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Minireview

The courtship of proteins: Understanding the encounter complex

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ABSTRACT

The formation of protein complexes involves an encounter complex, in which proteins show few specific interactions and assume many orientations. Recent kinetic and structural studies have shed light on this elusive state. It is generally dominated by electrostatic interactions, although hydrophobic interactions can play a role. During the encounter phase the proteins remain largely solvated. In extreme cases, the proteins only form an encounter complex, and in many other complexes, the encounter state constitutes a significant amount (5% or more), indicating that the energy difference between encounter and productive complexes is small. Thus, the encounter complex represents an essential part of protein complexes.

1. Introduction

Communication is an essential part of complex organisations, be it human society or a living cell. Proteins communicate via physical interactions. To transfer an informative message, specificity is required. Proteins recognize partners through short-range biophysical interactions, like hydrogen bonding, van der Waals forces and the hydrophobic effect, in a binding interface that usually represents a small fraction of the total surface of the protein. It is by no means a trivial task for a protein to find the binding site on the partner and align with it its own binding site to achieve the correct interactions. The process of complex formation comprises at least two steps. Upon meeting a partner protein, first an encounter complex is formed, that either proceeds towards the final complex or dissociates again. The nature of the encounter state has been elusive for a long time, due to a lack of experimental methods to probe it. Recently, new tools to study this preliminary step in complex formation have been reported and with it comes a changing view of this state. I will present a summary of the relevant theoretical considerations and then discuss the experimental results on the encounter state of protein complexes.

2. Diffusion through liquids

The diffusion of macromolecules through an aqueous solution is very different from diffusion through a gas. The latter can be compared with billiard balls, moving with constant speed over trajectories that are much longer than their radius, before bumping into another ball [2]. Diffusion through a liquid is better compared with a giant, 20 m diameter ball in a playground very crowded with children. It feels pushing forces from all sides, and thus, it is displaced as a consequence of statistical probability that at a given point in time the forces in one direction are stronger than in the opposing direction (Fig. 1A). The trajectory of displacement is extremely short because of constant collisions with children. An interesting situation arises when the ball hits a wall. No children push the ball away from the wall, because there are none between the wall and the ball, but children still push it against the wall (Fig. 1B). Therefore, the ball will remain close to the wall for a prolonged period of time. Translation along the wall and rotational movements are still possible. Analogously, a macromolecule pushed around by water molecules moves in a Brownian way through the solution. When it collides with another macromolecule, the two will stay together for a certain time and during the time of this macrocollision, diffusional and rotational movements lead to multiple microcollisions, allowing the macromolecule to sample a certain surface area of the partner. The duration of such a macrocollision is about R^2/D, with R being the sum of the radii of the macromolecules and D the diffusion constant [2], resulting in lifetimes in the ns range for proteins [3].

It is important to realize that the macrocollisions described here require no interaction forces between the macromolecules [3]. The lifetime of the macrocollision will often not be sufficient for a macromolecule to find a small binding site on the partner and

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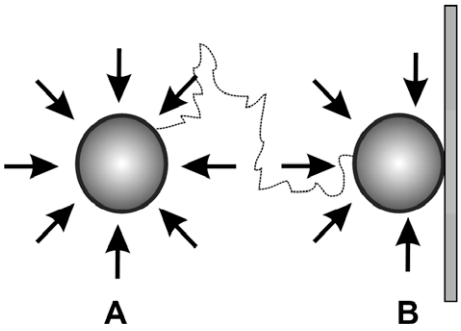

Fig. 1. A macromolecule experiences collisional forces from solvent molecules (arrows) and shows a Brownian movement (line) through the solution (A) until it collides with a wall or other macromolecule. The remaining forces push it onto the wall (B).

the fraction of macrocollisions that results in a productive complex will be small. The probability of forming a productive complex can be increased in two ways. First, the lifetime of the macrocollision can be extended. Second, the site of initial contact can be influenced to reduce the surface area that needs to be searched. In both mechanisms, electrostatic forces play an important role. The first mechanism is known as reduction in the dimensionality of the diffusional search [4]. An interaction force that is non-specific, i.e. does not lead to binding at a specific site, keeps the macromolecules in proximity for a prolonged time, allowing a more extensive search of the surface of the partner by translational and rotational movements. A well-known example is non-specific binding of proteins to DNA. The negative charge of DNA attracts positively charged proteins, without providing a specific interaction site. In this way, the protein can search the DNA for its specific binding sequence more extensively before it dissociates again. It has been demonstrated theoretically that a reduction of the dimensionality, in particular when going from three dimensions (3D) to two (2D), speeds up the search process [4].

