CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)

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Project Number

S0409

Project Title

A Tripeptide Based Study of the Role of Flanking Amino Acids in DOPA Mediated Mussel Adhesion

Abstract

Objectives/Goals

Mussel adhesion is achieved through secreted proteins such as mfp3 and mfp5, which have a high proportion of the modified amino acid Dopa. Dopa is suspected to be a key player in adhesion due to its ability to bind reversibly to Fe3+ and to be oxidized to quinone. The redox potential of the Dopa-quinone system favors quinone at the pH of sea water; however quinone is absent in mfps indicating that Dopa's oxidation is suppressed when it is a part of mfp proteins. This study employs a tripeptide model system to investigate the hypothesis that amino acids flanking Dopa affect its ability to be oxidized to quinone and to bind Fe3+.

Methods/Materials

Published sequences of mfp3 and mfp5 of marine mussels were analyzed to design tripeptides (GYG, KYK, NYN, GYK, GYN, RYN, RYG and GGG where Y is Dopa). Spectrophotometric assays were used to test the peptides at 80µM and 40µM concentration at pH 7 and 8.3 for their capacity to bind Fe3+ and get Dopa oxidized. Experiments were performed to study iron binding, auto oxidation, oxidation in the presence of mushroom tyrosinase, and the oxidizing agent NaIO4. Experiments were also carried out to study the competition between oxidation and iron binding.

Results

Results indicate that Dopa alone was best; the presence of flanking amino acids reduced the extent of both Dopa oxidation and iron binding. Auto oxidation was not seen at any pH. In general, oxidation was better at pH 8.3 while iron binding was better at pH 7. Competition studies show that presence of an oxidizing agent reduced iron binding; however oxidation was enhanced in the presence of Fe3+ especially for peptides containing asparagine. The nature of flanking amino acids affects both the extent of conversion of Dopa and its fate. Tripeptides such a GYG and RYG show robust oxidation and iron binding at both pHs. RYG always prefers oxidation and NYN iron binding.

Conclusions/Discussion

Flanking amino acids clearly affect DOPA mediated mussel adhesion by controlling the extent to which Dopa gets oxidized and binds to iron. An interesting observation is that Mytilus californianus, a Pacific mussel subject to harsh environment, achieves optimum adhesion by employing robust motifs in mfp3 while using motifs that specialize in iron binding or oxidation in mfp5. Future work could focus on studying whether the relative distribution of mfp3 and mfp5 is a function of iron concentration and oxygen levels.

Summary Statement

My project uses a tripeptide model system to demonstrate that flanking amino acids affect DOPA mediated mussel adhesion significantly, both by affecting DOPA's oxidation and its ability to bind to iron.

Help Received

Dr. Les Miranda (Amgen) gave peptides; used lab equipment at CSUCI under the supervision of Mike Mahoney and Cathy Hutchinson; Athol Wong communicated with CSUCI; mother helped understand concepts, assisted me in repetitive steps of the assay such as pipetting etc. to optimize lab time.