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Solution NMR Spectroscopy of the Human Vasopressin V2 Receptor, A G Protein-Coupled Receptor

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The human genome encodes at least 800 unique G protein-coupled receptors (GPCRs), which share a common membrane topology of seven transmembrane segments.[1] GPCRs serve as the primary sensors for human olfaction, taste, and vision, play a host of critical roles in human physiology,[2,3] and are the targets for a substantial fraction of all known drugs.[4] Despite their importance, only a *single* high-resolution structure of a GPCR has been determined, that of the dark (signaling-off) state of the bovine photoreceptor, rhodopsin, first solved by X-ray crystallographic methods in 2000.[5,6] While solution NMR spectroscopy is now established as a method for determining the structures of polytopic membrane proteins,[7−10] the feasibility of approaching the structures of GPCRs by this method has not been clear. Previous solution NMR applications to GPCRs have been limited to (i) characterization of GPCR fragments and isolated extramembrane domains,[11−14] (ii) examination of ligands bound to GPCRs,[15−18] and (iii) probes of selectively labeled rhodopsin to address specific structural, dynamic, and mechanistic issues[19−21] (see also refs 22 and 23). Here, results are presented for a uniformly isotopically labeled GPCR that indicate a promising outlook for application of solution NMR methods to structural studies of intact receptors.

The human vasopressin V2 receptor (V2R, Figure S1 in the Supporting Information) is a 371 residue GPCR that plays critical roles in kidney-based fluid homeostasis.[24,25] Human V2R is subject to mutations that lead to inherited diabetes insipidus (DI)[24,26] and is a drug target for both DI[27] and polycystic kidney disease.[28] Choice of *Escherichia coli* as the expression host for V2R was based on three considerations: (i) uniform isotopic labeling required for NMR of large-membrane proteins is convenient in *E. coli*; (ii) V2R function is known not to be dependent upon the presence of post-translational glycosylation or palmitoylation;[29−31] and (iii) a number of GPCRs have previously been functionally expressed in *E. coli*.[32]

The 41 kDa human vasopressin V2 receptor was expressed at 37 °C in *E. coli*. Under these conditions, the protein is expressed into inclusion bodies. Despite screening many different detergent mixtures, unfold/refold protocols, and NMR sample conditions, we were unable to find conditions in which 37 °C expressed V2R exhibits more than 50 amide resonances in $^{1}H,^{15}N$-TROSY[33] spectra. This led us to search for conditions for expressing V2R in a manner that avoids formation of inclusion bodies. Expressing the protein in *E. coli* cultured at 12 °C led to high-level production (ca. 5 mg of V2R per liter of culture) of the protein into membranes.[34] Following screening of methods, we developed a protocol in which membrane extraction and protein purification were carried out using lyso-myristoylphosphatidylcholine (LMPC).[34] Among a variety of detergents tested, LMPC yielded the best-resolved NMR spectrum. This detergent has previously been shown to solubilize V2R and a number of other GPCRs in a manner that maintains native function, at least in the presence of added lipid.[35] That a lysolipid was found to yield optimal NMR results mirrors the conclusions of Girvin and co-workers regarding the relative merits of various detergents for use in NMR studies of other membrane proteins.[36] We also observed that LMPC-solubilized V2R yielded better spectra at pH 5.0 than at higher pH and also that 15 °C yielded better results than 5 or 25 °C. Glutaraldehyde cross-linking followed by gel electrophoresis suggests that the protein is primarily monomeric under NMR conditions. Samples remained stable for at least a couple of weeks at 15 °C.

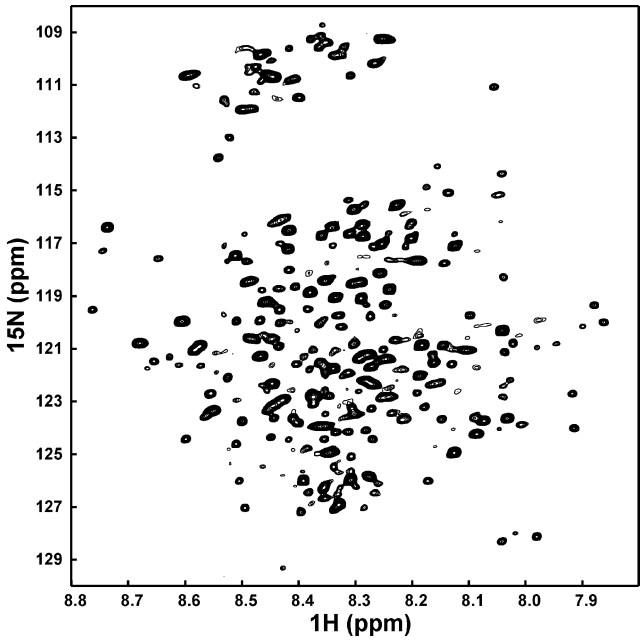

Figure 1. The 600 MHz $^{1}H,^{15}N$-TROSY[33b] spectrum of 0.5 mM U−$^{2}H,^{15}N$−V2R in LMPC micelles at pH 5.0 and 15 °C; 128 increments were acquired, with 16 scans per *t*1 point.

