Understanding protein complex formation: the role of charge distribution in the encounter complex

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English summary

The traditional description of the protein complex formation as direct binding of two proteins at their binding sites, in a “lock and key” process, has nowadays been replaced with a much more dynamic model. Often, two proteins free in solution are initially attracted to one another by electrostatic interactions. In the large majority of cases proteins do not interact directly with their stereospecific binding sites, but collide with different regions of their surface. In the presence of favorable electrostatic interactions, one protein interacts with the surface of the second one in an ensemble of very dynamic configurations, referred to as the encounter complex. The encounter complex can be productive when the electrostatic interaction guides the proteins to the stereospecific complex, or the encounter complex can be futile when the two proteins dissociate before forming the final complex. Electrostatic interactions are particularly important for the encounter complex as they are the main forces guiding the process, pre-orientating the proteins and minimizing the area to sample before reaching the stereospecific binding site. Short range hydrophobic interactions can also have a role in the formation and stabilization of the protein complex. Because the encounter complex occupies only a fraction of the protein complex lifetime and it is extremely dynamic, it is invisible to conventional structural biology techniques. The development of paramagnetic relaxation enhancement (PRE) NMR, a technique with a very high sensitivity to lowly populated states, enabled the observation of these dynamic encounters. The technique measures the relaxation induced by a paramagnetic tag attached on the surface of a protein, on nuclei of a second protein that approaches the tag.

Transient complexes, such as redox complexes, are evolutionary optimized to perform very efficient reactions and exhibit a high population of encounter complex. Thus, they represent an excellent subject for study of the encounter complex. In this thesis we chose to work on a well characterized electron transfer (ET) complex formed by cytochrome *c* (Cc) and cytochrome *c* peroxidase (CcP) from *Saccharomyces cerevisiae*. The biological function of the Cc:CcP complex is to protect the cell from oxidative stress, reducing hydrogen peroxide to water. The complex formation is guided by electrostatic interactions between the positively charged binding site of Cc and the negatively charged one of CcP. The encounter complex was successfully predicted by Monte Carlo simulations based on electrostatic interactions. PRE experiments showed that the encounter complex is concentrated on the CcP binding site, sampling only 15 % of the protein surface. The fraction of encounter complex state is the 30 % on the complex lifetime, while the 70 % is in the stereospecific complex.

The aim of the work described in this thesis is to question the importance of the charge distribution on the protein surface for the formation of the active complex. To test the hypothesis that the charge distribution on these proteins is evolutionary optimized for the most efficient protein complex formation, we modified the charge distribution of the CcP surface, producing five CcP variants. The CcP mutants were designed by combining the addition of a new negative patch on a lateral side or on the opposing side of the

stereospecific site for Cc, and the neutralization of the native negative charges in the stereospecific binding site.

In **Chapter 3** the CcP variant with an additional negative patch on a side of CcP relative to the stereospecific binding site (CcP_B) is analyzed through Monte Carlo simulation, PRE and stopped flow experiments to characterize the encounter complex formed with Cc and test the effect of the new charges on the formation of the active complex. Monte Carlo simulations, based solely on electrostatic interactions, show that Cc is strongly attracted to the added negative patch. PRE experiments confirm the Monte Carlo simulations results, showing that the new patch enlarges the area sampled by Cc in the encounter complex with CcP. Interestingly, the measurement of the association rate of the complex through stopped flow experiments shows that the added charges on CcP_B do not affect the association with Cc negatively. On the contrary, the formation of the Cc:CcP_B complex is more efficient than the wild type complex. This suggests that the encounter complex between Cc and CcP_B is a productive encounter complex and that the specific charge distribution on the surface of CcP is not critical for the formation of the active complex. In **Chapter 4** we characterized the complexes formed by Cc and the other CcP variants. Monte Carlo simulations show that Cc in strongly attracted to the added negative charges of the CcP variants, especially in absence of the native charges in the stereospecific binding site. Stopped flow experiments show that the association rate of the Cc:CcP complex is strongly slowed down when the native negative charges in the CcP binding site are neutralized. Surprisingly, the addition on a negative patch on the CcP surface enhances the association rate with Cc regardless of its localization. The finding that adding negative charges even on the opposite side of the binding site improves the affinity with Cc suggests that the overall charge of the CcP surface is more important for the active complex formation than a particular charge distribution. In **Chapter 5** we wondered if this could be caused by the prevalence of the stereospecific complex (70 %) over the encounter state population (30 %). The equilibrium between these two states is sensitive to point mutations in the binding sites. In particular the mutation of the Arg13 in Ala in the Cc binding site enhances the encounter complex from 30 % to 80 %, and causing a 100 fold decrease in the binding constant of the protein complex. To test how a prevalence of encounter complex population would affect the association rate of the complex, we studied the complexes formed by Cc R13A and the two variants of CcP, wt (CcP_A) and CcP_B. The chemical shift perturbations (CSP) of the complexes formed by Cc R13A are significantly smaller than in presence of wild type Cc due to the increased dynamics. Similar to the results reported in chapter 3, the PRE experiments show that Cc R13A visits the added charges on the surface of CcP_B, enlarging the surface sampled in the encounter complex. Finally, kinetics experiments revealed that the addition of the negative charges on CcP_B does not affect the association with Cc R13A, which is slightly higher than in presence of CcP_A at low ionic strength. Nevertheless, the Cc R13A complexes displayed a 5-7 fold reduction of the association rate in a wide ionic strength range. This indicates that the balance of the

dynamics in a protein complex, between stereospecific and encounter complex populations, is critical for the efficiency of electron transfer complexes.

Chapter 6 discusses the results and the techniques presented in this thesis.