Biochimica et Biophysica Acta 810 (1985) 123–131
Elsevier

BBA 41865

Plastocyanin conformation: the effect of the oxidation state on the pK_a of nitrotyrosine-83

G.P. Anderson, J.E. Draheim and E.L. Gross *

Department of Biochemistry, The Ohio State University, Columbus, OH 43210 (U.S.A.)

(Received May 21st, 1985)

Key words: Plastocyanin; Chemical modification; Electron transport; Redox potential; Protein conformation

Plastocyanin treated with tetranitromethane was nitrated at a single location, Tyr-83. Tyr-83 and its neighboring negative charges have been implicated as a binding site for positively charged redox agents (Chapman, S.K., Watson, A.D. and Sykes, A.G. (1983) J. Chem. Soc. Dalton Trans. 1983, 2543–2548). No effect was observed on either the plastocyanin midpoint redox potential or its reaction kinetics with P-700$^+$ and cytochrome *f*. This makes nitration an ideal spectroscopic probe for monitoring changes in the environment of Tyr-83. The pK_a of the nitrotyrosine was 8.6 and 8.3 for reduced and oxidized plastocyanin, respectively, indicating that the charge on the copper atom is 'felt' at Tyr-83. The high pK_a value for both forms indicates that Tyr-83 is in a negatively charged environment, near residues Nos. 42–45 and Nos. 59–61. The extinction of the nitrotyrosine chromophore at 360 nm was not affected by a change in redox state. However, the ellipticity of this transition was greater for the oxidized form, indicating that environment of Tyr-83 is dependent upon the charge on the copper atom. This suggests an electrostatically driven conformational change at Tyr-83. A conformational change at Tyr-83 could regulate the binding of plastocyanin with its reaction partners in order to promote smooth electron transport.

Introduction

Plastocyanin is a 10.5 kDa 'blue copper' protein functioning in chloroplast electron transport. It has been postulated that plastocyanin acts as a mobile electron carrier shuttling electrons between the cytochrome b_6/f complex and Photosystem I which contains P-700 [2–4]. The crystal structures of the oxidized, reduced, and apo forms of poplar plastocyanin have been determined [5–7]. A computer drawn picture of oxidized poplar plastocyanin is shown in Fig. 1 which displays the normal front face view of the protein. To provide a reference some of the residues have been labeled with their sequence number.

It has been proposed that there are two binding sites for the plastocyanin reaction partners [1,8,9]. One site is at His-87 near the 'top' of the molecule. This is the site at which ferricyanide oxidizes the protein. Another binding site is in the region near Tyr-83 at the 'east face' of the plastocyanin molecule. This is the site at which $[Ru(NH_3)_5(pyridine)]^{2-}$ reduces plastocyanin [10]. The binding site near Tyr-83 also contains several negatively charged residues, numbers 42–45 and 59–61. These residues appear to form a negative 'patch'. This can be easily observed in Fig. 2 which displays an 1.1 nm thick slice of a rotated view of plastocyanin such that the 'top' of the protein is nearest the reader.

The negatively charged residues, numbers 42–45

* To whom correspondence should be sent.

Abbreviations: CD, circular dichroism; DEAE, diethylaminoethyl; DPCC, diphenylcarbonyl chloride; Chl, chlorophyll; Cyt, cytochrome; PS I, Photosystem I.

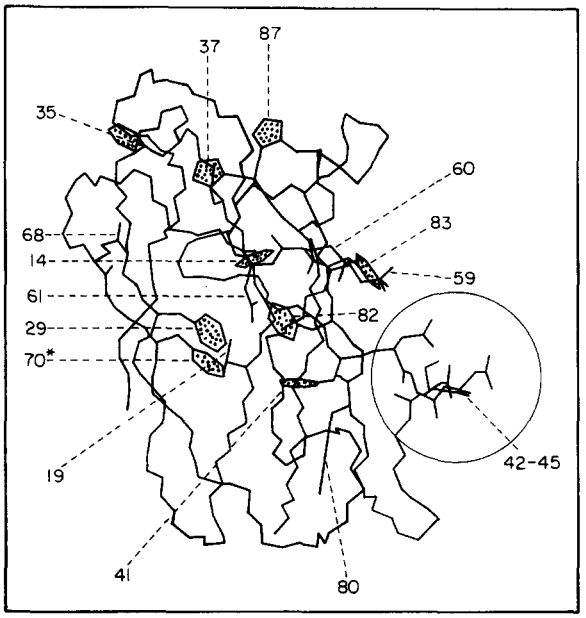

Fig. 1. Computer graphics representation of oxidized poplar plastocyanin. The X-ray coordinates for oxidized poplar (*Populus nigra*) plastocyanin were obtained from the Brookhaven Protein Data Bank and were displayed using a commercial graphics program. The peptide backbone, the histidine ligands to the copper, the tyrosine and phenylanaine residues are shown. Also shown are the highly conserved residues at positions Nos. 42–45 and 59–61 which form a negative patch near Tyr-83.

