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NMR Studies on Transient Protein Complexes: Perspectives

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Abstract It is generally understood that protein–protein interactions proceed via transient encounter complexes that rapidly evolve into the functional stereospecific complex. Direct detection and characterization of the encounter complexes, however, been difficult due to their low population and short lifetimes. Recent application of NMR paramagnetic relaxation enhancement first visualized the structures of the encounter complex ensemble, and allowed the characterization of their physicochemical properties. Further, rational protein mutations that perturbed the encounter complex formation provided a clue to the target search pathway during protein–protein association. Understanding the structure and dynamics of encounter complexes will provide useful information on the mechanism of protein association

Keywords NMR, paramagnetic relaxation enhancement, encounter complex, target search pathway

Introduction

Stereospecific protein–protein interaction is a key to many biological processes such as enzyme catalysis, immune response, and cell signaling. Specific protein interaction involves the diffusion-controlled collision of two proteins to form a nonspecific transient encounter complex ensemble, followed by two-dimensional search process leading to the final complex (Figure 1). The formation of early encounter complexes greatly facilitates the association of two proteins, so that proteins can promptly accomplish the reactions of interest within a given time frame. The existence of encounter complexes has been long predicted by simulation, but their transient nature and low population made it difficult to characterize their structures and interactions.[1, 2]

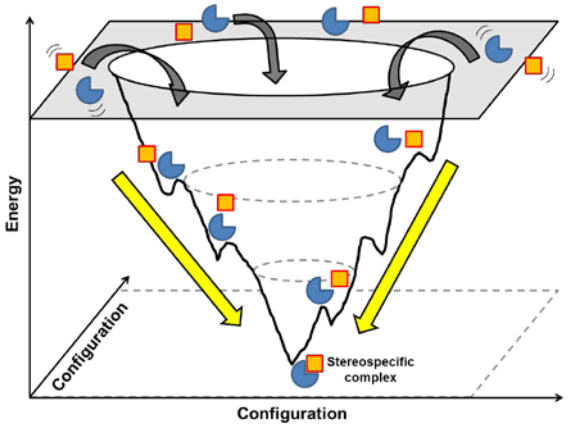

Figure 1. A cartoon diagram of the process of specific protein complex formation via an ensemble of encounter complexes. Two proteins (*blue* and *orange*) are weakly associated to form an encounter complex, and find their way toward the specific complex by two-dimensional search on the surface through translational and rotational diffusion. A few of possible search pathways are depicted by *yellow* arrows.

Recent application of NMR paramagnetic relaxation

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enhancement (PRE) first allowed the detection of the encounter complexes during protein–DNA and protein–protein association. NMR spectroscopy has traditionally been an insensitive technique. The exquisite sensitivity of PRE, however, combined with streamlined mutagenesis and recombinant protein production enabled the detection of the species of less than 0.5% of population. Here we highlight recent development of NMR techniques to character the encounter complexes, and address the questions answered and unanswered to date.

Measurement and interpretation of PRE

Paramagnetic relaxation originates from the interaction between a nucleus and an unpaired electron. The magnitude of paramagnetic relaxation is much larger than nuclear dipolar relaxation owing to the large gyromagnetic ratio of an unpaired electron. PRE is obtained by subtracting R_1 or R_2 relaxation parameters between a spin-labeled paramagnetic sample and an unlabeled diamagnetic sample. Paramagnetic samples are obtained by conjugating EDTA-Mn^{2+} or nitroxide groups that contain an unpaired electron to the surface-engineered cysteine residue. Increased relaxation rates of the paramagnetic sample compared to the diamagnetic sample represent the PRE rates. PRE can be obtained from ^{13}C and ^{15}N as well as ^{1}H, but ^{1}H PRE is mostly employed to derive distance information, because ^{1}H PRE is significantly larger than ^{13}C and ^{15}N PREs (proportional to the square of the gyromagnetic ratio), and also the interpretation ^{1}H PRE is straightforward. PRE data from R_1 or R_2 relaxation rates result in Γ_1 or Γ_2, respectively. The interpretation of ^{1}H-Γ_1 PRE data, however, require accurate information on the internal motion correlation times of individual amide groups, so that ^{1}H-Γ_2 PRE data are most widely used for structure calculation.[3] The pulse sequence and practical aspects of ^{1}H-Γ_2 PRE measurement are described in detail elsewhere.[4] In the fast exchanging system, chemical shifts and relaxation properties are obtained as a population-weighted average between the participating species. If the PRE rate of a transient species is large enough and in fast exchange with the predominant species, the PRE can be reported in the signals of the major species in equilibrium. For example, if a minor state of 0.5% population has a PRE rate of 5,600 s^{-1} (PRE from a 8 Å distance using a Mn^{2+} spin label) in equilibrium with the major state via the exchange rate constant of > 4,000 s^{-1}, PRE of > 10 s^{-1} would be measured. As the exchange rate becomes higher, PRE up to 28 s^{-1} is expected to be monitored. In this manner, PRE provide spatial information in a dynamic system as well as in a static system. When experimental PRE data are not consistent with the calculated PRE data from a single structure, the discrepancy can be interpreted as the presence of transient minor states, which can be encounter complexes during protein association, or conformational intermediate states during domain motions.

