Variable photosystem I binding affinities of cytochrome c_6 amino acid replacement mutants

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Summary

Photosystem I (PSI) is a transmembrane protein complex that uses photoexcitation to transport electrons across the membrane through an electron transport chain for production of NADPH. It is dependent on soluble electron carriers such as plastocyanin, and in cyanobacteria, also cytochrome c_6 as an electron source. These electron carriers have to attach to PSI to efficiently reduce its primary electron acceptor P700. Recent findings by the Zouni group (Kölsch et al., 2018) have identified a number of amino acid residues of cytochrome c_6 that may strongly affect its binding to PSI *(Fig 1.)*. Our aim is to investigate the role of these residues on the binding affinity of cytochrome c_6 to PSI by replacing them and measuring the effect on oxygen reduction by PSI.

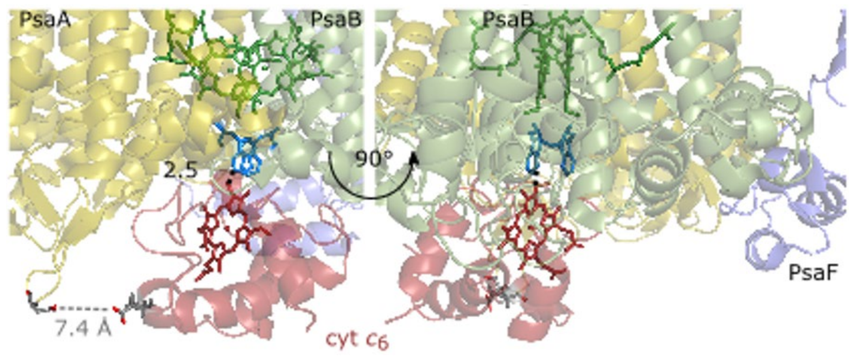


Figure 1. Model for binding of cytochrome c_6 from (Kölsch et al., 2018).

Expression and purification

BL21(DE3) cells containing the pEC86 vector for the heme cofactor and a plasmid encoding for cytochrome c_6 with different amino acid replacements were grown in selective LB medium for bulk production of cytochrome c_6 variants. Some optimization was also performed where strains were grown at variable temperatures and IPTG concentrations *(Fig 3a.)*.

The cytochrome c_6 variants were His-tagged and expressed in the periplasm to facilitate extraction by osmotic shock. The protein extracts were then validated using SDS-PAGE *(Fig 3b.)* purified using Ni-NTA columns (which select for His-tagged proteins) and concentrated using Centricon concentrators *(Fig 2.)*. Finally, the concentration was estimated based on the extinction coefficient for absorbance at 553nm *(Fig 4.)*.

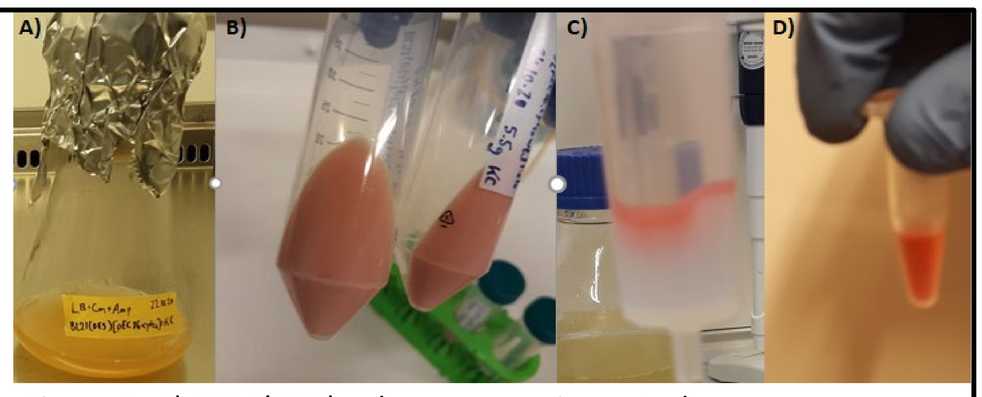


Figure 2. A) BL21(DE3) culture expressing cytochrome c_6. B) Pelleted culture. C) Extracted cytochrome c_6 loaded on Ni-NTA column. D) Purified and concentrated cytochrome c_6 (final product).

