New and Notable

Recalled to Life: Resurrection of Diffusion-Enhanced Fluorescence Energy Transfer

Robert H. Fairclough
Department of Neurology, University of California, Davis, California

On pages 1302–1335 of this issue is a triplet of biophysical studies on the acetylcholine receptor (AChR) from the Pedersen lab addressing the role of long-range electrostatic potentials in the binding of agonist and in determining channel pore properties. The first experimental determination of the electrostatic potential at the acetylcholine binding sites and through the ion channel pore (1,2) followed the introduction of the substituted cysteine accessibility method (SCAM) by Akabas et al. (3). The SCAM method relies on measuring the rates of covalent modification of cysteine-substituted mutants with methanethiosulfonates of different charges. However powerful this technology, the subject of the analysis is always a mutant with an introduced titratable charge that might alter the measured local potential. With an alternate paradigm, Meltzer et al. (4–6) have addressed the question of electrostatic potential at fixed sites on the AChR using diffusion-enhanced fluorescence energy transfer (DEFET), a technique pulled experimentally from the theory of Steinberg and Katchalski (7) by Thomas et al. (8) working in the Stryer lab back in the late 1970s. Initial applications of this technique seemed intent on using the quenching of long fluorescence lifetimes of chelated lanthanide donors to measure the distance of closest approach of a donor to an acceptor in a fluorescence energy transfer experiment. Soon thereafter came the realization and application of this technique to assess the electrostatic potential in the vicinity of an acceptor placed on a macromolecule (9–11) by measuring the long lifetimes of differently charged chelates of a lanthanide ($+1, 0, -1$ net charge) in the presence of the acceptor bound to a macromolecule. Since the mid 1980s, the technique seems to have remained dormant waiting for the right opportunity to spring back to life. The Meltzer studies applying this scheme to three critical sites on the AChR represent this reawakening.

Among the many charms of the AChR that press for understanding, the difference in agonist affinity at the two binding sites and the cation specificity of the AChR channel are certainly up high on the list. Certainly the advances in cryo-electron microscopy structural studies (12) have enabled new molecular dynamic calculation of electrostatic effects on receptor properties, but the limitations of the resolution of these electron microscopy studies and the missing components in the derived model compromise many attempts at this time. In the studies presented in this issue, Meltzer et al. provide DEFET as well as other kinetic determinations of electrostatic potential at the two agonist sites and in the neck of the ion channel pore. They further build a homology model of the AChR combining the high-resolution crystal structure of the acetylcholine binding protein (13) with the transmembrane structure of the AChR from Miyazawa et al.(14), and use this model with the nonlinear Poisson-Boltzmann equation to calculate the electrostatic potential about the AChR as a function of ionic strength. This electrostatic potential is then used to calculate the expected rate constants of DEFET for the differently charged Tb^{3+} chelates and the acceptors in the different locations. These rate constants, in turn, are used to calculate the potential at the site of the bound acceptors for the DEFET experiments. This scheme provides a bold head-to-head comparison of the experimentally-determined DEFET potentials to those predicted by the model, the calculated DEFET rate constants, and subsequent computed DEFET potentials. The results are presented for an acceptor in the three sites. The potentials determined at the two agonist sites, the $\alpha\gamma$-site and the $\alpha\delta$-site, at low ionic strength are -60 mV and -26 mV, respectively, and are computed to be -62 and -16 mV, respectively. These potentials at physiological ionic strength are -27 and -14 mV and contribute slight differences in agonist affinity at the two sites of -0.6 and -0.3 kcal/mole stabilization of agonist/AChR complexes at the $\alpha\gamma$- and $\alpha\delta$-sites, respectively. The good news is the rough agreement between the experimentally determined potentials and the computed potentials and the previous measurement of the low ionic strength potential using SCAM of -80 mV by Stauffer and Karlin (1). However, in the desensitized state, the affinity of the agonist at the $\alpha\delta$-site is threefold higher than that at the $\alpha\gamma$-site. Computing with Poisson-Boltzmann the potential within the binding pocket reveals a -87 mV potential at the $\alpha\delta$-site and a -42 mV at the $\alpha\gamma$-site, which goes some distance to explain the greater agonist affinity at the $\alpha\delta$-site compared to that at the $\alpha\gamma$-site. So the long range (through buffer) electrostatic effects are small but measurable, and lead to a higher concentration of cations just outside the $\alpha\gamma$-site, but the smaller negative potential within this site gives rise to a lower affinity for the positive-charged agonists than at the $\alpha\delta$-site.

A similar approach to the situation in the neck of the ion channel presents a slightly different picture but is intriguing as well. Meltzer et al. (4,5) evaluate the potential in the neck of the channel by three schemes: 1), by DEFET directly using the ratios of different Tb-chelate bimolecular rate constants, they find the potential to be -14 mV at physiological ionic strength; 2), by computation via Poisson-Boltzmann directly, they find the potential here to

Submitted May 15, 2006, and accepted for publication May 18, 2006.

Address reprint requests to Robert H. Fairclough, E-mail: rhfairclough@ucdavis.edu.

