Spectroscopic and Functional Characteristics of Ferredoxin from Clostridium pasteurianum after Substitution of the Inorganic Sulfur by Selenium and Tellurium

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At present there are only a few proteins known, which contain selenium in vivo (1). In vitro it is possible to replace the inorganic sulfur in $Fe_2S_2^*$-clusters by selenium (2). Although the $Fe_2Se_2^*$-clusters were less stable, these seleno-proteins showed full activity in biological tests.

For bacterial type ferredoxins which contain $Fe_4S_4^*$-clusters no reconstitutions with selenium were reported. The substitution of the inorganic sulfur in the $Fe_4S_4^*$-clusters by the higher homologues could give an interesting insight into the relationship between functional and spectroscopic properties of these redox-active proteins.

The selenoferredoxin has the same molecular weight (M = 5800) as the native protein but the stoichiometry shows that only one $Fe_4Se_4^*$-cluster is incorporated into the protein whereas native ferredoxin contains two $Fe_4S_4^*$-clusters.

The oxidized protein is ESR-silent as the respective native protein. The fully reduced form shows at liquid helium temperature an ESR spectrum ($g \approx 1.94$) with nearly rhombic symmetry ($g_1 = 2.099$, $g_2 = 1.937$, $g_3 = 1.884$) (3). This spectrum corresponds to that of the half-reduced (S = 1/2) native ferredoxin, whereas the fully reduced ferredoxin shows a spectrum of a S = 1 system (4). An additional resonance appears at g = 5.164. The temperature behaviour of this signal is in contrast to that of the high-field resonance, i.e. it can be measured at 77 K. This resonance is not observed in the oxidized state and must therefore belong to the cluster. The interpretation of this signal as a half-field resonance of a magnetically coupled two-cluster system with S = 1 (3) is to be excluded because of the cluster stoichiometry and of the field position of this resonance. It might be possible that this resonance belongs to a low-lying excited state with S = 3/2.

The Mößbauer spectrum of the oxidized protein shows one quadrupole pair with a splitting of 1.35 mm/s and confirms the non-magnetic state. The splitting correlates with that found in the sulfur protein.

The reduced selenoprotein shows a magnetic hyperfine splitting.

This finding supports the ESR results.

If one compares the prompted and the delayed fluorescence respectively of intact algae with those observed with isolated chloroplasts in the presence of ferredoxin and selenoferredoxin respectively, it can be shown that the fluorescence behaviour is more similar to intact algae in the presence of the selenoprotein. The activity of the selenoprotein in the phosphoroclastic system is decreased to 1/6. Whereas native ferredoxin is the better electron donor in the phosphoroclastic system, selenoferredoxin is the better electron acceptor in the photosystem I.

To our surprise the isolated ferredoxin reconstituted with tellurium showed no ESR spectrum in the reduced state. But ESR signals are observed in the oxidized protein. The measured g-values (g_1 = 4.62, g_2 = 4.304, g_3 = 4.06) and a resonance at g = 9.45 are indicative for a high spin iron ($^6S_{5/2}$) with rhombic symmetry. The resonance at g = 9.45 originates from a transition between the two sublevels of the lowest Kramers doublet. because of its increasing intensity with decreasing temperature. The signals at g = 4.3 belong to the middle Kramers doublet. Thus the interpretation for these signals given earlier can now be excluded (3).

The Mößbauer-spectrum of the oxidized form shows the hyperfine structure of a S = 1/2 system. These findings and the UV absorption spectrum of the oxidized protein support the conclusion that under the conditions of reconstitution no cluster with tellurium as bridging ligands were formed but instead of this a rubredoxin-type complex. The protein reconstituted with tellurium acts neither as electron acceptor for photosystem I nor as an electron donor in the phosphoroclustic system which is in correspondence with the fact that no $Fe_4Te_4^*$-cluster is formed.

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