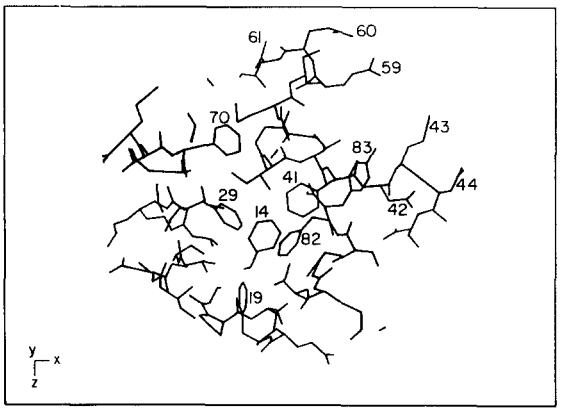

Fig. 2. Slice of plastocyanin containing Tyr-83 and near-by negative charges. A 1.1 nm thick slice of plastocyanin containing Tyr-83 and the associated negative charges was made and was rotated so that it is viewed from the top of the molecule. All amino acid side chains are included and Tyr-83, the negative charges at residues Nos 42–44 and 59–61 and the phenylalanine residues are specifically labelled.

and 59–61, are conserved in plastocyanin from higher plant species, but are replaced with neutral or positively charged residues in plastocyanin from cyanobacterial species [2]. In addition to Tyr-83, spinach plastocyanin contains two other tyrosine residues 70 and 80. In poplar plastocyanin, Tyr-70 is a phenylalanine residue [2]. Higher plant plastocyanins do not contain tryptophan.

In solution, the protein portion of the plastocyanin molecule appears to be flexible, changing its tertiary conformation in response to changes in pH, ammonium sulfate concentration, redox state, and chemical modification with ethylenediamine. This was determined on the basis of its near-ultraviolet absorption, circular dichroic and fluorescence spectra [11,12]. In contrast, the environment of the copper atom in oxidized plastocyanin remained invariant.

A fluorescence study of plastocyanin nitrated at Tyr-83 indicated that Tyr-83 participates in the redox-state-dependent conformational changes in spinach plastocyanin [12]. This is interesting in view of its role as a possible binding site. A redox-state-dependent binding site conformation could induce differential binding of the oxidized and reduced forms of plastocyanin with its reaction partners Cyt *f* and P-700$^+$. This in turn would promote smooth electron transport since plastocyanin is believed to be a mobile electron carrier [2–4]. It is the purpose of this article to document the properties of nitrotyrosine-plastocyanin and to use this point modification to examine the effect of the plastocyanin redox state on the local environment of Tyr-83.

Methods

Nitration of plastocyanin. Tetranitromethane adds a nitro group to the *ortho* position of accessible tyrosine residues [13,14]. The protonated form of nitrotyrosine exhibits transitions at approx. 270 nm and 360 nm. Upon deprotonation, the 360 nm transition shifts to 428 nm. The pK_a for a typical nitrotyrosine is approx. 7.0.

Spinach plastocyanin was isolated according to previously published procedures [12] and suspended in 50 mM Tris-HCl (pH 8.5) + 100 mM NaCl. The plastocyanin concentration was ad-

justed to 0.3 mM and initially oxidized with excess ferricyanide. A 10 μl aliquot of tetranitromethane was then added to the plastocyanin sample every 10 min until the total tetranitromethane added was 50 μl.

The reaction was quenched by gel filtration after a 5% decrease in absorbance at 597 nm occurred. Nitrated plastocyanin was separated from excess tetranitromethane on a Sephadex G-25 column equilibrated with 10 mM sodium succinate (pH 6.0). Nitrotyrosine-plastocyanin was stored at lower pH values to prevent denaturation.

FPLC purification of nitrotyrosine-plastocyanin. The above procedures result in a mixture of singly modified nitrotyrosine-plastocyanin, unmodified control plastocyanin, and denatured (*apo*-)nitrotyrosine-plastocyanin. This mixture was reduced by sodium ascorbate and desalted on a Bio-Gel P-10 column equilibrated with 10 mM sodium succinate. It was then separated using a Pharmacia FPLC with the MONO Q anion exchange column (Fig. 3). This column was equilibrated with 25 mM Tris-HCl (pH 8.2) and eluted with a linear NaCl gradient. The isolated nitrotyrosine-plastocyanin

