REDUCTION OF PLASTOCYANIN BY SOLVATED ELECTRONS IN NON-AQUEOUS MEDIA

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1. Introduction

In the elucidation of the mechanism of electron transport in enzymatic redox reactions, and that between electron-carrier proteins, the use of solvated electrons and the study of their interaction with proteins is becoming increasingly important. In most studies solvated electrons have been generated by pulse radiolysis, i.e., by irradiation of aqueous solutions by high-energy electrons or γ-rays [1–3]. However, solvated electrons are known to be unstable in aqueous media. Their stabilization, which is necessary for more detailed studies, may be achieved in some non-aqueous media such as liquid ammonia, ethylene diamine and hexamethyl phosphoramide (HMPA). In fact, one method to produce solvated electrons is to dissolve alkaline metals in these organic solvents which then exhibit dark blue colour and display the optical and EPR spectra characteristic for solvated electrons [4,5].

In a recent paper [6] we were the first to describe the interaction of solvated electrons with erythrocuprein, a copper-containing enzyme exhibiting superoxide dismutase activity, in non-aqueous media. As a continuation of this work the present paper describes the reaction of solvated electrons with a copper-containing electron-carrier protein, plastocyanin, in two non-aqueous media.

2. Materials and methods

Plastocyanins were isolated from cucumber (*Cucumis sativus*), goosefoot (*Chenopodium album*) and garden cress (*Lepidium sativum* L.). The optical and EPR spectra of all preparations were similar [7,8]. The preparations were electrophoretically homogeneous with spectral indices, A_{278}/A_{597}, of 1.4–1.6. Plastocyanins were transferred from the aqueous media to organic solvents by the following procedure. Proteins were precipitated from the aqueous solution by the addition of acetone. The traces of acetone were removed either by a stream of dry nitrogen or under vacuum. When the light blue powder of plastocyanin was redissolved in water, the optical and EPR spectra of the resulting solutions were found to be similar to those of the initial preparation. The powder was easily dissolved in anhydrous dimethylsulfoxide (DMSO) and formamide as well. However, its solubility in HMPA was limited. The organic solvents were dehydrated and distilled by conventional methods immediately before use [9,10]. The water contents of DMSO, formamide and HMPA were 10^{-2}%, 2×10^{-2}% and 10^{-2}%, respectively. Solvated electrons were generated by dissolving 0.4 g metallic sodium in 15 ml HMPA [6].

Aliquots, 0.05 ml, of this solution were used for the titration of the protein dissolved in 3 ml DMSO, formamide or water; the concentration of the protein was 2 mg/ml. The titration was carried out anaerobically in Thunberg type cuvettes at 22°C. Immediately after the addition of the aliquots of solvated electrons the optical and EPR spectra of the reaction mixtures were recorded.

EPR spectra were obtained at 77°K on an instrument operating at X-band. The experimental conditions were: microwave frequency 9.12 GHz; amplitude modulation 6 G; power 10 mW. Optical spectra were recorded in 10 mm cells.

3. Results and discussion

It was found that the optical and EPR spectra of plastocyanin in organic solvents are strikingly different from those of the aqueous solutions. Figure 1 and 2 show the optical and EPR spectra of plastocyanin from *Chenopodium album* in aqueous and organic solvent media, respectively. Similar changes were observed for the other two plastocyanins. As may be seen from fig.1, the protein solution in organic media is not blue because in these media the band with λ_{max} 597 nm, typical for the aqueous solutions of plastocyanins, disappears. However, in organic solvents a new absorbance band appears with a maximum at 340 nm. The EPR spectrum of axial symmetry with two g-factors, typical of the aqueous solutions of plastocyanin, was drastically changed in organic solvents. The shape of the spectrum observed in organic media suggest a shift to a rhombic symmetry with three corresponding g-factors. The parameters of EPR spectra of plastocyanin in aqueous and organic media are listed and compared in table 1. As may be seen, the hyperfine splitting in the low field region (A_{II}) of the EPR spectrum was increased more than 3 times in organic media as compared with the aqueous solution. Thus, optical and EPR spectra indicate that in organic solvents the environment of the copper in plastocyanin is altered. Nevertheless, plastocyanins in both aqueous and organic solutions were found to be easily reduced with solvated electrons. The addition of small amounts of solvated electrons (0.05–0.1 ml/3 ml protein solution) resulted in a partial decease in the intensity of EPR spectra. The addition of higher amounts of solvated electrons (0.15–0.2 ml) has led in both cases to the disappearance of EPR spectra of the protein. No EPR spectra were observed after the addition of a large excess of solvated electrons (0.5–1.0 ml).

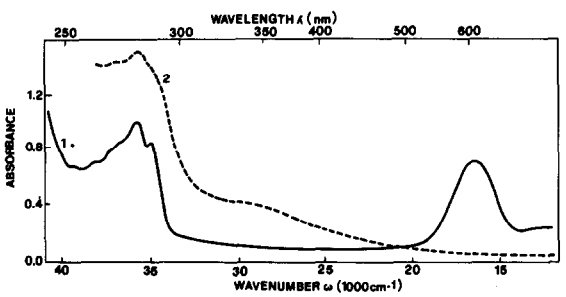

Fig.1. Comparision of optical spectra of plastocyanin in water (1) and DMSO (2).

