yielded preliminary structures for the metarhodopsin II-bound peptide segment. Work continues in this laboratory to better define the dark- and light-bound structures and yield further understanding of this protein interface.

CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

Introduction

How cells detect their environment, respond to it and communicate these responses to other areas of the cell or the entire organism are key research areas in biological chemistry today. There are a variety of routes through which this information is communicated - hormones, neurotransmitters, growth factors, and ion channels that are selectively activated. All of the routes involve a receptor that can specifically discriminate and respond to chemical signals from outside the cell. In many cases the receptors are not themselves ion channels or enzymes, but are coupled to specific enzymes to produce the desired cellular response. There are several superfamilies of receptors that appear to have very similar structures within each superfamily. The largest superfamily of receptors are coupled to amplifier proteins called guanine-nucleotide-binding-proteins, or G proteins, and are called G protein coupled receptors (GPCR).

Overview of G proteins

A review by Simon et al states that over 100 different G protein coupled receptors had been found in mammals by 1990(1). Current estimates are that there are nearly 900 different GPCR in all species including insects and fungi (98). A classic review by Gilman (2) in 1987 classifies and defines G proteins on the basis of their structure and functions. His functional definition of G proteins is that they act as intermediates in transmembrane signalling by means of receptor-induced GTP binding. The signalling pathway always consists of the sequence: Receptor → G protein → Effector. As a structural definition, G proteins discovered to date are either heterotrimers, consisting of α, β, and γ subunits or so-called "small G proteins"(1) which appear to be homologous to a section of the heterotrimer α subunit. These small G proteins regulate cell growth, protein secretion and intracellular vesicle interaction (3). This discussion will focus on the heterotrimeric G proteins, the class in which the G proteins involved in vision are found. Among the receptors for the heterotrimeric G proteins, virtually all are thought to have a heptahelical transmembrane structure. The heterotrimeric G proteins are thought to exist in two distinct states depending on the state of excitation of the receptor to which they are bound (1).

Currently, the α subunits are believed to be key differentiators between G proteins, whereas the β and γ subunits are more similar to one another. Among all

the known α subunits, 20 % of the amino acids are fully conserved (4) and the α subunits can be grouped into several different classes based on amino acid and functional similarities (Table 1; reference 1).

Table 1. G protein designations and functions.

α Subunit Designation	Function
G_s	stimulatory regulator of adenylyl cyclase
G_i	inhibitory regulator of adenylyl cyclase
G_q	stimulates phosphatidylinositol-specific phospholipase C
G_{12}	little currently known about function
G_t	stimulates cGMP phosphodiesterase

Features of the $G\alpha$ subunits to be noted are in the NH_2 and COOH terminal regions. The NH_2 terminal region is believed to be involved in the interaction between the α and $\beta\gamma$ subunits and the receptor; and the extreme C terminal region is thought to be involved with the recognition of specific receptors (1). A recent paper (5) changed three of the four C terminal residues in a $G\alpha_q$ sequence to match those of a $G\alpha_{i2}$ sequence and changed the receptor specificity to that of $G\alpha_{12}$.

The literature indicates that much less is known about the function and structure of the β and γ subunits. The review article by Simon et al (1) states that four distinct $G\beta$ subunits have been found in mammals, with over 80 % of their amino acid sequences conserved. Tamir et al (6) investigated five different sources of

$G\beta\gamma$ and found only two highly conserved β forms. It is also postulated that the 30-40 initial amino acids on the NH_2 terminal end of the β subunit is responsible for the interaction with the γ subunit (1). As for the γ subunit, more diversity has been found here, with up to seven different $G\gamma$ sequences found (6). Tamir and co-workers hypothesize that the $G\beta$ subunits contain a common recognition site for various $G\alpha$ subunits, while the $G\gamma$ subunit is involved in receptor specificity.

In this research project the G protein involved is often referred to in the literature as transducin or G_t and is involved in the light activation of cGMP phosphodiesterase (PDE) in retinal rod outer segments (ROS). G_t is considered to be a distinct type of G_i protein by some researchers and it acts as an intermediate between the transmembrane rhodopsin and the enzyme PDE bound to the cytoplasmic surface of the membrane. Rhodopsin is able to function as a single photon detector in retinal rods by virtue of G_t, which acts as a pre-amplifier of the light signal and PDE which acts as a power amplifier for the output signal of the receptor cell via the cGMP cascade to be discussed further.

The Rhodopsin - G_t Interaction

The rhodopsin - G_t system has many advantageous features for study by modern biophysical techniques. A key advantage of the system is the availability and relative ease of isolation of the rhodopsin protein and G_t. Rhodopsin, along with its associated lipids, can be rather easily isolated from bovine retinas (7). Since the proteins in the rod outer segment are approximately 70% rhodopsin (8), it is relatively

easy to obtain rhodopsin in milligram amounts. Rhodopsin consists of two parts: the protein opsin and the small chromophore retinal, which is closely related to vitamin A(9, 10).

If ease of obtaining rhodopsin is one of the advantages of this system, a major complexity of this system is the photocycle of rhodopsin. Shown in Figure 1 is a diagram for the early events in the photocycle for rhodopsin, showing some of the major intermediates, structures and absorption maxima of the chromophore at each intermediate state (11). Several other intermediates have been described, including a metarhodopsin III (MIII) intermediate that can follow metarhodopsin II (MII) under appropriate temperature and pH conditions (12).

