Dynamics in photosynthetic transient complexes studied by paramagnetic NMR spectroscopy

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English Summary

The living cell is an open and dynamic system that continuously adapts to both inner and external stimuli. The cellular steady state is maintained thanks to the efficient balance between the different biochemical pathways, most of which involve interactions between proteins. Protein association requires the initial formation of a transient intermediate, defined as encounter complex, which can either dissociate or evolve to a final, productive complex. The formation of the encounter complex was described as resulting from Brownian diffusion and long-range electrostatics, whereas short-range interactions take place in the final complex. Thus, a productive protein complex implies the selective recognition between the specific interaction partners, determined by the surface properties of the individual proteins. In order to understand the mechanisms that control molecular recognition the complex formed by Cyt *f* and Pc from the cyanobacterium *Nostoc* was studied. These proteins are redox partners within the oxygenic photosynthetic chain in plants, green algae and cyanobacteria. As a consequence of the electron transfer function, the complex shows a transient nature, resulting from low specificity and surface complementarity between Cyt *f* and Pc. Another important transient feature of the complex is represented by the high K_D measured in several Cyt *f*-Pc complexes, which have been characterized by either kinetic or NMR studies. The presence of an extended hydrophobic patch surrounding the redox centers is a common feature to both Cyt *f* and Pc among different organisms. On the contrary, the electrostatic surface properties significantly vary between different organisms and seem to influence the final orientation and the degree of dynamics within the complexes. In the particular case of *Nostoc*, Cyt *f* and Pc have an overall negative and positive charge, respectively. The key techniques used in this thesis are NMR spectroscopy and computational methods, which allowed for the description of both the dynamic and structural aspects of Cyt *f*-Pc complex formation.

For the first time, the interaction between Cyt *f* and Pc from *Nostoc* was investigated by paramagnetic relaxation enhancement (PRE). Three sites around the putative binding site for ET on Cyt *f* were selected for spin label attachment. The positions of the mutations were designed on the basis of the solution structure of the wild type complex, as determined by taking advantage of the pseudocontact shifts (PCS) generated by the haem of Cyt *f* on Pc nuclei. It was found that the complex is highly dynamic, suggesting a significant population of the encounter complex. These early results suggested the existence of the complex in multiple orientations and, consequently, also indicated the need to understand the distribution of the encounter complex to provide a complete description of the association between Cyt *f* and Pc.

For the detection of Cyt *f*-Pc encounter complex, the initial data set was extended to nine spin labels, distributed over a wide area on Cyt *f* surface. The measurements of PREs and their analysis showed that Pc samples an extended portion of Cyt *f* surface. The similarity between the PRE patterns observed in the presence of spin labels attached near to the haem of Cyt *f* indicated that Pc samples the Cyt *f* surface with a single patch. Electrostatic interactions are thought to pre-orient Pc with the hydrophobic patch towards Cyt *f* and to favor the contact of the proteins to form the encounter complex, which is then stabilized by hydrophobic interactions. The observed PRE data in the fast exchange regime are population weighted averages of the PREs for Cyt *f*-Pc

complex in both the encounter and final states. The observed PRE therefore provide only qualitative information about the encounter complex. The visual representation of the encounter complex was obtained by using theoretical models. MC-dock predicts the formation of a complex solely on the basis of the electrostatic surface properties of the interacting proteins. The encounter complex is represented by a Boltzmann distribution of complexes according to their electrostatic-interaction energy. MC simulations of Cyt *f*-Pc encounter complex did not provide a model in agreement with the experimental PREs, indicating that electrostatic interactions alone cannot describe the formation of this encounter complex. In the ensemble docking method, the driving force for complex formation is given by the observed PREs. The resulting encounter complex represents a probability distribution of Cyt *f*-Pc orientations, which account for the experimental data. This encounter complex has a diffusive nature, in which Pc can diffuse on the non-polar region of Cyt *f* by overlap with its own hydrophobic patch. Long-range electrostatics mainly contribute to the pre-orientation of Pc towards Cyt *f*, as indicated by the PRE analysis, and hydrophobic interactions in the formation and stabilization of the encounter complex. The diffuse nature of the encounter complex suggests that in this system a final, well-defined orientation of the complex could be not a fundamental requirement for the ET function. In fact, the efficient turnover required for rapid ET through the photosynthetic redox chain precludes the formation of a tight complex and favors the existence of an ET active complex in multiple orientations. The dual role of hydrophobic interactions either in the formation of the encounter complex and in the stabilization of the final complex suggests a small energy barrier between the encounter and the final complex, favoring the smooth transition between the two energetic states. In the light of these observations, a new model to describe protein association has been proposed, in which the formation of the encounter complex presents a relatively flat energy landscape during all phases of the association, without a clear distinction between the encounter and the final complex.

The non-physiological complex formed by *Nostoc* Cyt *f* and *Phormidium laminosum* Pc was found to have an intermediate affinity between the physiological complexes of *Nostoc* and *Phormidium laminosum*. ^{Ph}Pc can be considered as a natural variant of ^{N}Pc, in which the overall charge is negative. The complex showed a "head-on" orientation, reminiscent of that found in the *Phormidium* complex, in which only the hydrophobic patch of Pc makes contact with the hydrophobic region surrounding the haem on Cyt *f*. The reduced electrostatic attraction also seems to favor a more diffusive distribution of the encounter complex than in *Nostoc*, suggesting a higher degree of dynamics within the cross-complex. Interestingly, despite the reduced electrostatic attraction between ^{N}Cyt *f* and ^{Ph}Pc, salt-titration experiments and MC simulations showed that electrostatic pre-orientation is still occurring and contributes to the association. These findings supported the new model proposed for protein complex formation, in which hydrophobic and electrostatic interactions together promote the association of Cyt *f* and Pc.