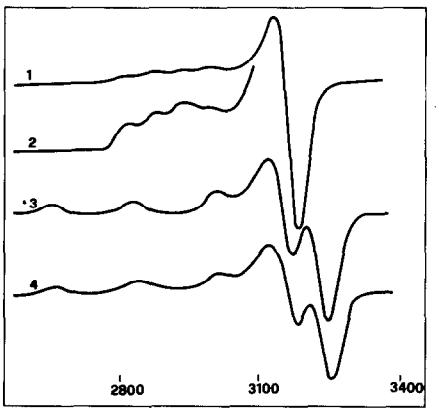

Fig.2. Comparision of EPR spectra of plastocyanin in water (1,2), DMSO (3) and formamide (4). Receiver gains for 1, 3 and 4 were the same. Receiver gain for 2 was 3-times higher.

It was found that in contrast to its aqueous solutions, plastocyanin reduced in organic media was autoxidable (fig.3). It is of interest to note that the addition of 30% water to plastocyanin solutions in organic

Table 1
Parameters of EPR spectra of plastocyanin

Parameters	In water-containing media	In formamide- and DMSO-containing media	After reoxidation in organic media
A_1 (A_{II})	60 G	175 G	215 G
g_1 (g_{II})	2.23_5	2.23_2	2.01_6
g_2 (g_m)	2.06_0	2.06_2	2.06_0
g_3		2.00_1	1.96_2

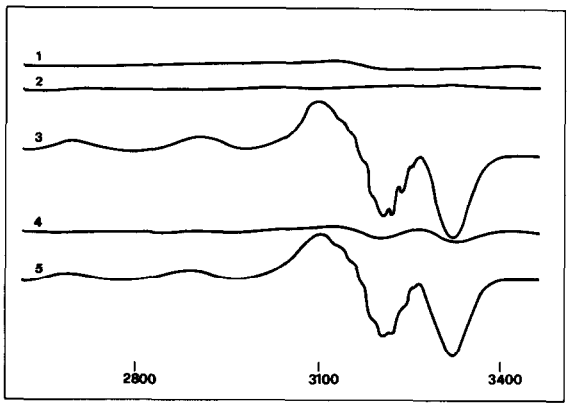

Fig.3. Changes of EPR of plastocyanin: spectra were recorded at the same gain. For other details see the text. (1) Plastocyanin dissolved in water after the addition of 0.15 ml solvated electrons. The spectrum of the original solution was the same as shown in fig.2-1. (2) Plastocyanin in formamide after the addition of 0.2 ml solvated electrons. The spectrum of the original solution was the same as shown in fig.2-3. (3) Sample 2 after treatment with oxygen for 20 min. (4) Sample 2 after treatment with oxygen for 1 min. (5) Sample 3 after addition of 30% water.

solvents had no effect on the autoxidation of the protein.

Thus, the present results show that the copper of plastocyanin is reduced with solvated electrons in aqueous as well as in organic media, however, the reduced protein is autoxidable only in organic media. These observations probably indicate that the redox potentials of the protein in aqueous and organic media are different. These differences may be due to the modification of the ligand environment of the copper in organic media rather than to differences in the dielectric constants of solvents. This follows from the fact that the EPR spectra of the protein in DMSO and formamide are similar, although the dielectric constants of these solvents are different (47 and 109, respectively). Also, the dielectric constant of water is 80, still the optical and EPR spectra of plastocyanin in aqueous solutions are distinctly different from those in the above mentioned organic solvents.

The copper of plastocyanins in aqueous media has an EPR spectrum suggesting axial symmetry with low hyperfine splitting (60 G). The protein in this medium is bright blue with intensive absorbance at 597 nm. Consequently, the copper of plastocyanins in aqueous media is referred to as 'type I copper' [11]. Although the ligand amino acids of type I copper are not yet known, the data available [12-15] indicate the possible significance of cysteine, tyrosine and histidine as ligand amino acids. The participation of water molecules in the binding with copper in plastocyanins can be ruled out from the results of proton relaxation rate measurements [16]. The data presented in this paper indicate that the environment of copper is changed in organic solvents. However, it is not yet clear whether this is due to changes in ligand amino acids in these media or it reflects just a change in symmetry of the ligand environment of the copper atom. Optical and EPR spectra of plastocyanins in organic media are characteristic for type 2 copper and, as reported here, the reduced form of plastocyanin, dissolved in organic media, is autoxidable. It has been found [17] that type 2 copper contains a water molecule in its first coordination sphere. On the other hand, type 1 copper has no coordinated solvent molecule. It may be suggested, on this basis, that in organic media the protein-bound copper became accessible for solvent molecules.

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