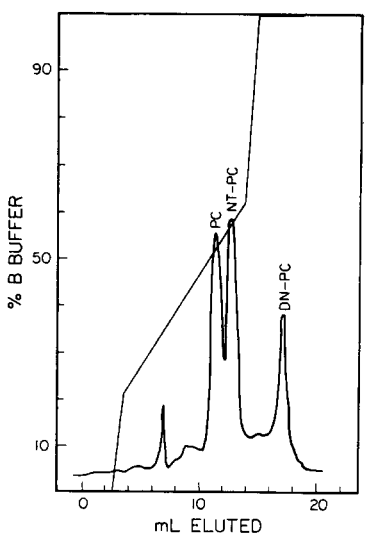

Fig. 3. FPLC separation of control plastocyanin (PC), nitrotyrosine-plastocyanin (NT-PC), and denatured (*apo*-)nitrotyrosine-plastocyanin (DN-PC). The products of the tetranitromethane reaction were separated by FPLC using a linear NaCl gradient in 25 mM Tris-HCl (pH 8.2). The B buffer was 25 mM Tris-HCl (pH 8.2) plus 0.5 M NaCl. Other conditions were as described in the Materials and Methods section.

contained a single nitrotyrosine. This was determined using the molar extinction coefficients of 2790 at 360 nm for nitrotyrosine 4900 at 597 nm for oxidized plastocyanin.

Location of modification. The location of the nitrotyrosine modification was determined by HPLC separation of the nitrotyrosine-plastocyanin tryptic peptides. Heat-denatured control plastocyanin and nitrotyrosine-plastocyanin were digested for 1 h with 1% DPCC-treated trypsin. The peptides were separated using reverse phase chromatography on a LDC HPLC with an Anspec RP-8 column. The peptides were eluted using 45% 9 : 1 acetonitrile/0.1% trifluoroacetic acid in water gradient. The identity of the peptides were determined using a Beckman 119 CL Amino Acid Analyzer.

Ethylenediamine modification of nitrotyrosine-plastocyanin. Nitrotyrosine-plastocyanin was chemically modified with ethylenediamine plus a water-soluble carbodiimide using the procedure of Burkey and Gross [15] with minor alterations. The ethylenediamine and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide concentrations were 20 and 5 mM, respectively. The reaction time was 1 h at 4°C. These conditions result in a mixture of forms containing between one and three molecules of ethylenediamine per plastocyanin, thus all forms have a net negative charge.

Isolation of Cyt f. Cyt *f* was isolated from turnip leaves by the procedure of Matsuzaki et al. [16]. The Cyt *f* had a purity index ratio of 0.3 ($A_{545.5}/A_{277}$) after Sephadex column chromatography. This was sufficiently pure for the kinetic experiments.

Measurement of the kinetics of the reaction of plastocyanin with P-700$^+$ and Cyt f. Rates of P-700$^+$ reduction were determined as previously described [15]. PS I was isolated according to the method of Shiozawa et al. [17]. PS I (at 15 μg/ml Chl) was incubated with up to 2 μM plastocyanin or nitrotyrosine-plastocyanin in a reaction mixture containing 10 mM sodium succinate (pH 6.0) + 5 mM $MgCl_2$. $MgCl_2$ titration curves were determined by titrating PS I (at 15 μg/ml Chl) and either 1 μM control plastocyanin or nitrotyrosine-plastocyanin in 10 mM Tris-HCl (pH 8.0) with up to 7 mM $MgCl_2$.

The rate of oxidation of Cyt *f* by control

plastocyanin and nitrotyrosine-plastocyanin was determined by using an Aminco DW-2a with a stopped-flow attachment. Second-order rate constants were obtained using equal concentrations of plastocyanin and Cyt *f* (1 μM) in 10 mM phosphate buffer (pH 7.0) + 100 mM NaCl. The oxidation of Cyt *f* was monitored by observing the decrease in absorbance at 421 nm with the reference wavelength at 650 nm. A reduced-minus-oxidized extinction coefficient of 1230 $M^{-1} \cdot s^{-1}$ for 421 nm was determined from the cytochrome *f* used for this study. The data were recorded using a Bascomb-Turner Model 2110 electronic recorder at a rate of one data point per ms.

Determination of the midpoint redox potential. The midpoint redox potentials of nitrotyrosine-plastocyanin and control plastocyanin were measured using a twin-electrode design in a thin-layer, optically transparent electrochemical cell [18]. The $E^{0\prime}$ values for plastocyanin obtained in mediated thin-layers were dependent upon the type of mediator used. Some mediators (most notably ferricyanide) were not electrochemically reversible at the surface of the gold electrode. The $E^{0\prime}$ obtained for both control plastocyanin and nitrotyrosine-plastocyanin using 1,1′-bis(hydroxymethyl)ferrocene as a mediator was +370 mV vs. a normal hydrogen electrode.

Near-ultraviolet absorption and circular dichroic spectra. The absorption and circular dichroic (CD) spectral measurements were performed using a Cary 118 C spectrophotometer and a Jasco 500A spectropolarimeter, respectively [11]. Plastocyanin (15–35 μM) was suspended in 10 mM concentration of the buffers indicated. Both the absorption and the CD of plastocyanin varied linearly with concentration between 15 and 100 μM, indicating that aggregation effects do not contribute to our results.

