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DISSERTATION SUMMARY

Using labeled mutant cytochromes for the examination of intraprotein electron transfer

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One of our purposes is to investigate the role of the protein matrix in electron transfer processes. To this end we measure the elecron transfer kinetics spectroscopically on various cytochromes of the mitochondrial respiratory chain.

To initiate electron transfer in cytochrome c we use a photoactive protein label, 8-thiouredopyrene-1,3,6-trisulfonate (TUPS) (Kotlyar et al. 1997). TUPS has two slightly different chemical forms, one can label surface lysines and the other one cysteines. Upon light excitation the triplet state of the dye forms with high quantum efficiency. The triplet has a long lifetime (0,5 ms) and low redox potential, therefore it is a strong reductant, capable of donating its electron to a variety of acceptors. We have shown that TUPS is also capable of acting as a photoactive electron acceptor, depending on the redox partner (Kotlyar et al. 2004).

The selected cytochrome c for electron transfer measurements is the horse heart protein, because it contains only two cysteine amino acids, which are inaccessible, since they participate in heme binding. Therefore single cysteines introduced on the surface of the protein allow exclusive labeling by TUPS and the initiation of electron transfer from different positions of the protein. In c-type cytochromes covalent heme binding is catalysed by the enzyme heme lyase during cytochrome c maturation. For that reason cytochrome c gene expression was performed together with the heme lyase gene. For expression of the cytochrome c and heme lyase genes we used the pBAD24 plasmid (Guzman et al. 1995), a member of a vector family containing the inducible and controllable powerful P_{BAD} promoter of the arabinose operon (*araBAD*). The proteins were heterologously expressed in BL21(DE3) strain of *E. coli*.

We have measured electron transfer on cytochrome c labelled on the K8 and K39 side chains, positioned at opposite sides relative to the heme cofactor, as well as on K8C and K39C introduced by site directed mutagenesis. These changes allow us to compare the electron transfer rates when the lysines are replaced by cysteines, thereby altering both the chemical nature and the length of the putative pathway between the electron donor and acceptor.

The experiments show us that the electron transfer between TUPS and the heme of cytochrome c deviates from the expected monoexponential kinetic behavior. Neither the overall rate, nor the individual exponential components of electron transfer, as followed by kinetic absorption spectroscopy, correlate with the length of the covalent link connecting the dye with the protein. Molecular dynamics calculations show that TUPS can approach the protein surface and occupy several such positions. This heterogeneity may explain the multiexponential electron transfer kinetics. The calculated optimal electron transfer pathways do not follow the covalent link but involve through space jumps from the dye to the protein moiety, effectively decoupling the length of the covalent link and the electron transfer rates (Tenger et al. 2005).

Our further goal is to measure electron transfer in the complex of cytochrome c and cytochrome c oxidase. In order to optimize the reduction of cytochrome oxidase, we were searching for the optimal label position on the surface of cytochrome c. We expressed the G1C, V3C, V11C, A15C, G23C and G34C mutants, and our preliminary measurements indicate that two of them (V11C and A15C) are fast enough to initiate efficient electron transfer in cytochrome c oxidase. This will enable us to investigate the details of the electron transfer route and the catalitic activity of cytochrome oxidase.

References

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