Darwinian biophysics: Electrostatics and evolution in the kinetics of molecular binding

J. Andrew McCammon[1]

Howard Hughes Medical Institute, Center for Theoretical Biological Physics, Departments of Chemistry and Biochemistry and Pharmacology, University of California at San Diego, La Jolla, CA 92093-0365

At the molecular level of biology, the competition for favorable outcomes has been shaped by evolution, just as in more familiar examples from ecological biology. At both levels, this competition is often based on raw speed. There are differences, of course. Most notably, a race between molecules is more often determined by diffusional dynamics than by inertial dynamics. The driving forces on molecules typically comprise electrostatic nudges rather than thundering hooves digging into soil. Electrostatic interactions can be surprisingly effective, however. The rate of degradation of the neurotransmitter acetylcholine by the synaptic enzyme acetylcholinesterase is known to be increased by a factor of up to a few hundred as a result of "electrostatic steering" of the positively charged acetylcholine molecule toward the predominantly negative active-site region of the enzyme (1). This tends to optimize the clearing and resetting of neuromuscular junctions and other cholinergic synapses, which offered a clear competitive advantage to our successful ancestors, relative to more sluggish individuals of their species who faced the same predators. Such selective pressures are also recorded in proteins at the next level of a hierarchy, in some of the venom molecules of snakes such as the green mamba that prey on small mammals in sub-Saharan East Africa. The green mamba toxin fasciculin-2 is a small protein whose positively charged surface is attracted to, and clamps down on, the active-site entrance of acetylcholinesterase, causing muscular activity of the unfortunate rat or other prey to cease. Here, again, the binding involves electrostatically steered diffusion, and the binding speed is increased by a factor of up to a few hundred by the electrostatic attraction between the proteins (1). Many other examples of electrostatically steered, diffusion-controlled processes are now known, including such familiar ones as the polymerization of actin (2, 3). In a recent issue of PNAS, a new article by Qin and Zhou greatly deepens our insight into these important processes, and extends the range of analysis to include reactions in which the rates may be influenced by events following the initial diffusional encounter (4).

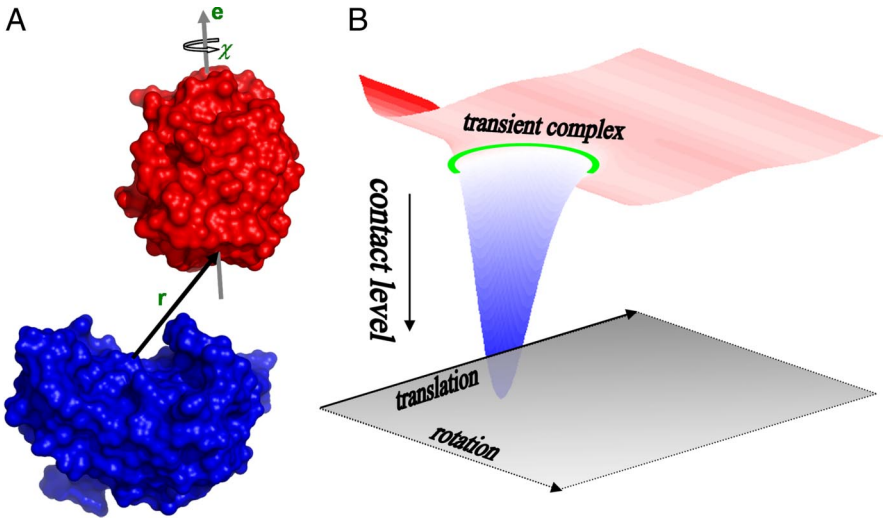

Fig. 1. Schematic illustration of concepts in the transient complex theory for the binding of 2 proteins. (*A*) The red protein approaches the blue protein, with resulting variations in translation (*r*) and orientation (e and χ). (*B*) The corresponding energy landscape. Long-range electrostatic steering forces may operate during the initial approach, leading to a gradual decrease in free energy as the proteins approach (red surface). Closer approach may lead to a more rapid decrease in free energy as contacts begin to form (blue funnel), starting with configurations in the transient complex region (green ring). [Reproduced with permission from ref. 9 (Copyright 2008, Wiley).]

