Dynamics in electron transfer protein complexes

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Summary

A vast majority of the biological processes is mediated by protein-protein interactions. Depending on the affinity of the interactions, the protein complexes range from static to transient. The static complexes are characterized by high affinity and low dissociation rate. The proteins involved in these complexes have high specificity and form a single well-defined complex. The transient complexes are characterized by low affinity, and high association and dissociation rates. The proteins involved in these complexes have low specificity and interact with multiple partners. The fast association and dissociation of the transient complexes is achieved by the formation of the encounter complex which exists in equilibrium with the well-defined complex. The equilibrium of the encounter complex and the specific complex varies among different complexes. Electron transfer processes requiring high turn over are carried out by the transient protein complexes.

The work described in this thesis focuses on the dynamics in transient complexes with an emphasis on the characterization of the encounter complex. Using NMR spectroscopy and Monte Carlo simulations we have studied the equilibrium of the encounter complex and the specific complex. We have also investigated how this equilibrium can be modulated by single interfacial mutations.

Chapter 2 describes the study of the binding of yeast cytochrome *c* (Cc) with *A. thaliana* L-galactono-γ-lactone dehydrogenase (GALDH) by NMR spectroscopy. The interaction surface of yeast Cc was mapped by the chemical shift perturbations of its residues, arising from its interaction with GALDH. The small size of the chemical shift changes shows that the complex is transient and of dynamic nature. The Cc residues involved in interaction with the GALDH are confined to a single patch around the haem edge of Cc. The interaction surface was compared with its other interaction partners. The results show that Cc utilizes a conserved surface to interact with its partners. We have also investigated the effect of a 9-fold surface charge mutant of GALDH on its interaction with Cc. The dissociation constants determined for the GALDH and its 9-fold mutant are 50 ± 10 μM and 44 ± 10 μM, respectively. The binding map of the 9-fold surface mutant is similar to the wild-type GALDH. Thus the interaction remains intact after removal of the 9 charge residues from the GALDH surface.

Although transient protein interactions are involved in a variety of biological processes, little is known about the principles underlying these interactions due to the difficulties associated with their structural characterization. Most of the knowledge related to protein recognition and binding in the transient protein complexes comes from the study of the stable and static complexes. It is not well understood whether the binding hot spots which are frequently found in static complexes also exist in transient complexes. In chapter 3 we have used yeast Cc and cytochrome *c* peroxidase (CcP) as model proteins to answer this question. Using NMR spectroscopy it is shown that the Cc R13 residue is a hot-spot in this transient complex, as the R13A mutation destabilizes the binding. We have also used a double mutant cycle and showed that the Cc R13 interacts with CcP Y39. These results provide evidence that the binding hot-spots could also govern the interactions in weak and transient complexes.

The process of protein complex formation is modeled by a two-step process. The proteins first approach each other by diffusion and associate in an encounter complex, followed by the formation of the specific complex. The encounter complex has been detected in a number of protein complexes. Visualization of the encounter complex has been a problem due to lack of the experimental methods to probe it. In chapter 4 we have characterized the encounter complex in the electron transfer complex of yeast Cc and CcP. Using paramagnetic relaxation enhancement (PRE) NMR spectroscopy we have experimentally mapped out the complete conformational space searched by CcP in the encounter complex. The encounter ensemble was also simulated by Monte Carlo simulations considering only the electrostatic interactions at atomic level. The PRE data combined with the theoretical simulations provided a more complete picture of the encounter complex than could be obtained by the individual methods. The results indicate that Cc samples only about 15% of the surface area of CcP in the encounter complex, surrounding the binding site. The relative populations of the encounter complex and the specific complex have been found to be 30% and 70%, respectively, with only 0.5 kcal/mol difference in the free energies between the two states. This delicate balance is supposed to ensure the fast electron transfer and a high turn over rate. The approach used offers a new method to determine the fraction of the encounter complex.

The population of the encounter complex can vary in different complexes. We have shown that the relative population of the encounter complex and the specific complex can be varied by single residue mutations in the interface (chapter 5). Using PRE NMR and Monte Carlo simulations, we have shown that the population of the encounter complex can be enhanced or reduced by single point mutations in the transient complex of the yeast Cc and CcP. The native complex exists as equilibrium of 30% of the encounter complex and 70% of the specific complex. A single R13K mutation increases the population of the encounter complex to 50%, and R13A mutation reverses the equilibrium of the two states, increasing the encounter complex to 80%. The T12A mutation decreases the population of the encounter complex to 10%. Thus by a careful choice of interfacial point mutations it is possible to modulate the occupancy of the encounter complex and the binding specificity in transient complexes.

Horse cytochrome *c* (hCc) forms a non-physiological complex with yeast cytchrome *c* peroxidase (yCcP). This complex is more dynamic than the physiological complex between yeast cytchrome *c* (yCc) and cytochrome *c* peroxidase. The structures of the two complexes differ in the relative orientation of the Cc molecules. The yCc has an Arg residue at position 13. In chapter 5 we have shown that the yCc-yCcP complex can be made more dynamic by mutation of this residue to Lys or Ala. Horse Cc has a Lys residue at the equivalent position. In chapter 6 we have investigated the role of this residue in controlling the specificity of the hCc-yCcP complex. The results reveal that K13R mutation turns the highly dynamic non-physiological hCc-yCcP complex into a specific one, resembling that of the yCc-yCcP complex. On the other hand, K13A mutation makes the complex more dynamic. Thus the specificity of the transient complexes can be changed by rational engineering of interface residues.