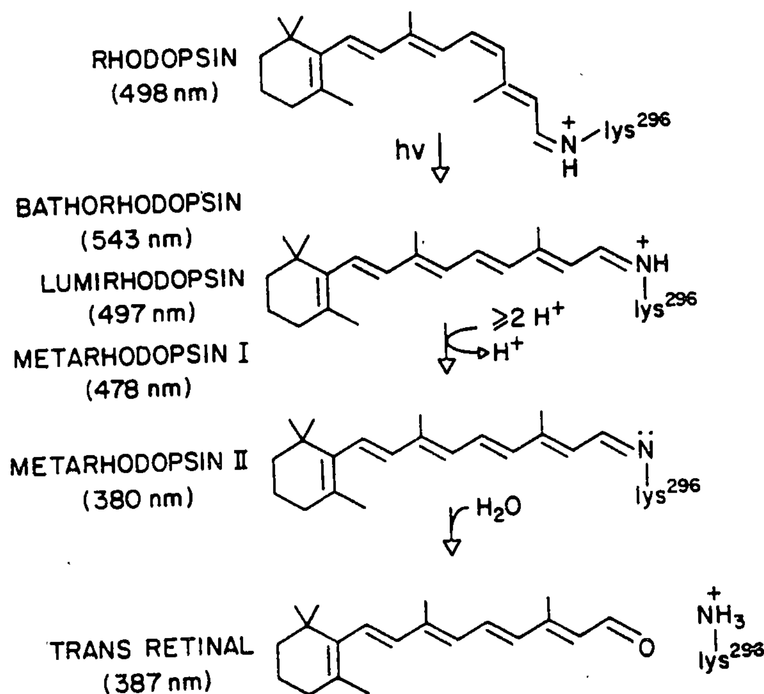

Figure 1. The rhodopsin photcycle. Shown are the changes in the chromophore structure and the absorption maximum of each intermediate. [Taken from Nathans (11)]

Another factor to be accounted for in the interaction of rhodopsin with the G protein is the physical structure of rhodopsin. Figure 2 gives a model of the rhodopsin protein within a rod outer segment, showing seven transmembrane helices that are thought to be present and a cut-away view of the approximate position of the chromophore within the protein (13).

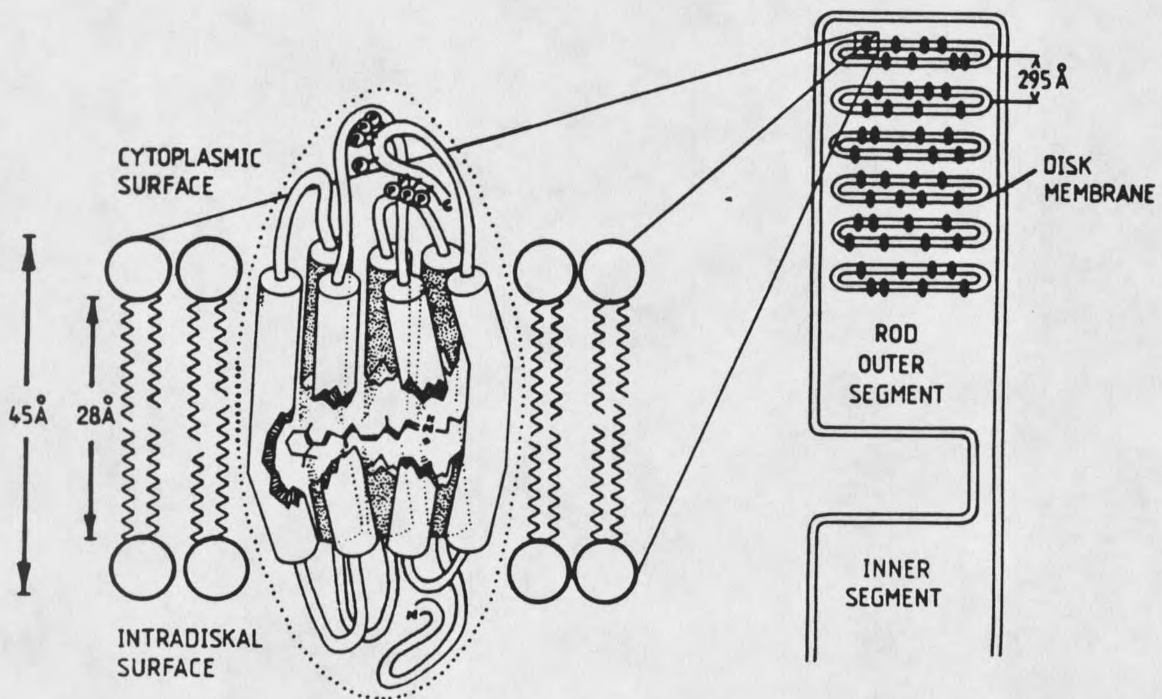

Figure 2. Cross-section of a rod outer segment. The drawing is not to scale. [Taken from Dratz and Hargrave (13)]

Figure 3 shows the primary sequence of bovine rhodopsin (13), and a model for the transmembrane topology, that is useful for further discussion of the interaction of rhodopsin with the G protein. The binding of photolyzed rhodopsin to a guanyl nucleotide binding protein with GTPase activity was first proposed by Fung and Stryer

(14), and is now generally accepted by researchers in this field (15). A diagram of a recent model for this binding is shown in Figure 4, and the complete cyclic GMP cascade that couples rhodopsin photolysis to an electrical response of the cell is shown in Figure 5.

Figure 3. Primary sequence of the rhodopsin protein. [From Dratz and Hargrave (13)]

The photolysis intermediate of rhodopsin that interacts with the G protein has been shown in a variety of studies to be MII, which is equivalent to the activated state rho* (or R* shown in Figure 5) originally referred to by various researchers (16). It has also been noted that the G protein shows some binding affinity to phospholipid