Studies on Cross-Linking and Protein-Protein Interactions of Adhesive Proteins from the Blue Mussel

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Abstract

The aim with this thesis work has been to investigate the cohesive and adhesive properties of byssal proteins from the common blue mussel, *Mytilus edulis*. The results are of importance for the development of strategies focused on preventing marine fouling as well as for applications in which the ability of the mussel glue to cure under water is utilized in biotechnological and medical applications.

The redox functional amino acid di-hydroxyphenylalanine (DOPA), which is present in the majority of the byssal proteins, is involved in the cross-linking reaction (curing process) and does most likely also contribute to the strong adhesive properties of the mussel glue. **Paper I and II** are concentrated on the adsorption and cross-linking behavior of the mussel adhesive protein, Mefp-1, investigated using the surface sensitive techniques: quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR) and ellipsometry. By comparing the adsorption behavior on a hydrophilic SiO_2 surface and a hydrophobic methyl-terminated gold surface it was shown that the structure of the adsorbed protein film is strongly influenced by the surface chemistry. On the hydrophilic SiO_2 surface Mefp-1 formed a rigidly attached and compact protein layer, whereas on the hydrophobic surface Mefp-1 formed an extended, flexible and water rich layer. This difference is attributed the high content of polar residues in Mefp-1 as well as the ability of DOPA to form strong complexes with Si(IV). Cross-linking, induced chemically using $NaIO_4$ and Cu^{2+} or enzymatically using mushroom tyrosinase, resulted in a significant increase in rigidity and a decrease in thickness of the protein layer. Upon chemically induced cross-linking there was a simultaneous release of coupled water, whereas for enzymatically induced cross-linking the release of coupled water was accompanied by binding of the enzyme, suggesting that the enzyme in it self may play an active role as a structural component of the glue. This question was further stressed in **paper III**, where biotin-doped Mefp-1 (b-Mefp-1) was coupled to a streptavidin functionalized lipid bilayer. The influence from Cu^{2+}, $NaIO_4$ and mushroom tyrosinase on the interaction between b-Mefp-1 and Mefp-2 was investigated using QCM-D. It was demonstrated that association between b-Mefp-1 and Mefp-2 could only be achieved when the cross-linking was induced by the enzyme. Moreover, the binding of the enzyme to immobilized b-Mefp-1 and subsequent binding of Mefp-2 was dependent on enzymatic activity. The absence of interaction between b-Mefp-1 and Mefp-2 in the presence of Cu^{2+} or $NaIO_4$ is most likely resulted by repulsion between the positively charged proteins, which was reduced by introduction of the negatively charged enzyme. However, even if the cross-linking reaction and various protein-protein interactions within the mussel glue could be efficiently probed with the techniques used in paper I to III, it was not possible to discriminate between different possible reaction pathways for the cross-linking reaction. In **paper IV**, it is demonstrated how attenuated total internal reflection Fourier transform infrared spectroscopy (ATR-FTIR) can contribute with detailed chemical information, allowing discrimination between Cu^{2+} mediated oxidation of DOPA followed by di-DOPA formation and complex formation between Cu^{2+} and DOPA. By comparing $NaIO_4$ induced oxidation of DOPA with Cu^{2+} induced cross-linking, it was demonstrated that the Cu^{2+} mediated increase in rigidity originates from complex formation between Cu^{2+} and DOPA, even if simultaneously occurring oxidation of DOPA could not be entirely ruled out.

Keywords: mussel adhesive protein, Mefp-1, Mefp-2, DOPA, cross-linking, protein adsorption, protein-protein interaction, mushroom tyrosinase, streptavidin, biotin, supported phospholipid bilayer, QCM, SPR, ellipsometry, ATR-FTIR.

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