The second mechanism is important for proteins with a charge dipole. As an example, in the complex of the redox proteins cytochrome *c* (Cc) and cytochrome *c* peroxidase (CcP), the search for the binding site is limited by dipolar preorientation of the proteins upon their approach. The encounter state consists predominantly of complexes in which the strongly positive patch of Cc and the negative side of the CcP face each other, as will be discussed in more detail later. Thus, this mechanism leads to a dramatic reduction of the area to be searched. At the same time the first mechanism will apply. The charge interactions will prolong the lifetime of the macrocollision allowing for a 2D search of the charged areas to find the productive orientation. In this way, the fraction of productive complexes can approach 100% of the macrocollisions. However, a protein surface is more irregular than DNA and it is less straight forward to create a smooth surface charge, in which all protein orientations have similar energy. It is probably for this reason that 'locking' of proteins in non-productive electrostatic complexes is observed at low ionic strength, at which the charge interactions can be very strong (see below).

The dominant role for electrostatic forces in the initial stage of complex formation is a consequence of its long-distance nature, in contrast to the short-range forces mentioned above that are responsible for specificity.

3. Two-step complex formation

The formation of a productive protein complex can be described by a two-step model

$$A + B \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} AB^* \underset{k_{-2}}{\overset{k_2}{\leftrightarrows}} AB \qquad (1)$$

where A and B are the free proteins and AB^* is the ensemble of orientations that precedes the productive complex AB. AB^* is defined here as the encounter complex. The macroscopic rate constant for formation of the productive complex is $k_{on} = k_1k_2/(k_{-1} + k_2)$, assuming a steady state for the concentration of AB^*, and for dissociation, $k_{off} = k_{-1}k_{-2}/(k_{-1} + k_2)$ [5].

In Fig. 2 energy diagrams are shown to illustrate various possibilities. The diagrams are shown as a 3D funnel, with the productive complex at the tip and the encounter complex as the broad area around it to emphasize that the latter represents an ensemble of orientations, with a similar energy level. The diagrams are drawn schematically for clarity but are less smooth and symmetric in reality.

Fig. 2A illustrates the macrocollision between two non-interacting macromolecules, with a lifetime of nanoseconds. The energy level of the complex is the same energy level as for the free proteins, because there is no stabilizing force. An energy barrier is shown but it may be that formation of this encounter complex is activationless, i.e. without any transition state. In Fig. 2B, electrostatic attraction between the proteins results in a stabilized encounter complex with a smaller set of possible orientations due to dipolar orientation. Whether this process involves a significant transition state is unknown. The first question that needs to be posed is

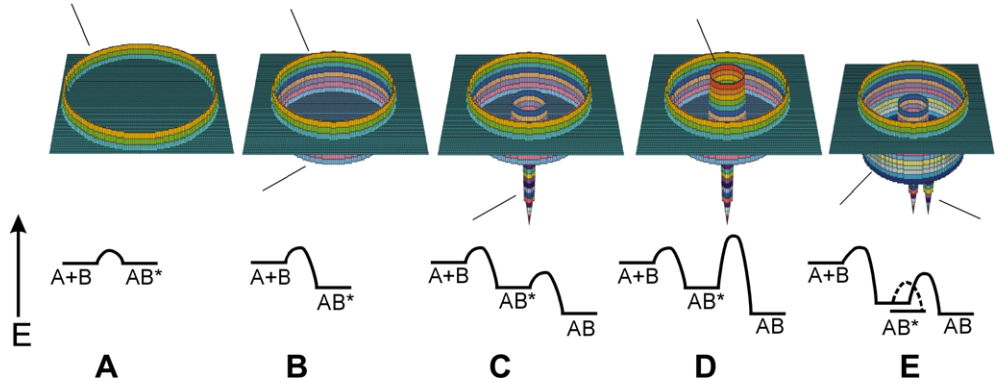

Fig. 2. Energy diagrams. (A) Encounter complex without favourable forces between the proteins. (B) Encounter complex with electrostatic attraction. The encounter complex is stabilized by charge interactions and the number of orientations is reduced by dipolar preorientation. (C) The encounter complex can proceed to a more stable productive complex via a low-energy transition state ($k_2 \gg k_{-1}$). (D) Like C, with a high-energy transition state ($k_2 \ll k_{-1}$). (E) An electrostatic encounter complex at low ionic strength, with highly stabilized encounter complex. The proteins can get trapped in a non-productive local energy minimum.