ABSTRACT

The copper containing protein plastocyanin is an important link in the electron transfer chain of oxygenic photosynthetic organisms, transferring electrons from the cytochrome b_6f complex to photosystem 1. Plastocyanin from spinach is a small protein made up of 99 amino acid residues and its fold is dominated by β-structure. Azurin is a member of the same protein family, the cupredoxins, sharing a greek-key motif β-sandwich fold and an intensely blue type 1 copper site. In this thesis X-ray crystallography has been used to study the structure of mutants of both these proteins to investigate their structure-function relationships. Plastocyanin from spinach was successfully crystallized for the first time and its structure determined to a resolution of 1.8 Å. The key to the successful crystallization was to stabilize a flexible loop by mutating a glycine in position 8 to an aspartic acid residue. The structure was found to be similar to that of poplar plastocyanin. However, significant differences were found in the small acidic patch, which is important for the interaction between plastocyanin and its redox partners. The G8D mutation also facilitated the successful crystallization and structure determination of the G8D/L12E double mutant to a resolution of 2.0 Å. It was found that its low reactivity towards photosystem 1 is explained by a small perturbation of the hydrophobic patch. The insertion of a disulfide bridge between positions 30 and 69 in spinach plastocyanin was found to increase the thermal stability of spinach plastocyanin by about 2 °C. The structures of the spinach plastocyanin double mutant K30C/T69C and triple mutant G8D/K30C/T69C were both determined to a resolution of 1.9 Å. The inserted disulfide bridge did not affect the structure but resulted in a new form of crystal packing for plastocyanin with the hydrophobic patches together in a head-to-head fashion. This arrangement has previously been found in most structures of azurin, where it has been proposed to provide a route for electron self-exchange. The resolution for the oxidized form of the spinach plastocyanin mutant G8D was extended to 1.35 Å and the structure of the reduced form was determined to 1.25 Å. A conformational change of the copper binding loop was found to be associated with the change in oxidation state. This change in conformation leads to a change in the shape of the hydrophobic patch of comparable magnitude to that found for the L12E mutant. Hence, this can have a comparable effect on the binding affinity to physiological redox partners. The C3S/S100P double mutation of azurin from *Pseudomonas aeruginosa* disrupts the naturally occurring disulfide bridge, thereby destabilizing the protein by ~20 kJM^{-1}, while the folding rate is barely affected. The structure, determined to 1.8 Å, reveals that the largest changes involve the side chains in the disulfide mutation site. The substitution of a serine in position 100 for a proline was found to break a weak hydrogen bond but to leave the structure essentially unchanged.

Keywords: azurin, electron transfer, plastocyanin, X-ray crystallography

ISBN: 91-628-6121-2