Plastocyanin concentrations were determined after each experiment using an extinction coefficient at 597 nm of 4.9 $mM^{-1} \cdot cm^{-1}$ after the addition of excess ferricyanide. Plastocyanin was oxidized using $K_3Fe(CN)_6$ which was subsequently removed by gel filtration on a Bio-Gel P-10 column. Plastocyanin was reduced using sodium ascorbate which was removed by gel filtration on a Bio-Gel P-10 column.

Determination of the pK_a of the nitrotyrosine derivative. The pK_a of the nitrotyrosine was determined for both oxidized and reduced nitrotyrosine-plastocyanin by measuring its absorption at both 360 and 428 nm as a function of pH between 6.0 and 9.5. The pH was adjusted by the addition of 0.1 M NaOH or HCl.

Computer graphics display of poplar plastocyanin. A graphic display of the 3-dimensional structure of oxidized poplar plastocyanin was made using the X-ray coordinates, obtained from the Brookhaven Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton NY 11973, U.S.A., together with a commercial engineering wireframe modeling graphics package IDEAS. Further details of the graphics methodology will be published elsewhere [19].

Materials. DEAE-cellulose and Bio-Gel P-10 were obtained from BioRad Laboratories. Tetranitromethane, Tris, and DPCC-treated trypsin were obtained from the Sigma Chemical Co. HPLC grade acetonitrile and water were obtained from Burdick & Jackson Laboratories Inc. All other chemicals were of reagent grade.

Results

Location of the nitrotyrosine derivative. Davis and San Pietro [14] reported an average of one nitrotyrosine residue per molecule for plastocyanin treated with tetranitromethane. FPLC separation (Fig. 3) of the products obtained by this procedure showed three separate peaks. These were identified as unmodified control plastocyanin, singly modified nitrotyrosine-plastocyanin, and denatured (*apo*-)nitrotyrosine-plastocyanin. This identification was made on the basis of the ratio of the nitrotyrosine absorbance at 360 nm to the copper center absorbance at 597 nm. Only the singly modified nitrotyrosine-plastocyanin was extensively studied.

The tryptic peptides of singly modified nitrotyrosine-plastocyanin were separated using HPLC. Only peptide 6, which includes Tyr-83, contained a nitrotyrosine. Therefore, Tyr-83 is the nitro modification site. This is reasonable, since Tyr-83 appears to be the most accessible tyrosine residue in poplar plastocyanin (Fig. 1).

The effect of nitration on the plastocyanin activity and redox potential. Several kinetic experiments

were performed to determine the effect of nitration on the activity of plastocyanin. The $E^{0'}$ obtained for both control plastocyanin and nitrotyrosine-plastocyanin using 1,1'-bis(hydroxymethyl)ferrocene as a mediator was +370 mV vs. a normal hydrogen electrode. Thus modification at Tyr-83 does not appear to affect directly the environment of the copper site. This is consistent with the lack of change observed in the visible absorption and CD spectra of oxidized plastocyanin upon nitration with tetranitromethane (see below).

The rate of P-700$^+$ reduction was measured at pH 6.0 for both control plastocyanin and nitrotyrosine-plastocyanin. At this pH nitration had no effect on either the K_m for the plastocyanin or the Mg^+ requirement. The phenolic hydroxyl group for the nitrotyrosine is protonated at pH 6.0. However, at pH 8.0 the K_m for the Mg ion requirement increased from 3.0 to 6.0 mM upon nitration of the plastocyanin. Similar results were obtained for pH 9.0 (not shown). The phenolic hydroxyl group is partially deprotonated at pH 8.0 and completely deprotonated at pH 9.0 (see below). These results are consistent with the need for shielding the additional negative charge [20].

The second-order rate constant for the reaction of Cyt *f* with plastocyanin was determined to be $5 \cdot 10^7$ $M^{-1} \cdot s^{-1}$. This rate is in good agreement with that of Takenaka and Takabe [21] who reported a rate of $1.1 \cdot 10^8$ $M^{-1} \cdot s^{-1}$. Rates of Cyt *f* oxidation were identical for control plastocyanin and nitrotyrosine-plastocyanin. These results show that formation of the nitrotyrosine-plastocyanin derivative does not cause a major alteration in the plastocyanin structure. In addition, the nitration of Tyr-83 does not alter the binding of plastocyanin with either of its reaction partners Cyt *f* and P-700. Since the plastocyanin activity is not affected, the nitro modification of Tyr-83 is an ideal spectroscopic probe for determining changes in the environment of the plastocyanin 'east face'.