Experimentally, diffusion-controlled reactions have been characterized by an inverse linear dependence of their rates on solvent viscosity. Further changes of rate with changes of salt concentration in the solvent signal attractive or repulsive electrostatic interactions between the reactants, which are diminished or "screened" by the salt. More detailed understanding of such reactions has been developed by computer simulations, typically using Brownian dynamics propagation of model reactants with the salt effects treated with approximate descriptions of the ionic atmosphere (5, 6). Reaction between the diffusing species is typically assumed to happen instantaneously on collision, if specified geometric criteria are attained (e.g., a reactive patch on one protein contacts a reactive patch on its partner). Such simulations have often predicted enhancements of ≈2–3 orders of magnitude in the rate constants for electrostatically attractive reactants, as mentioned above.

Qin and Zhou (4), following a line of work initiated some years ago by Zhou (7, 8), have elaborated a quasi-analytic formulation of rates of electrostatically steered reactions, whose rates may be influenced by diffusion (Fig. 1). The reaction is considered to involve 2 steps, the formation of a transient complex, which then either reverts to separated reactants or converts to form products. If the rate of reversion is slow relative to product formation, one has effectively a diffusion-controlled process. Otherwise, the reaction may settle into a steady state characterized by "preequilibrium" kinetics. Formally, the equation for the rate constant in the transient complex theory resembles that in familiar elementary formulations of transition state theory: The rate is equal to a basal rate constant multiplied by a Boltzmann factor. However, although there is resemblance in form, the contents are fundamentally different (9). Transition state theory applies to processes that involve climbing over an energy barrier, whereas the transient complex theory deals with processes in which the rate-

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[1]E-mail: jmccammon@ucsd.edu.

limiting step is the diffusional search for specific separations and orientations between 2 molecules. The basal rate constant is just the rate constant for reactants to achieve transient complex configurations in the absence of steering forces; this is determined by use of Brownian dynamics simulations. The effects of the steering forces are collected into the Boltzmann factor, with an electrostatic free energy that gauges the enrichment of the transient complex population. The Boltzmann factor in transient complex theory typically corresponds to rate enhancement; in contrast, the Boltzmann factor in transition state theory typically leads to rate reduction. The transient complex formulation has been shown to be quite accurate as long as the complex configurations are not too widely dispersed in space (7, 8).

In the new work, Qin and Zhou (4) have studied a most remarkable reaction: the destruction of ribosomes by ribonuclease toxin enzymes from the group that includes such notorious examples as ricin. The particular toxin studied by Qin and Zhou is restrictocin, which is known to cleave the sarcin/ricin loop of the ribosomal 23S–28S rRNA. The rate constant for this reaction at low ionic strengths is increased by an astonishing factor of ≈3 million because of electrostatic attraction between the enzyme and the ribosome. Using the transient complex formalism, Qin and Zhou were able to show that the rate constant for binding to the isolated sarcin/ricin loop is increased by a factor of ≈300 due to electrostatics, very similar to what has been seen in earlier studies of electrostatically steered reactions. The additional factor of 10,000 was shown to result from 2 synergistic effects: electrostatic interaction with the more distal part of the ribosome, and a small displacement of the location of the transient complex relative to the sarcin/ricin loop, to a region where the local electrostatic interaction with the loop is significantly enhanced. The latter displacement in this case results from short-range interactions with nearby ribosomal proteins that reorient the restrictocin slightly, bringing three of its basic residues closer to the negatively charged loop. For the restrictocin–ribosome system, the 2 steps of the forward reaction were found to be, first, the formation of the transient complex, and second, a conformational change of the loop in an apparently induced fit step concomitant with the formation of the stable complex. Beyond the findings for the particular system studied, Qin and Zhou note that the strengthening of electrostatic attraction between reactants by repositioning the transient complex with short-range interactions can be an important complement to the strengthening of long-range electrostatic interactions in producing very fast binding processes.

The Boltzmann factor in transient complex theory typically corresponds to rate enhancement.

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