*Commentary*

# "Light" Reading: Targeting Tryptophans in Cyclic Nucleotide–gated Channels

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Our knowledge of the crystal structures of two related ligand binding domains (Weber and Steitz, 1987; Su et al., 1995) and a related $K^+$ channel pore–forming region (Doyle et al., 1998), together with detailed knowledge of function, makes cyclic nucleotide–gated (CNG) channels a unique model system for studying allostery in proteins. Already, we have learned much about the molecular basis for ligand specificity (Varnum et al., 1995), how subunits interact (Gordon and Zagotta, 1995c; Liu et al., 1998; Shammat and Gordon, 1999), and the features that distinguish one subfamily of CNG channel subunits from the next (Goulding et al., 1994; Gordon and Zagotta, 1995b).

One approach that has been used successfully with CNG channels, and many other types of proteins, is that of perturbing function by altering amino acid side chains. Through site-directed mutagenesis, any individual amino acid or group of amino acids can be replaced with natural or even unnatural amino acids. In CNG channels, this method has led to the elucidation of the mechanism of calmodulin modulation (Chen and Yau, 1994; Varnum and Zagotta, 1997), the determination of the binding site for external divalent cations (Root and MacKinnon, 1993; Eismann et al., 1994) and for local anesthetics (Fodor et al., 1997), the revelation of a role for the C-linker region in coupling ligand binding to opening of the pore (Gordon and Zagotta, 1995a,b; Zong et al., 1998; Paoletti et al., 1999), and identification of the molecular basis of ligand discrimination (Varnum et al., 1995). A second method for altering side chains (actually the first to be used to modify proteins; Means and Feeney, 1971) is to use reagents that modify channels after they have been translated and assembled. This method has been used to examine state-dependent changes in reactivity or accessibility (Gordon et al., 1996; Sun et al., 1996; Gavazzo et al., 1997; Brown et al., 1998, 2000; Becchetti et al., 1999; Matulef et al., 1999; Shammat and Gordon, 1999) and the proximity between domains distant in the primary sequence (Gordon et al., 1997).

Two papers in this issue combine old and new technology to probe the energetic coupling between ligand binding and the allosteric conformational change in CNG channels (Middendorf and Aldrich, 2000; Middendorf et al., 2000). Before the advent of site-directed mutagenesis, the induction of covalent modifications in amino acid side chains by ultraviolet light was one of the few available tools for altering protein structure (Vladimirov et al., 1970; Grossweiner, 1976). Middendorf and colleagues applied this technique to CNG1 (rod) and CNG2 (olfactory) channels. They found that exposure to UV light had complex effects on channel function: decreasing the maximal response to cGMP, increasing the response to low concentrations of cGMP, and decreasing the limiting slope of the dose–response relation for activation by cGMP. The wavelength dependence of the channel's UV sensitivity was consistent with that expected for the modification of tryptophan residues. A variety of experiments determined that each subunit probably contains a small number of tryptophan targets, each of which was modified through a one-photon mechanism and contributed toward altering the energetics of channel activation in a graded manner. Although they could not localize which tryptophan residues in the channel were the targets of UV modification, there clearly were two distinct classes of tryptophan targets. Modification of one class of tryptophan inhibited the channels, decreasing the response to high concentrations of cGMP. Modification of the second class of tryptophan potentiated the channels, increasing the response to low concentrations of cGMP. A decrease of the limiting slope of the cGMP dose–response relation to less than one also was observed.

To examine the interaction between the energetics of channel activation and the effects of UV modification, the authors altered channel function in three ways: altering the primary sequence (CNG2 channels compared with CNG1 channels), using two agonists with different efficacies (cAMP compared with cGMP), and applying a potentiator of CNG1 channel activation (the divalent transition metal $Ni^{2+}$). These experiments revealed an inherently different energetic cost for modifying the tryptophan targets in CNG2 than in CNG1. Finally, three types of models of activation were exam-


J. Gen. Physiol. © The Rockefeller University Press • 0022-1295/2000/08/223/3 $5.00
Volume 116 August 2000 223–225
http://www.jgp.org/cgi/content/full/116/2/223

ined: an independent Hodgkin-Huxley (HH) model (Hodgkin and Huxley, 1952) in which binding of cyclic nucleotide to a given subunit independently drives the opening conformational change in that subunit; a Monod-Wyman-Changeux (MWC) model (Monod et al., 1965) in which the binding of successive cyclic nucleotides produces an exponential increase in the favorability of the opening conformational change; and a Coupled Dimer (CD) model (Liu et al., 1998), in which the channel consists of a pair of dimers, each of which undergoes HH-type activation, although the two subunits within each dimer conform to MWC behavior. By comparing predictions of these models with their data, the authors found that both the MWC model and the CD model could produce adequate descriptions of channel behavior; the HH model could not.

Modifying tryptophan residues with UV light has several advantages. As the most highly conserved amino acid, efforts to substitute another amino acid for tryptophan using site-directed mutagenesis often results in nonfunctional proteins. This, in fact, was the case for every combination of two tryptophans that Middendorf and colleagues attempted to alter in the CNG channel sequence. Thus, using UV light can be a way to trick a protein into substituting something else for a tryptophan, with the caveat that only a small number of substitutions are possible and the experimenter cannot control which one will result. Their highly conserved nature makes tryptophans excellent subjects for this type of analysis—if they are so important to channel function, altering their structure is almost sure to perturb channel function.

As a general approach, the utility of using UV light to modify tryptophans in ion channels is limited by a few technical issues. One issue is that the *Xenopus* oocytes used for expression in this study contained a conductance that was activated by UV light. This slightly voltage-dependent, cation-selective conductance increased exponentially with cumulative UV light dose, and did not saturate within the range of the amplifier used. This is not likely to be a limitation unique to the oocyte expression system; similar UV-activated conductances have been reported in several mammalian cell lines (Mendez and Penner, 1998; Hsu et al., 1999; Wang et al., 1999). Another point to consider is that modification of tryptophans with UV light works best when only one type of photoproduct results from UV absorption. This case represents the simplest scenario in which the wavelength of the UV light affects the probability of photon absorption but not the efficacy or nature of the subsequent photochemistry. Finally, modifying tryptophans with UV light will be of most benefit in proteins that have a very small number of endogenous tryptophans. The greater the number of tryptophan targets, the greater the likelihood that more than one will be modified by UV light, and, thus, the greater the difficulty in eliminating all the tryptophans using site-directed mutagenesis.

UV light can be an important tool in our quest for understanding the structural basis for ion channel function. As with any technique, it has its own strengths and weaknesses. By taking advantage of its specificity (wavelength-dependent affect) and unique ability to target mutagenesis-resistant residues, photomodification could be used to gain insights into the importance of tryptophans, in particular ion channels and other proteins.

*Submitted: 7 July 2000*
*Revised 7 July 2000*
*Accepted: 11 July 2000*

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