Thermodynamic Bias for Functional Specificity of the Different Haems of the Small Tetrahaem Cytochrome *c* from *Shewanella oneidensis*

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The gram negative bacterium *Shewanella* is remarkable in its respiratory versatility, being able to couple its metabolism to the respiration of a variety of different electron acceptors, making this genus a potential candidate for applications in bioremediation and also microbial fuel cells. Detailed knowledge on how its respiratory proteins function will allow an enhanced application of these bacteria.

When growing under anaerobic conditions, these bacteria contain in their periplasmic space an abundant small tetrahaem cytochrome *c* (STC). Due to its small size (12 kDa), localization (soluble) and the fact that within the *Shewanella oneidensis* specie its physiological role still remains to be identified, this cytochrome is an interesting candidate for functional studies.

A thermodynamic characterization of the STC from *Shewanella oneidensis* MR-1 (*So*STC) was performed over a wide pH range (5.5 – 9.0), enclosing the physiological pH, using NMR and UV-Visible spectroscopy. NMR data collected using partially oxidized samples and redox titrations obtained from UV- Visible spectroscopy, allowed the discrimination of the redox- and redox-protonation interactions (Redox-Bohr effect) that occur during the titration of the haems in the physiological pH range. The results demonstrate that electrostatic effects dominate the haem-haem interactions, and that the protonation of ionisable centres in the protein produce a considerable effect on the redox properties of the haems in the physiological pH range.

This detailed thermodynamic characterization of *So*STC shows that of all the possible microscopic redox states accessible by this protein, only a few show dominant populations. On this basis a functional pathway was proposed for the redox activity of the *So*STC in the physiological pH range, where reduction and protonation are thermodynamically coupled.

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