The 2-D TROSY spectrum of V2R under optimized conditions is shown in Figure 1. The identity of the protein represented in this spectrum was confirmed as intact V2R by mass spectroscopy. Moreover, a negative control sample prepared from cells induced at 12 °C in the same manner as for V2R, but using a V2R gene-empty plasmid, yielded a spectrum in which there were no amide peaks. The V2R spectrum is reproducible from preparation to preparation. Over 250 peaks out of an expected 349 amide peaks are observed in Figure 1. Resolution is quite encouraging, suggesting that making backbone resonance assignments for many sites in the protein is feasible. Further support for this notion was provided by a set of 3-D spectra (HCNA, HN(CO)CA, HNCACB, HN(CO)CACB), in which ca. 230 complete residue spin systems ($^{1}H_{amide}/^{15}N_{amide}/^{13}C_{\alpha}/^{13}C_{\beta}/^{13}CO$) could be identified. From these data, it was possible to make some tentative resonance assignments, as exemplified in Figure 2 for part of the extracellular loop connecting transmembrane segments 3 and 4.

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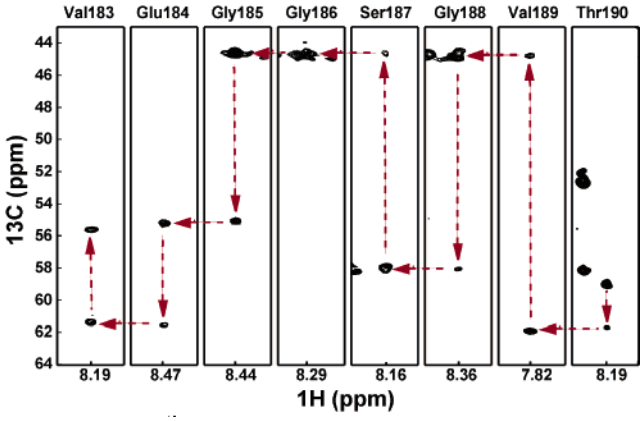

Figure 2. Selected ^{15}N planes from an 800 MHz 3-D TROSY−HNCA of V2R collected using the same conditions as given in Figure 1, but with uniform ^{2}H,^{15}N,^{13}C-labeling; 64 t_1 (^{13}C) and 56 t_2 (^{15}N) points were collected, with 8 scans per increment.

In the absence of perdeuteration of V2R, the number of peaks that could be detected was reduced by a factor of about 2 from what is observed in Figure 1. This is what might be expected as a result of deuteration-induced reduction in the dipolar relaxation pathways from side chain protons to amide ^{15}N/^{1}H. However, the quality of TROSY spectra was not higher than that for conventional HSQC spectra at either 600 or 800 MHz, and typical 600 MHz amide ^{15}N transverse relaxation times were surprisingly long (on the order of 200 ms). This suggests a high degree of global and/or local mobility associated with the amides giving rise to the peaks in Figure 1. One possibility is that most of the resonances of Figure 1 (and corresponding 3-D data sets) arise from mobile extramembrane loops and termini, rather than from the transmembrane domain.[37] However, even the ability to characterize the extramembrane domains of intact GPCRs and their interactions with cognate ligands and effector proteins using solution NMR would represent an important development in structural biology.

The sample preparative conditions described here[34] represent a basis for additional sample optimization. Immediate goals for V2R include observation of an even higher percentage of amide peaks. Also, while LMPC is known to support the functionality of native V2R (provided that some lipid is also present[35]), this has yet to be carefully tested for recombinant V2R. It is very possible that further optimization of sample conditions will be required to attain a favorable match between receptor function and NMR spectral quality. In any case, the results of this work offer a promising platform from which to stage future efforts to tackle the structure of V2R using NMR or other methods. There is also hope that the approach of coupling methods for expressing the receptor into membranes rather than into inclusion bodies, with use of a biochemically mild detergent at all stages of sample preparation, will prove to be applicable to other GPCRs.

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Supporting Information Available: Sample preparative methods and topology/sequence diagram for V2R. This material is available free of charge via the Internet at http://pubs.acs.org.

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