Dynamics in electron transfer protein complexes

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Concluding remarks

NMR chemical shift perturbation analysis

NMR chemical shift perturbation (CSP) analysis has been frequently used to study the characteristics of transient protein complexes. Interaction between proteins is accompanied by changes in the chemical environment of the nuclei at the interface. These changes can be followed in [^{1}H, ^{15}N] HSQC experiments if one of the partners is ^{15}N labelled. Chemical shift perturbations have been used throughout this work to map out the binding interface (chapter 2) and the dynamics (chapter 2, 3, 5 and 6) of the complexes. The size of the chemical shift changes has been suggested to correlate with the dynamics of the protein complexes[29,30,124,185]. In the specific complexes, proteins spend most of the time in a single well-defined orientation resulting in large size of the chemical shift changes. The dynamic complexes constitute a considerable fraction of the encounter complex, consisting of multiple orientations. The chemical shift changes are averaged over all these orientations resulting in a small size.

In this work we provide strong evidence to support the earlier suggestions of the relation between overall size of chemical shift perturbations and degree of dynamics in the complex by PRE analysis and Monte Carlo simulations. It is revealed that the size of the chemical shift perturbations correlates with the population of the encounter complex. We confirm that NMR chemical shift perturbations can be used as a reliable diagnostic tool to study the dynamics in protein complexes. The higher the population of the encounter complex, the smaller the size of chemical shift perturbations and vice versa. The highly dynamic transient complexes described in this study include R13A yCc-yCcP, R13K yCc-yCcP, K13A hCc-yCcP and wt hCc-yCcP, while the specific complexes include wt yCc-yCcP, T12A yCc-yCcP and K13R hCc-yCcP.

Paramagnetic relaxation enhancement

Following the elegant work by Tang et al.[20] and Volkov et al.[19], PRE NMR spectroscopy has proved to be a powerful technique for detection of the minor states in transient complexes. The paramagnetic probe can be engineered anywhere on the

protein surface via cysteine residue. Due to dependence of the paramagnetic effect on r^{-6} between the paramagnetic center and the observed nucleus, even lowly populated states in which the partner approaches the spin label for a short time can be detected. In this study we have successfully applied PREs to detect the encounter complex. For the first time, we have mapped out the conformational space searched by the proteins (Cc-CcP) during the encounter complex (chapter 4). PRE NMR spectroscopy has also enabled us to observe the effect of interface mutations on the dynamics of the Cc-CcP complex. Because PREs are sensitive to the minor changes, PRE NMR spectroscopy has been successfully applied to investigate the effect of interface mutations on the equilibrium of the encounter complex and the specific complex (chapter 5).

PREs can be converted into distance restraints which can be used to determine the solution structure of the complex by dynamic docking. This method has been used to determine the solution structure of yeast Cc-CcP complex[19]. The approach involves the molecular docking of the proteins using intermolecular distance restraints obtained from a paramagnetic center attached to the protein via single disulphide bond. Such paramagnetic center is highly mobile in the three dimensional space due to the freedom of rotation around the single bond. This puts a limit to the accuracy and precision of the determined solution structure of the complex. In our structure calculation of the hCc-yCcP complex, the determined structures differ with rms deviation of 3.8 Å (chapter 6). As this approach works with a limited resolution, it cannot be used to get the interface details. Moreover, the minor changes in the structure due to the mutations cannot be observed with this method. However, the dynamic problem of the paramagnetic probe can possibly be solved by two point attachment of the probe. Double armed probes are more rigid and have limited mobility[201]. Application of such probes can enhance the accuracy of the determined structure.

Computational methods to study the encounter complex

Although PRE NMR spectroscopy has proved to be a powerful technique to detect the minor species in solution, it is not possible to visualize the individual orientations as the method yields a value that is an overall average of all orientations. Numerous computational methods have been applied to simulate the encounter complex[23,102,103,185].

According to a number of theoretical[102,103,173] and experimental[6,174,175] studies, the encounter complex exhibits a few short range interactions and is predominantly driven by non-specific electrostatic interactions. The specific complex is dominated by short range interactions, like van der Waals interactions and hydrogen bonding. Thus, the encounter complex can be simulated by the specifically defining these interactions between the proteins. We have successfully simulated the encounter ensemble of Cc-CcP complex using Monte Carlo simulations considering the electrostatic interactions only (chapter 4, 5 and 6). The simulated ensemble provides the distribution of one protein around the other, making it possible to look into the individual orientations and the regions of higher density. It also gives the area sampled by the proteins in the encounter complex.

The computational methods combined with the experimental data provide more information compared to the individual approaches. Recently Kim et al.[23] have combined replica exchange Monte Carlo simulations with the PRE data to characterize the encounter complex. We have been able to characterize the encounter complex by combining the Monte Carlo simulations with the PRE data (chapter 4 and 5). The two approaches combined offer a new approach to get relative populations of the specific complex and the encounter complex.

Encounter complex

The concept of the encounter complex has been supported by a number of experimental and theoretical studies[17]. However, the encounter complex had not been characterized due to its diffusive nature and the lack of the methods to probe it. The encounter complex is dominated by the electrostatic interactions. The long range electrostatic interactions keep the proteins in close proximity and pre-orient the reacting proteins. The encounter complex reduces the conformational search for the specific binding site and hence enhances the association rate. At the same time the long range electrostatic interactions in the encounter complex destabilize the specific complex and increase the dissociation rate. This provides a right equilibrium between the fast association and high turn over of the complex required in transient interactions.

An efficient electron transfer requires the redox centers to be separated by less than 16 Å[177]. For large protein complexes (chapter 4), most of the orientations in the encounter complex separate the electron transfer centers by a distance larger than 16 Å, making these orientations inefficient for fast electron transfer. In these complexes, the formation of the specific complex becomes compulsory as it brings the redox centers within the required distance. The encounter complex provides the required balance between association and dissociation required for the efficient functioning. In the small electron transfer complexes, most of the orientations in the encounter complex bring the redox centers within the required distance and thus the role of the specific complex becomes less important. In some cases the specific complex formation is not even required[26].

Nature has established equilibrium between the relative populations of the encounter complex and the specific complex. This equilibrium varies in different electron transfer complexes. Generally the smaller complexes have higher population of the encounter complex, and the bigger complexes have more population of the specific complex. The equilibrium can, however be shifted towards either direction by interface mutations as was demonstrated in chapter 5 and 6.