The effect of oxidation state and ethylenediamine chemical modification of the pK_a of the nitrotyrosine chromophore. Previously, redox-state-dependent changes in the plastocyanin conformation which were thought to be electrostatically driven by the change in charge on the copper atom have been observed [11,12]. If this is true, there should be a change in the electric field around Tyr-83 which can be monitored by determining the pK_a of the nitrotyrosine chromophore as a function of redox state. This was accomplished by varying the pH between 6.0 and 9.5 and determining its effect on the nitrotyrosine-plastocyanin absorbance at 360 and 428 nm. These are the wavelength absorption maxima for the protonated and unprotonated forms of nitrotyrosine, respectively. The spectra for both the protonated and deprotonated froms are the same as those observed by Davis and San Pietro [14].

The reduced and oxidized forms of nitrotyrosine-plastocyanin exhibited pK_a vlaues of 8.6 and 8.3, respectively. These were both much higher than the pK_a of 7.0 observed for the model tyrosine tripeptides, which were used as a control (see also Ref. 13). This indicates that Tyr-83 is in a very negatively charged environment, consistent with its location near residues Nos. 42–45 and 59–61.

Furthermore, the environment of Tyr-83 becomes less negative upon oxidation of the plstocyanin molecule, consistent with the increase in positive charge at the copper site. If the high pK_a of both oxidized and reduced plastocyanin is due to the presence of these nearby negative charges, then chemical modification of these charges with ethylenediamine should change the pK_a of the nitrotyrosine. Chemical modification with ethylenediamine and a water-soluble carbodiimide replaces negative charges with positive charges [13,15,22]. This results in a mixture of forms in which those containing 1–3 molecules of ethylenediamine per plastocyanin dominate, changing the charge character of the negative patch. Ethylenediamine modified nitrotyrosine-plastocyanin exhibited a pK_a for its nitrotyrosine chromophore of 8.1 and 7.75 for its reduced and oxidized forms, respectively. Therefore, the high pK_a value for nitrotyrosine-plastocyanin must be due in part to the effect of these nearby negatively charged residues. Secondary effects due to changes in the conformation of plastocyanin upon ethylenediamine modification cannot be ruled out. The pK_a of denatured (*apo*-)nitrotyrosine-plastocyanin was 7.2 similar to the model compounds. Thus the 3-dimensional structure near Tyr-83 is also an important factor in maintaining the high pK_a in

nitrotyrosine-plastocyanin.

Near-ultraviolet absorption and CD spectra. Fig. 4A shows the near-ultraviolet absorption spectra of oxidized and reduced nitrotyrosine-plastocyanin and control plastocyanin at pH 6.0. Oxidized and reduced control plastocyanin both exhibit transitions between 250–290 nm. The transitions at 278 nm and 284 nm are attributed to tyrosine residues, while the transitions between 250–270 nm are attributed to phenylalanine residues [23]. The extinction in the 250–290 nm region increases upon reduction as previously described [11,24]. No more than 20% of the increase in extinction at 278 nm and none of that at 260 nm can be due to charge-transfer transitions [24]. When protonated, the nitrotyrosine chromophore in nitrotyrosine-plastocyanin exhibits transitions at 360 nm and 270 nm. The 360 nm transition is very broad with a bandwidth at half-maximum of approx. 60 nm. Upon deprotonation this transition shifts to 428 nm.

Fig. 4B shows the absorption difference spectra (nitrotyrosine-plastocyanin minus control plastocyanin) of both the oxidized and reduced forms. Changing the redox state does not alter the extinction of the 360 nm nitrotyrosine transition. However, there is a small change at approx. 270 nm. There are several possible explanations which are not mutually exclusive: (1) the extinction of the 270 nm nitrotyrosine transition changes upon reduction of the copper atom; (2) the extinction due to Tyr-83 (in control plastocyanin) changes upon reduction; (3) nitration at Tyr-83 alters the 278 nm extinction of the two remaining unmodified tyrosine residues (Nos. 70 and 80). The visible absorption spectrum (not shown) of oxidized nitrotyrosine-plastocyanin and control plastocyanin were identical. This indicates that the incorporation of a nitro group at Tyr-83 does not directly perturb the geometry of the oxidized copper center.

Fig. 5A shows the near-ultraviolet CD spectra of oxidized and reduced control plastocyanin and nitrotyrosine-plastocyanin. Control plastocyanin exhibits positive ellipticity at approx. 252 nm in the phenylalanine spectral region and negative ellipticity at approx. 280 nm in the tyrosine spectral region. Upon reduction there are significant changes in the near-ultraviolet CD spectra which have been previously discussed in detail [11,24]. In addition, there are small positive CD bands of 300–340 nm which are most likely due to charge-transfer transitions, possibly involving the histidine ligands [24].

