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Formation of transient protein complexes

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The encounter complex of two proteins is a dynamic intermediate state that guides proteins to their binding site, thus enhancing the rate of complex formation. It is particularly useful for complexes that must balance a biological requirement for high turnover with the need for specific binding, such as electron transfer complexes. Here, we describe the current methods for studying and visualizing encounter complexes. We discuss recent developments in mapping the energy landscapes, the role of hydrophobic interactions during encounter complex formation and the discovery of futile encounter complexes. These studies have not only provided insight into encounter complexes of electron transfer proteins, but also opened up new questions and approaches for studying encounter complexes in other weakly associated proteins.

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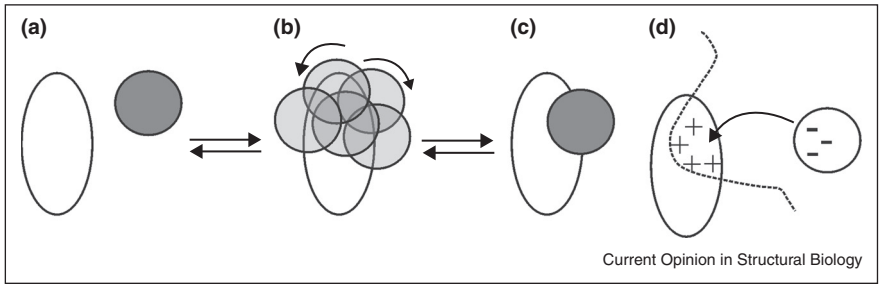
Introduction

Historically, protein complex formation has been viewed as a simple two state process in which the proteins are either free and unbound or bound in a static, specific complex. However, this view has changed to include an intermediate stage, commonly known as the encounter complex (Figure 1a–c). The encounter complex is comprised of an ensemble of low energy, weakly associated conformations that are in a dynamic equilibrium with a specific complex. In this loosely associated state, the proteins are free to rotate and reorient themselves, sampling each other's surfaces and increasing the number of contacts, until optimal binding geometry is reached and the complex can proceed to the tightly bound, active state [2]. The encounter complex is stabilized mainly by long-range electrostatic interactions and desolvation forces, with short-range interactions generally becoming important later in specific complex formation [3]. The electrostatic forces start to work already when the proteins approach each other, resulting in a pre-orientation in the electric field (Figure 1d). If the charges are placed correctly on the surface, this process increases the chance that the proteins collide with the binding sites in close approximation. The charge attraction also prolongs the lifetime of the encounter. Thus, the encounter complex can aid in specific complex formation by reducing the surface area on the two proteins that needs to be searched before the binding site is found as well as by extending the lifetime of diffusional collisions between the two proteins [4]. However, a consequence of charged patches on a protein surface is that the partner can bind in many different orientations with more or less the same decrease in free energy [5].[1] For formation of a specific complex, a single orientation is required with a much lower free energy than that of similar orientations, which is usually achieved by multiple short-range interactions (van der Waals, H-bonding, hydrophobic contacts and specific salt bridges). Consequently, stabilization of the encounter complex enhances complex formation, but at the same time can counteract the formation of a specific complex. This dilemma is particularly relevant for weak complexes, such as formed between electron transfer (ET) proteins.

The encounter state plays an important role in complexes in which the biological requirement of a high turnover rate must be counterbalanced with the demand of forming a specific interaction, which is the case for many ET proteins. This review will mainly discuss ET complexes, because much work has been done to study their encounter states, although examples of other, higher affinity complexes will also be given. Interestingly, the nature of the encounter complex varies for each ET complex, depending on the exact physiological requirements of the protein complex. In particular, the fraction of the proteins bound in the encounter state versus the productive state can differ markedly between complexes, depending on the specificity of binding in the productive state [6]. For example, the encounter complex for cytochrome (cyt) *c*–cyt *c* peroxidase complex comprises 30% of the population [7••,8•] and this population can readily be shifted with targeted point mutations at the interface of the

[1] This type of binding was dubbed the Velcro model of binding [5], because it resembles pieces of Velcro, which can bind with similar strength in many orientations. However, that metaphor is also deceptive, because Velcro halves need to be separated completely before another orientation can be formed, whereas it is believed that the proteins in the encounter complex constantly rearrange within the complex without complete dissociation, although no direct evidence for that assumption is available.