© 2006 by the Biophysical Society

0006-3495/06/08/1143/02 $2.00

doi: 10.1529/biophysj.106.087015

be −70 mV; and 3), finally by calculating the DEFET bimolecular rate constants for the differently charged chelates and taking the ratios, they find a potential between −40 and −20 mV depending on chelate size. So the potentials estimated by calculated DEFET rate constants (from −40 to −20 mV) may underestimate the Poisson-Boltzmann calculated potential (−70 mV) by as much as a factor of two or three. And the potential calculated from computed chelate rate constants (from −40 to −20 mV) is roughly double the potential calculated from measured rate constants (−14 mV) for the three chelates. This last potential suggests that the outer ring of charge in the neck is not the primary determinant of ion selectivity.

So Meltzer et al. (4–6) have their hands on significant tools to measure the electrostatic potential at various sites and determine the role played by through-buffer electrostatics on anion/cation discrimination. The DEFET technique offers a wealth of opportunity to probe the local electrical environment almost wherever one can attach an acceptor dye. The main experimental hurdle to overcome is to be able to assess whether dipole-dipole resonance energy transfer is the sole quenching mechanism or whether collisional quenching is playing a role in the experimental system. Finally, the analysis will improve with models for computing the electrostatic potential that include components of the AChR not yet included: sugars, protein bound divalent cations, and helical dipoles, all of which could have substantial effects on the actual experimental DEFET rates and are not yet included in the calculated potentials from which calculated DEFET rates are computed and potentials estimated.

Meltzer et al. (4–6) have reintroduced an old biophysical paradigm and with many of the new computational tools at hand have indicated the wonderful potential for extracting the essence of the biophysical identity of macromolecular systems: the electrical potential about and throughout a macromolecule. We now have with SCAM and DEFET two techniques that with refinement offer the ability to determine the electrostatic identity of biological macromolecular assemblies. Hello, proteome.

REFERENCES

1. Stauffer, D. A., and A. Karlin. 1994. Electrostatic potential of the acetylcholine binding sites in the nicotinic receptor probed by reactions of binding-site cysteines with charged methanethiosulfonates. *Biochemistry.* 33:6840–6849.
2. Pascual, J. M., and A. Karlin. 1998. State-dependent accessibility and electrostatic potential in the channel of the acetylcholine receptor. Inferences from rates of reaction of thiosulfonates with substituted cysteines in the M2 segment of the alpha subunit. *J. Gen. Physiol.* 111:717–739.
3. Akabas, M. H., D. A. Stauffer, M. Xu, and A. Karlin. 1992. Acetylcholine receptor channel structure probed in cysteine-substitution mutants. *Science.* 258:307–310.
4. Meltzer, R. H., M. M. Lurtz, T. G. Wensel, and S. E. Pedersen. 2006. Nicotinic acetylcholine receptor channel electrostatics determined by diffusion-enhanced luminescence energy transfer. *Biophys. J.* 1315–1324.
5. Meltzer, R. H., W. Vila-Carriles, J. O. Ebalunode, J. M. Briggs, and S. E. Pedersen. 2006. Computed pore potentials of the nicotinic acetylcholine receptor. *Biophys. J.* 1325–1335.
6. Meltzer, R. H., E. Thompson, K. V. Soman, X.-Z. Song, J. O. Ebalunode, T. G. Wensel, J. M. Briggs, and S. E. Pedersen. 2006. Electrostatic steering at acetylcholine binding sites. *Biophys. J.* 1302–1314.
7. Steinberg, I. Z., and E. Katchalski. 1968. Theoretical analysis of the role of diffusion in chemical reactions, fluorescence quenching, and nonradiative energy transfer. *J. Chem. Phys.* 48:2404–2410.
8. Thomas, D. D., W. F. Carlsen, and L. Stryer. 1978. Fluorescence energy transfer in the rapid-diffusion limit. *Proc. Natl. Acad. Sci. USA.* 75:5746–5750.
9. Meares, C. F., S. M. Yeh, and L. Stryer. 1981. Exchange interaction contribution to energy transfer between ions in the rapid-diffusion limit. *J. Am. Chem. Soc.* 103:1607–1609.
10. Wensel, T. G., and C. F. Meares. 1983. Electrostatic properties of myoglobin probed by diffusion enhanced energy transfer. *Biochemistry.* 22:6247–6254.
11. Wensel, T. G., C. F. Chang, and C. F. Meares. 1985. Diffusion-enhanced lanthanide energy transfer study of DNA-bound cobalt(III) bleomycins: comparisons of accessibility and electrostatic potential with DNA complexes of ethidium and acridine orange. *Biochemistry.* 24:3060–3069.
12. Unwin, N. 2005. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J. Mol. Biol.* 288:765–786.
13. Brejc, K., W. J. van Dijk, R. V. Klaassen, M. Schuurmans, J. van der Oost, A. B. Smit, and T. K. Sixma. 2001. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature.* 411:269–276.
14. Miyazawa, A., Y. Fujiyoshi, and N. Unwin. 2003. Structure and gating mechanism of the acetylcholine receptor pore. *Nature.* 423: 949–955.