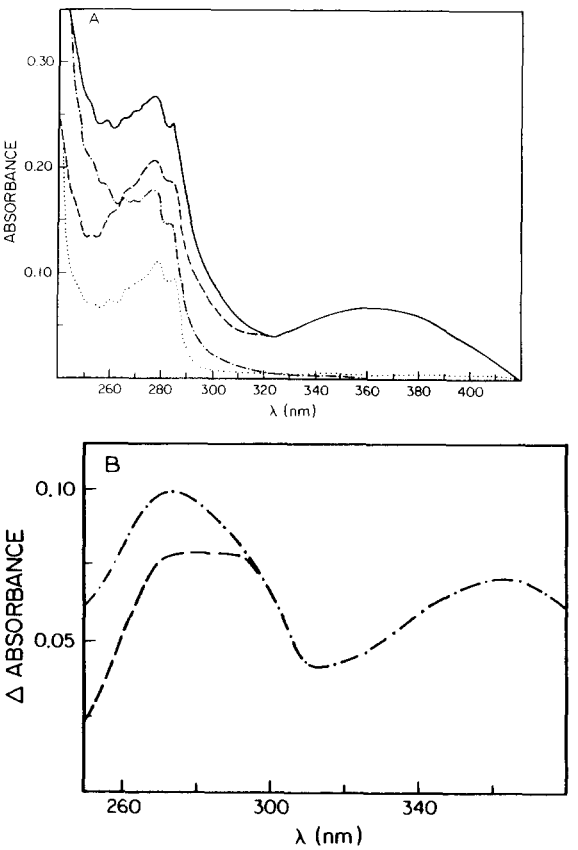

Fig. 4. (A) Near-ultraviolet absorption spectra of control and nitrotyrosine-plastocyanin as a function of oxidation state. Near-ultraviolet absorption spectra were determined for oxidized and reduced control and nitrotyrosine-plastocyanin suspended in 10 mM sodium succinate (pH 6.0). The absorbance spectra were normalized to 20 μM concentrations of plastocyanin. Other conditions were as described in the Materials and Methods section. ·–·–·, reduced control plastocyanin; ·····, oxidized control PC; ———, reduced nitrotyrosine-plastocyanin; — — —, oxidized nitrotyrosine-plastocyanin. (B) Near-ultraviolet difference absorption spectra for oxidized nitrotyrosine-plastocyanin minus oxidized control plastocyanin and reduced nitrotyrosine-plastocyanin minus reduced control plastocyanin. Conditions were as described for Fig. 4 and in the Materials and Methods section. — — —, reduced nitrotyrosine-plastocyanin minus reduced control plastocyanin; ·–·–·, oxidized nitrotyrosine-plastocyanin minus oxidized control plastocyanin.

Nitrotyrosine-plastocyanin has a broad negative CD band centered at 340 nm with a bandwidth at

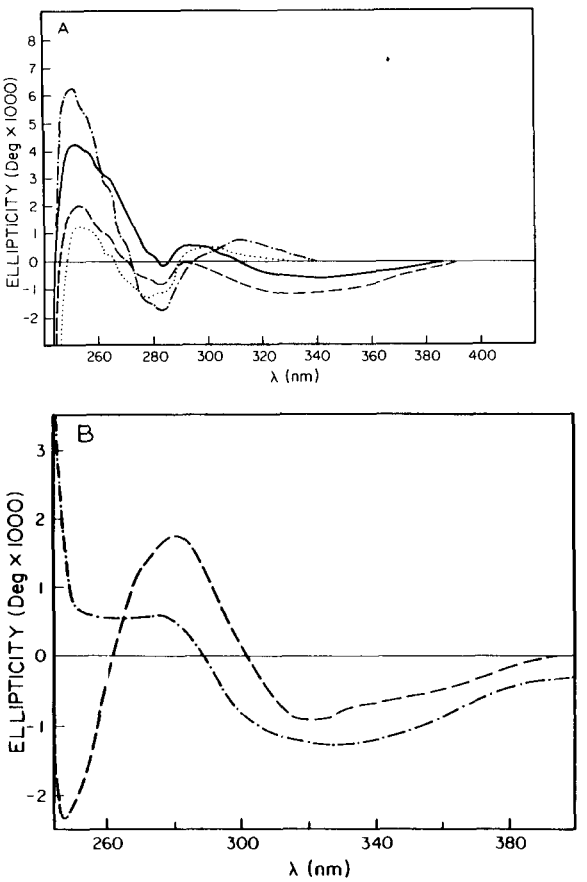

Fig. 5 (A) Near-ultraviolet CD spectra for control and nitrotyrosine-plastocyanin as a function of oxidation state. Control and nitrotyrosine-plastocyanin were incubated in 10 mM sodium succinate (pH 6.0). Ellipticities were normalized to 20 μM concentrations of plastocyanin. Other conditions were as described in the Materials and Methods section. ———, reduced nitrotyrosine-plastocyanin; ·–·–·, reduced control plastocyanin; — — —, oxidized nitrotyrosine-plastocyanin; ·····, oxidized control plastocyanin. (B) Near-ultraviolet difference CD spectra for oxidized nitrotyrosine-plastocyanin minus oxidized control plastocyanin and reduced nitrotyrosine-plastocyanin minus reduced control plastocyanin. Conditions were as described for Fig. 5 and in the Materials and Methods section. — — —, reduced nitrotyrosine-plastocyanin; ·–·–·, oxidized nitrotyrosine-plastocyanin minus oxidized control plastocyanin.

