New technique helps researchers determine amino-acid charge

December 14 2005

Measurements of the ion-current through the open state of a membrane-protein's ion channel have allowed scientists at the University of Illinois at Urbana-Champaign to obtain a detailed picture of the effect of the protein microenvironment on the affinity of ionizable amino-acid residues for protons.

The findings, reported in the Dec. 15 issue of *Nature*, are expected to be welcome news for chemists and biophysicists, both experimentalists and theoreticians, because they have previously relied on theoretical estimations to predict protonation states -- whether an amino acid is charged or not. Appropriate experimental benchmarks also had been lacking.

All cells have membrane proteins that form channels to allow water and/or ions to pass through. Malfunctions have been linked to such problems as hypertension, abnormal insulin secretion, abnormal heart conditions and brain seizures. As a result, these membrane proteins are often targeted by drug treatments.

Previous approaches didn't provide sufficient resolution to let researchers accurately detect the association and dissociation of protons to and from individual amino-acid residues in real time.

Using the patch-clamp technique, the researchers were able to probe the electrostatic properties of the inner lining of the ion-channel's pore, and, from there, they inferred the rotational angle of the pore-lining alpha-

helices in the open state.

In this case, researchers focused on the muscle nicotinic acetylcholine receptor, a membrane protein that mediates voluntary muscle contraction.

"Our paper has implications that are specific to this receptor, but many of the findings can be extended to several other membrane proteins," said Claudio Grosman, a professor of molecular and integrative physiology at Illinois.

"We are working with the open state of the ion channel, and we now know how the helices that line the pore are oriented. This was not known before," Grosman said. "Previous work has told us how the helices are oriented in the closed state."

A major problem in understanding the relationship between structure and function in proteins, and the impact that electrostatics have on them, Grosman said, is not knowing the protonation state of ionizable residues. Protons, he added, are so small that they cannot be detected even with X-ray crystallographic approaches.

For the study, Grosman and colleagues used protein engineering. They mutated each residue of the pore's lining with basic amino-acid residues, which can acquire a positive charge upon binding protons and become neutral upon releasing them.

"One thing is the proton affinity of an amino-acid residue when the amino acid is dissolved in a lot of water in, say, a glass beaker; another thing is the affinity for protons in the complex microenvironment presented by a membrane protein," Grosman said. "For the first time, we were able to measure proton-transfer events at the single-proton, single-amino-acid level, in real time. Chemists will be happy to see this."

In addition to providing an extensive set of proton-affinity values for basic residues, which differed greatly from those found in a "glass beaker," the findings also discounted a long-held theory that the rotation of the pore-lining helices underlie the mechanism of opening and closing of the nicotinic receptor ion channel. The data, Grosman said, indicate that such rotation is minimal, if any.

Source: University of Illinois at Urbana-Champaign