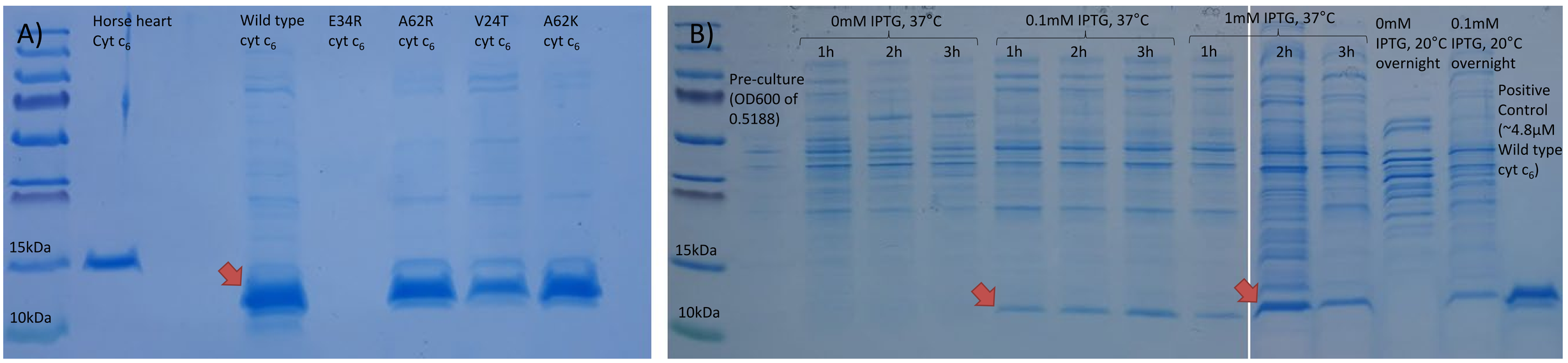
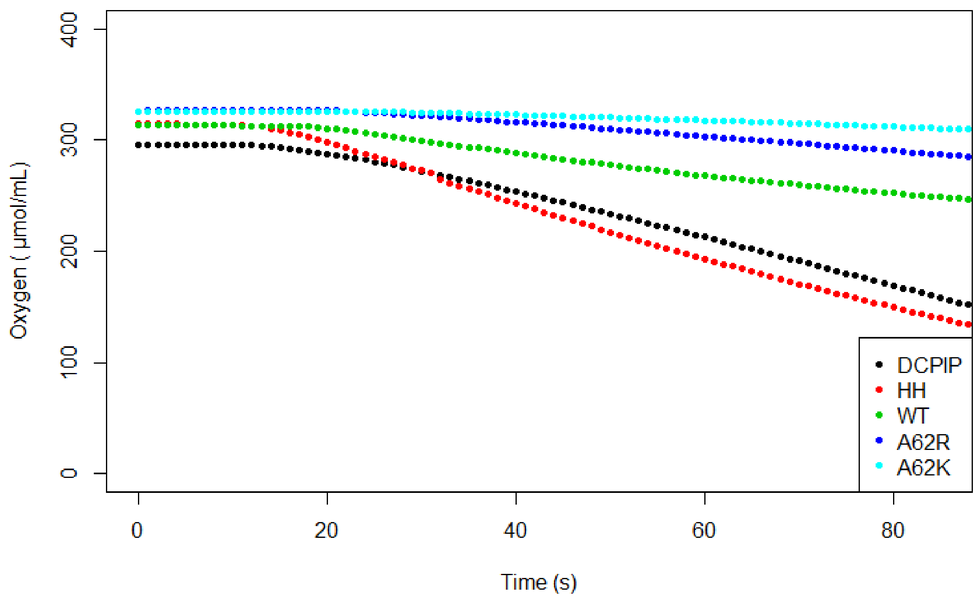