half-maximum of approx. 60 nm. This CD band can only be due to the nitrotyrosine chromophore, since it is not observed in the control plastocyanin spectra. The ellipticity at 340 nm increases upon oxidation. The ellipticity of a transition is dependent upon both its oscillatory and rotatory strength, while the extinction of a transition is dependent only upon its oscillatory strength. Since the ellipticity of the nitrotyrosine transition varies with plastocyanin redox state, the environment of Tyr-83 must be sensitive to the charge on the copper atom. The center of this CD band is blue shifted 20 nm from the center of the main absorption band of nitrotyrosine. One possible explanation is multiple transitions. If there were two close lying overlapping transitions of unequal oscillatory and rotatory strength, then one would not expect the absorption and CD spectra to exhibit the same maxima. Preliminary results concerning the pH dependence of the CD bands (not shown) favor this interpretation. The visible CD spectrum of oxidized plastocyanin was not altered by treatment with tetranitromethane providing further evidence that the geometry of the oxidized copper center was not perturbed.

Fig. 5B shows the difference CD (nitrotyrosine-plastocyanin minus plastocyanin) for both the oxidized and reduced forms. In addition to the spectral changes at approx. 340 nm, there are also changes in the difference CD spectra in the aromatic region usually associated with transitions due to phenylalanine and tyrosine residues. However, these changes are difficult to interpret. There is a positive extremum in the difference CD spectrum at 280 nm which is probably due to a higher energy nitrotyrosine transition. These results also suggest that the environment of Tyr-83 is dependent upon the redox state of the plastocyanin molecule.

Discussion

Nitration of Tyr-83 in spinach plastocyanin does not affect either the reaction kinetics of plastocyanin with Cyt *f* and P-700$^+$ or the plastocyanin midpoint redox potential. Thus it is an ideal spectroscopic probe for monitoring changes in the environment of Tyr-83. Previously, it was shown that the local environment of Tyr-83 is sensitive to the plastocyanin redox state [12]. The results of the pK_a study indicate that the environment of Tyr-83 is sensitive both to nearby negative charges and to the redox state of the copper center. This can be interpreted as either a direct electrostatic effect or an electrostatically driven conformational change

in the plastocyanin molecule. This latter interpretation is supported by the results of our previous study of the fluorescence of control and nitrotyrosine-plastocyanin [12] which showed a decrease in the relative quantum yield for the fluorescence of Tyr-83 upon oxidation of the protein. This was interpreted as a decrease in the hydrophobicity of the environment of Tyr-83 [26]. However, purely electrostatic effects cannot be distinguished from changes in the plastocyanin tertiary conformation.

There are two possible explanations for the observed change in the ellipticity of the nitrotyrosine transition with plastocyanin redox state: (1) changing the charge on the copper atom directly perturbs the environment near Tyr-83 through an electrostatic mechanism; (2) changing the charge on the copper atom induces a change in the plastocyanin tertiary conformation which is 'felt' at Tyr-83. According to the first explanation, changing the electrostatic field due to the charge on the copper atom will alter the asymmetric electric field applied at the nitrotyrosine. This in turn will alter the optical activity of the nitrotyrosine transitions through the one-electron mechanism. The one-electron mechanism of optical activity is a state mixing mechanism whereby an electrically allowed transition is mixed with a magnetically allowed transition in the presence of an asymmetric static perturbing field [25]. An asymmetric physical twist can also produce the same effect. Both transitions become optically active with opposite polarity. According to the second explanation, the ellipticity of the nitrotyrosine transitions can be affected by either the one-electron mechanism or by the coupled oscillator mechanism of optical activity. The coupled oscillator mechanism is obviously dependent upon the precise geometry of the nitrotyrosine chromophore and any other chromophore to which it is coupled. Presently, it is impossible to distinguish between the two possible explanations. Considering the size of the plastocyanin molecule, it is likely that changing the plastocyanin tertiary conformation will also affect the electrostatic field at the nitrotyrosine. In addition, changing the charge at the copper atom will also probably perturb the local conformation of the nitrotyrosine chromophore.

