Redox Properties of Tryptophan and Tyrosine: Effects of the Protein Matrix

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Essentially nothing is known about the reduction potentials of amino-acid radicals in proteins. This is despite the frequency and importance of the enzymes employing amino-acid redox chemistry in biology. A key reason for this lack of information about the basic chemistry of amino-acid redox cofactors is the simple fact that their reduction potentials typically are so high that the oxidation of other cofactors, the protein matrix itself and even bulk water interferes with measurements. The "redox inert" design of the α_3W and α_3Y proteins was specifically directed to address some of these issues in order to facilitate electrochemical measurements on tryptophan and tyrosine residues. The potential of the single buried Trp-32 in α_3W is highly oxidizing relative to tryptophan free in solution. A density functional theory analysis suggests that the increase in potential is partly due to the Lys/Trp cation-π interaction present in this system. This effect will be described.

The redox properties of a tyrosine appear to be strongly influenced by the micro-environment surrounding the phenol OH group. Again, there is virtually nothing known experimentally about the nature of this effect in proteins. To address this issue, we have used the α_3Y system to investigate how varying the degree of solvation of a phenol OH group influences the pK_a and the potential of the aromatic molecule. The buried Tyr-32 in α_3Y was exchanged to a Cys to which 2-mercaptophenol, 3-mercaptophenol or 4-mercaptophenol was covalently bound. By attaching the structurally different mercaptophenols to Cys-32, the phenol OH group was rotated from a position at the protein surface towards more solvent-shielded positions in the interior of the protein. The structural and electrochemical properties of the mercaptophenol proteins will be described. In order to isolate the effects of the protein matrix on the phenol redox properties, the data derived from the protein samples are compared to data derived from the three mercaptophenols when bound to an uncharged cysteine derivative in aqueous buffer.