Figure 3. A) SDS-PAGE containing purified cytochrome c_6 variants used in preliminary oxygen reduction measurements. Some of the products had very low concentrations and were omitted from activity measurements. B) SDS-PAGE containing impure wild type cytochrome c_6 extracts from optimization experiments, grown at varying temperatures and IPTG concentrations. IPTG seems to have a significant effect on expression, but with 1µM IPTG it seems to decrease after 3h. Additionally, incubation at 20°C over night does not seem to increase the purity of the extraction significantly. Red arrows point out some cytochrome c_6 bands.

Activity assay

Effects of cytochrome c_6 amino acid replacements on oxygen reduction by PSI was measured with a Clark electrode *(Fig 5.)*. A saturating concentration of sodium ascorbate was used as a primary electron donor. Purified PSI and cytochrome c_6 variants were placed in this solution with methyl viologen added as an intermediate between PSI and oxygen. Oxygen reduction activity of PSI was triggered by the application of saturating light.



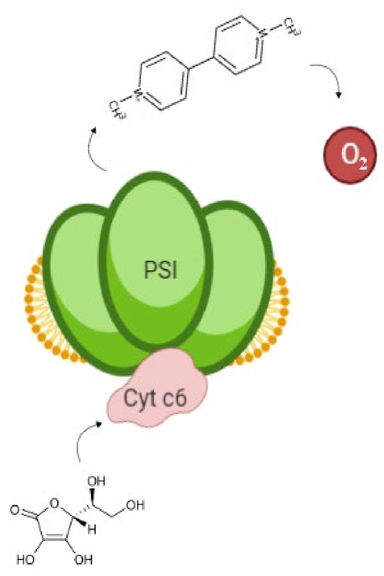


Figure 5. Preliminary results from Clark electrode measurements. DCPIP and horse heart cytochrome c_6 (HH) were used for reference. Interestingly, amino acid replacement mutants seemed to have lower activity than the wild type. To the right is a diagram showing the sequence of electron flow from ascorbic acid to oxygen.

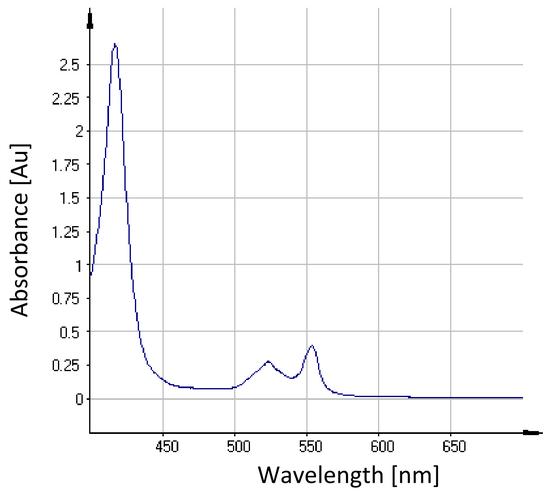


Figure 4. Absorbance spectrum of purified cytochrome c_6. Note the small peak at ~553nm.

Findings

As of now, we have some preliminary results showing that the A62R and A62K mutants actually have lower binding affinity than the wild type. This will have to be explored further with the additional mutants and more measurements.

The optimization experiments indicate that IPTG may be used to increase expression of cytochrome c_6 considerably, and that reasonably high expression can be achieved by incubation at 37°C for 3 hours. Incubation at a lower temperatures over night does not seem to increase the purity of the protein extract.

References

KÖLSCH, A., HEJAZI, M., STIEGER, K. R., FEIFEL, S. C., KERN, J. F., MUH, F., LISDAT, F., LOKSTEIN, H. & ZOUNI, A. 2018. Insights into the binding behavior of native and non-native cytochromes to photosystem I from Thermosynechococcus elongatus. *J Biol Chem,* 293**,** 9090-9100.

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