Structure and dynamics of encounter complexes

PRE measurement of the complex between a transcription factor and DNA first reported the presence of the encounter complexes.[5] The binding mode of the transient complexes was similar to that of the specific complex, and the target search process of the protein revealed intramolecular and intermolecular translocations between cognate and non-cognate DNA sites. Subsequently, two independent groups visualized the encounter complexes in a bacterial phosphotransferase complex and in a yeast electron transfer complex based on PRE measurement.[6,7] The encounter complexes were comprised of an ensemble of loose nonspecific complexes in equilibrium with the specific complex. In particular, the complex of N-terminal domain of enzyme I (EIN) and HPr in bacterial phosphotransferase system has been extensively studied to characterize the physicochemical nature of the encounter complexes. When HPr was spin-labeled at different locations, intermolecular ^{1}H-Γ_2 PRE rates measured on EIN showed a large discrepancy from the calculated PRE data obtained from the solution structure of the EIN:HPr complex.[8] Ensemble refinement using rigid-body simulated

annealing to minimize the differences between experimental and calculated PRE data showed that encounter complexes of overall 10% population and 20 conformers are required to explain the observed PRE data.

It is notable that the distribution of HPr in the encounter complexes correlated with the surface electrostatic potential of EIN. The encounter complexes were highly populated along the negative surface charges of EIN. Since encounter complexes are much less compact than the specific complex, long-range electrostatic interactions likely drive the encounter complex formation. Measurement of PRE with salt titration demonstrated that encounter complexes were more sensitive to the ionic strength than the specific complex, indicating that electrostatic interaction is indeed important in the encounter complexes formation.[9] Interestingly, computational approach using replica exchange simulations using EIN and HPr showed similar correlation for the binding interface of nonspecific encounter complexes, which supports the main role of electrostatic interactions for the early encounter events.[10]

The encounter complexes can be classified into distinct families according to their structural characteristics and their mechanistic contribution toward protein association. Monte Carlo simulation suggested the presence of structurally distinct encounter complexes such that some encounter complexes are more diffuse than others, and a small number of structured nonspecific complexes lead to the specific complex in the funnel-shaped energy landscape of complex formation.[10] Monitoring PRE at different stoichiometric ratios of EIN and HPr revealed distinct encounter complexes according to their roles in protein association.[11] Some encounter complexes were localized around the active site so that small rotational and/or translational diffusion lead to the final specific complex. On the other hand, some encounter complexes coexisted with the specific complex to form a ternary complex, so that they can recharge the active site rapidly when the specific complex is dissociated. It is inferred that encounter complexes are heterogeneous in their structure and dynamics, and a full description of the encounter complex ensemble awaits systematic studies on diverse protein complexes.

Mapping protein target search pathways

Encounter complexes visualized by PRE are in equilibrium with the free states and/or the final specific complex. PRE profiles thus do not distinguish the encounter complexes that settle down to the final complex from those that do not. PRE measurement and thermodynamic analysis based on rational mutagenesis that affects the encounter complex formation has proved useful to address this question and identify the role of encounter complexes along the target search pathway.[12] Encounter complexes can be described as productive if they contribute to the final specific complex, and non-productive if they do not (Figure 2). The productive encounter complexes are in equilibrium with the free state and also with the specific complex. They largely form the reaction coordinates that lie between free and complex states, and thus represent the associative target search pathway. On the other hand, non-productive complexes are in equilibrium only with the free state, but not with the specific complex. Thus, non-productive complex represent the dissociative target search pathway.

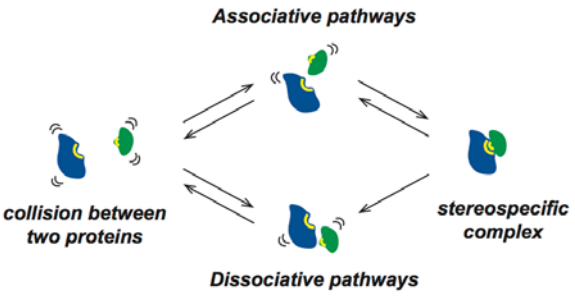

Figure 2. A schematic diagram of protein association. Two proteins (*blue* and *green*) associate two form a functional stereospecific complex by way of an encounter complex ensemble. The encounter complexes that form the reaction coordinate of the associative target search pathway represent productive encounter complexes, whereas those in the dissociative pathway represent non-productive encounter complexes.

The mutagenesis can be rationally designed to choose potential hot-spot region that influence the encounter complex formation without affecting the specific complex structure. Mutant proteins selected by this strategy are subject to structural, dynamic, and thermodynamic analysis, and removed when the specific complex structure is perturbed. If reduced encounter complex formation is correlated with reduced thermodynamic association, the mutation region likely recruits productive encounter complexes. If reduced PRE does not correlate with the binding, encounter complexes over the region are probably on the dissociative pathway. Employing several mutants enabled the description of potential protein target search pathways between EIN and HPr. The result indicated that initial encounter complex formation is directed by electrostatic interactions, but the target search toward the specific complex requires short-ranged noncovalent interactions as well as the electrostatic interaction.

Concluding Remarks

The showcase examples demonstrated that PRE is a valuable tool to report on the transient protein complexes. There still remain many questions about the target search process of a protein complex. We still do not know if there is a preferred target search pathway, if there is cooperativity in the target search process, and if the search path can be controlled to increase or decrease the binding affinity. We also note that PRE can be employed to study transient minor state of protein complexes that involve conformational change or oligomer formations.[13,14] Future studies using engineered mutants will illustrate the target search process in more detail, and provide a way to control the search process.

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