The conformational changes required could be quite small, but still be significant with respect to the interaction of plastocyanin with its reaction partners. The change in charge at the copper center could cause a subtle conformational change in the Tyr-83 region which could cause binding or release of the reaction partner. This would be particularly useful for a mobile electron carrier. Conversely, binding of the reaction partner could alter the electrostatics of the surface producing a conformational change which could alter the electron transport properties of plastocyanin. This would be analogous to the effect of ethylenediamine modification which affects not only the pK_a of the nitrotyrosine but also the plastocyanin near-ultraviolet absorption and CD spectra [11] and midpoint redox potential [15,22]. Protonation of the negative charges on the plastocyanin 'east face' also influence the midpoint redox potential and electron transport properties of the copper center of plastocyanin [1,10].

Conclusions

Plastocyanin treated with tetranitromethane was nitrated at a single location, Tyr-83. The pK_a of the nitrotyrosine indicated that the environment of Tyr-83 is highly negatively charged due in part to the nearby negative 'patch' (residues Nos 42–45 and 59–61). The nitrotyrosine pK_a was also sensitive to the plastocyanin redox state, becoming more negative upon reduction. These changes can be interpreted as either a direct electrostatic effect due to the copper atom or an electrostatically driven conformational change.

Acknowledgments

We thank Dr. J. Duane of the Engineering Graphics Department at the Ohio State University for help in developing the computer graphics programs. We would also like to thank S.L. Ketchner, C.H. Lee and D.G. Sanderson for their invaluable assistance in various aspects of this research. This research was supported in part by Grant R01 GM 30560 from the National Institutes of Health.

References

1 Chapman, S.K., Watson, A.D. and Sykes, A.G. (1983) J. Chem. Soc. Dalton Trans. 1983, 2543–2548

2 Boulter, D., Haslett, B.G., Peacock, D., Ramshaw, J. and Scawen, M.D. (1977) Int. Rev. Biochem. 13, 1–39
3 Olsen, L.F. (1982) Biochim. Biophys. Acta 682, 482–490
4 Takano, M., Takshashi, M. and Asada, K. (1982) Arch. Biochem. Biophys. 218, 369–375
5 Freeman, H.C. (1981) Coord. Chem. 21, 29–51
6 Guss, J.M. and Greeman, H.C. (1983) J. Mol. Biol. 169, 521–563
7 Garrett, T.P.J., Clingeleffer, D.J., Guss, J.M., Rogers, S.J. and Freeman, H.C. (1984) J. Biol. Chem. 259, 2822–2825
8 Cookson, D.J., Hayes, M.T. and Wright, P.E. (1980) Nature 282, 682–683
9 Farver, O., Shahak, Y. and Pecht, I. (1982) Biochemistry 21, 1885–1890
10 Chapman, S.K., Sanemasa, I. and Sykes, A.G. (1983) J. Chem. Soc. Dalton Trans. 1983, 2549–2553
11 Draheim, J.E., Anderson, G.P., Pan, R.L., Rellick, L.M., Duane, J.W. and Gross, E.L. (1985) Arch. Biochem. Biophys. 237, 110–117
12 Gross, E.L., Anderson, G.P., Ketchner, S.L. and Draheim, J.E. (1985) Biochim. Biophys. Acta 808, 437–447
13 Means, G.E. and Feeney, R.E. (1971) Chemical Modification of Proteins, Holden-Day, San Francisco, CA
14 Davis, D.J. and San Pietro, A. 91979) Anal. Biochem. 95, 254–259
15 Burkey, K.O. and Gross, E.L. (1981) Biochemistry 20, 5495–5499
16 Matsuzaki, E., Kamimura, Y., Yamasaki, T. and Yakashiji, E. (1975) Plant Cell Physiol. 16, 237–246
17 Shiozawa, J.A., Alberte, R.S. and Thornber, J.P. (1974) Arch. Biochem. Biophys. 165, 388–397
18 Sanderson, D.G. and Anderson, L.B. (1985) Anal. Chem., in the press
19 Duane, J.W., Rellick, L.M. and Gross, E.L. (1985) Eng. Design Graphics J., in the press
20 Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 74, 174–181
21 Takenaka, K. and Takabe, T. (1984) J. Biochem. 96, 1813–1821
22 Burkey, K.O. and Gross, E.L. (1982) Biochem. 21, 5886–5890
23 Donovan, J.W. (1979) in Physical Principles and Techniques of Protein Chemistry (Leach, S.J., ed.), Part A, pp. 102–170, Academic Press, New York
24 Draheim, J.E., Anderson, G.P., Duane, J.W. and Gross, E.L. (1986) Biophys. J., in the press
25 Moscowitz, A. (1965) in Modern Quantum Chemistry (Sinanoglu, O., ed.), Academic press, New York
26 Gaincotti, V., Quadrifoglio, F., Cowgill, R.W. and Crane-Robinson, C. (1980) Biochim. Biophys. Acta 624, 60–65