Transient interactions between photosynthetic proteins

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Concluding remarks

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NMR chemical shift perturbation analysis

Throughout this work NMR titration experiments have been used as a tool to study protein properties and several aspects of transient protein interactions. Typically, a ^{15}N-labelled protein is presented with for instance a change in pH or the addition of a partner protein or peptide. The resulting changes in the 2D [^{1}H, ^{15}N] NMR spectrum are followed and resonances are recorded at every step. This technique has been used extensively in our group and proved to be very useful to characterise transient protein complexes[8,9].

In Chapter II this technique is used to investigate the protonation behaviour of a histidine copper ligand. Previously, crystallography and 1D NMR spectroscopy studies suggested that histidine protonation does not occur in fern Pc[77,85]. We show that 2D NMR spectroscopy can overcome aggregation problems and provides a detailed picture of the protonation event that occurs at a lower pK_a than expected for Pc.

It has been suggested before that the size of the chemical shift changes that arise due to the addition of the partner protein can be used as a diagnostic tool for the dynamics in a transient protein complex[33,35,38]. This work supports that view (Chapter III). A new approach was used that creates an ensemble of randomly distributed orientations in the complex between mutant Pc and cyt*f*. The average PCS are calculated for each ensemble to determine which ensemble of orientations corresponds to the experimental PCS. Results show that changes on the surface of *P. hollandica* Pc can increase the dynamics, which was previously inferred from the decrease in size of the chemical shifts changes (Chapter III and reference 87).

Dynamics in transient protein complexes

The two-step model proposed for transient protein complex formation (Fig. 1.1) has slowly emerged from increasing amounts of experimental data and theoretical studies. The first step of protein association has been extensively studied and the role of electrostatic interactions is well established. The second stage, characterised by an

equilibrium between the dynamic encounter and the well-defined state has come into the focus of recent research, for which paramagnetic NMR techniques are indispensable. The approach of creating an ensemble of orientations was used to study the Pc - cyt*f* (Chapter III) and cytc_6 - cyt*f* complex (Chapter IV). In the latter case the PCS data were complemented with intermolecular paramagnetic relaxation enhancement (PRE) data, caused by five spin labels on cyt*f*. This type of paramagnetic data is fundamentally different from PCS. It has been used to determine the conformational space occupied by the cytochrome *c* – cytochrome *c* peroxidase complex, which is a mostly well-defined complex[31]. Both complexes studied in this work are found to be balanced between encounter and well-defined state, which complicates the characterisation of the interaction. This is illustrated by the violations of the PRE restraints from five spin labels, which cannot be met all at the same time. This is a strong indication of the dynamic nature of the complex. The creation of an ensemble of orientations around a starting structure works well for the *P. hollandica* mutant Pc in complex with cyt*f* (Chapter III), for which the single orientation determine for the wt complex is most likely the centre of the ensemble. In the case of *Nostoc* cytc_6 - cyt*f* it is likely that the distribution of orientations in the ensemble is less symmetric. This suggests that the use of methods that do not require a starting structure, such as Brownian dynamics, might be more successful in precisely describing the encounter state of this complex.

Surface interactions in the Pc - cytf complex

The mutation of residues in the interface between *P. hollandica* Pc and cyt*f* has a profound effect on the dynamics of the complex, indicative of the role that geometric compatibility plays in complex formation (Chapter III). In Chapter V the interaction of a positively charged peptide with the negative eastern patch of Pc shows why this peptide can inhibit the electron transfer from cyt*f*. The addition of hydrophobic residues to the peptide does not significantly change the interaction with Pc. From these results it can be concluded that surfaces of electron transfer proteins are designed to interact specifically with their partners, keeping a fine balance between hydrophobic, electrostatic and solvent interactions (Chapter III and V).

The Pc - cytf complex in vivo

The work described here is all performed on proteins that were removed from their original environment. *In vitro* studies on proteins and protein complexes has delivered a wealth of knowledge on their function and on the mechanisms involved. While drawing conclusions from *in vitro* work, however, it is not only important to keep in mind that there is a difference with the cellular environment, but also to assess how these differences effect the system under investigation. Macromolecular crowders have been used to determine to what extent the *in vitro* data can be representative for *in vivo* behaviour of bio-macromolecules. The addition of the high molecular weight crowder Ficoll70 to the Pc - cyt*f* complex does not cause significant changes in the binding (Chapter VI) or overall reaction rate[238]. It is in agreement with the view that these type of crowders form a 'low-viscosity', porous medium. Whether this is the most appropriate model for the cellular environment in general and the lumen in which the Pc - cyt*f* complex functions remains to be seen.