Figure 1



Model of protein complex formation. **(a)–(c)** During complex formation, free proteins (a) first form a weakly bound transient intermediate, the encounter complex (b), before proceeding to the final specific complex (c). **(d)** As the binding partners approach each other, long-range electrostatic interactions between charged residues on the protein surfaces pre-orient the proteins for complex formation.

specific complex to as low as 10% or as high as 80% [9]. Similar results have been shown for the myglobin–cyt b_5 complex [10–12] and the plastocyanin–cyt *f* complex [13]. Furthermore, some complexes have been shown to exist purely in a productive encounter complex, never proceeding to a specific complex, such as the adrenodoxin–cyt *c* complex [14] and myglobin–cyt b_5 complex [15–18].

This paper will give a brief overview of the current methods for studying and visualizing ET encounter complexes. It will also mention the latest developments in mapping the energy landscapes of these associations as well as discuss the increasing recognition of the role that hydrophobic interactions play in encounter complex formation. Finally, complexes that are not optimized for fast complex formation, resulting in 'futile' encounters, will be considered.

Methods to study encounter complexes

Kinetics

During kinetic experiments, some form of spectroscopic change is measured in a time-resolved manner. This change can be a consequence of a chemical reaction, such as an ET reaction. Under certain conditions the observed rate depends not (only) on the ET reaction itself but is influenced by the type of complex formed or the rate of complex formation; thus, indirectly, information about the encounter complex can be extracted. In this manner, stopped flow experiments have been used to study the effects of changes in protein surface charges as well as buffer ionic strength on the electron transfer rate in the cyt *f*–plastocyanin complex. This complex was shown to exist in multiple conformations [19–22] including minor states that had not been observed in previous NMR studies [23]. Likewise, transient absorption kinetics measurements have been used to study the complex of zinc-substituted myoglobin and cyt b_5. Using flash photolysis to observe triplet-quenching and ET, the complex was shown to exist purely in the encounter state [18,24].

The association rate of complex formation can also be detected directly, if it leads to a change in Trp fluorescence. In combination with mutagenesis studies it has been possible to characterize interactions between specific residues in the encounter complexes of several proteins [25•]. For kinetic data to be interpreted with respect to the encounter complex in such cases, the complex formation must be considered as an intramolecular chemical reaction. So strictly speaking, it is the transition state (peak in the energy landscape) between the encounter and specific states that is probed in such kinetic experiments, whereas in the structural studies discussed below, the wells in the energy landscape are studied. Because the transition state is closer to the specific complex than the encounter state is, kinetic and structural studies are complementary.

NMR studies

Various NMR observables can be used to characterize encounter states. However, it should be realized that each of these represents an average of all the states of the protein complex in equilibrium, because exchange between these different conformations usually occurs much faster than the NMR time scale [4]. Nevertheless, it has been shown that the magnitude of backbone amide chemical shift changes upon complex formation is a good indicator for the degree of dynamics within the protein complex, and thus the population of the encounter complex, at least if it is dominated by electrostatic interactions. A possible explanation for this observation is that in the encounter complex the proteins remain solvated and thus the chemical environment of the amides hardly changes, resulting in very small perturbations, while in the specific complex, the solvent layer is changed more drastically, causing large perturbations of the amide resonances [15,26,27].

More recently, paramagnetic NMR has proven to be a powerful technique for studying lowly populated states such as the encounter complex. Paramagnetic effects are

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