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Characteristics of Cu deficiency-induced inhibition of photosynthetic electron transport in spinach chloroplasts

Magdolna Droppa [a], Jiři Masojidek [a,*], Zsuzsanna Rózsa [a], Adam Wolak [b,**], László I. Horváth [b], Tibor Farkas [c] and Gábor Horváth [a]

[a] *Institute of Plant Physiology,* [b] *Institute of Biophysics and* [c] *Institute of Biochemistry, Biological Research Center, Szeged (Hungary)*

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In the present work we studied the effect of Cu deficiency on spinach chloroplasts. We found that in spinach the electron transport was inhibited as reported previously for sugar beet (Droppa, M., Terry, N. and Horváth, G. (1984) Proc. Natl. Acad. Sci. USA (1984) 81, 2369–2373). The breakpoint of the Arrhenius plot of the whole electron-transport activity was shifted from +6°C to +12°C in Cu-deficient chloroplasts. A similar effect could be observed with a spin-labelled probe, when the rotational correlation time was plotted vs. the reciprocal temperatures. This indicates that the membrane fluidity might be changed by Cu deficiency. The lipid/protein ratios were similar in both control and deficient chloroplasts. On the other hand, the saturated/unsaturated ratio of phosphatidylcholine (PC), phosphatidylglycerol (PG) and sulpholipids (SL) was increased but that of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) decreased. We conclude that Cu deficiency does not change the entire membrane fluidity but rather the lipid composition of the microenvironment of some electron-transport components. The inhibition of Photosystem II electron transport in Cu-deficient chloroplasts was characterized by thermoluminescence and 2-dimensional gel electrophoresis. It was found that Cu deficiency shifted the main peak of the glow curve from +18°C to +8°C, similar to that of DCMU-poisoned chloroplasts. Two apoproteins of the 29 kDa polypeptide disappeared in Cu-deficient chloroplasts which indicates that this polypeptide has a regulatory role in ensuring the normal electron flow between Q_A and Q_B.

* Permanent address: Institute of Microbiology, CSAV, 379 81 Trebon, Czechoslovakia.

** Permanent address: Department of Physics, Jagellonian University, Kraków, Poland.

Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DCIP, dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DQH_2, duroquinol; LHC, light-harvesting chlorophyll *a*/*b*-containing complex; *p*BQ, *p*-benzoquinone; PS I and II, Photosystem I and II; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TL, thermoluminescence; Q_A and Q_B, 'primary' and 'secondary' acceptors of PS II; 16-SASL, 16-(4′, 4′-dimethyloxazolidine *N*-oxyl) stearic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; SL, sulpholipids; Mes, 4-morpholineethanesulfonic acid.

Correspondence: G. Horváth, Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, Szeged, H-6701 Hungary.

Introduction

Copper is known to be an essential microelement for the development of most algae and higher plants [1,2]. The involvement of Cu in photosynthesis was first suggested by Green et al. [3] who found that Cu-chelating agents markedly inhibited the photosynthetic CO_2 fixation. The discovery of plastocyanin, the Cu-containing protein of chloroplasts, provided a plausible explanation for the Cu requirement of the photosynthetic electron transport [4–7]. Plesnicar and Bendall [8] concluded that plastocyanin is the only Cu-containing compound that is involved in photosynthetic electron transport. This conclusion was based on their finding that the total amount of Cu present in chloroplast of barley was equal to that of plastocyanin Cu [8]. However, in many other plant species the Cu content of chloroplasts was significantly higher than the plastocyanin-associated Cu [8,9]. The role of this excess Cu has not been elucidated albeit that results indicate that Cu may have additional roles in photosynthetic electron transport.

Anderson et al. [10] found that a relatively high proportion of Cu was present in the $10000 \times g$ particles of digitonin-fractionated spinach chloroplasts. Subsequent ESR studies demonstrated that 0.8 atom Cu/100 molecules of chlorophyll was present in isolated PS II particles of spinach [11]. Holdsworth and Arshad [12] succeeded in isolating a Cu-Mn-pigment-protein complex which was part of PS II and might be involved in the water-splitting reaction.

Other research with Cu chelators also suggests that Cu may have additional roles in the photosynthetic electron-transport chain. Salicylaldoxime, a Cu chelator used by Trebst [13] and Katoh and San Pietro [14], proved to be ineffective as a Cu chelator of plastocyanin in PS I but it did inhibit a site in PS II [15]. Later, Barr and Crane [16] showed that salicylaldoxime interacted preferentially with Mn on the water-splitting side of PS II, implying that Cu was probably not involved. The same authors, however, found that other Cu chelators did inhibit PS II reactions [16] suggesting that Cu may influence electron transport at sites additional to plastocyanin. The specific requirement for Cu in the restoration of PS II transport in Tris-washed potato tuber chloroplasts was also demonstrated [17]. Measuring the activity of various fractions of the photosynthetic electron transport in Cu-deficient chloroplasts indicates that Cu-deficiency inhibits not only PS I reaction but PS II electron transport as well [9,18,19].

Very recently, using various levels of Cu deficiency in sugar beet, it was shown that Cu influenced the photosynthetic electron transport at two new sites in addition to plastocyanin [20,21]. (1) Mild Cu deficiency inhibited electron transport between PS II and PS I. It was assumed that the fluidity of the thylakoid membranes might be altered by the decreased Cu content. (2) At severe deficiency PS II electron transport was also inhibited; Cu was found to be a constituent of PS II and its absence caused the loss of two PS II polypeptides in the chloroplast membranes. Since these observations were obtained with sugar beet chloroplasts only, it is necessary to verify them with further experiments carried out on other plant species. On the other hand, further characterization of these new inhibitory sites may help us to understand the role of excess Cu in the photosynthetic membranes.

In the present work, we studied the effect of various levels of Cu deficiency on spinach chloroplasts. In agreement with our earlier results [20], mild Cu deficiency inhibited electron transport between the two photosystems prior to the PS I inhibition induced by plastocyanin depletion. We concluded that Cu deficiency changed the lipid microenvironment of some electron transport components. Severe Cu deficiency was found to inhibit PS II electron transport which was associated with the absence of the 29 kDa and 13.5 kDa polypeptides. Thermoluminescence characteristics of deficient chloroplasts indicate that the inhibition may be located on the acceptor side of PS II.

Materials and Methods

Plant Material. Spinach (*Spinacia oleracea* cv. Popeye) was cultivated hydroponically in growth chambers at 18°C/14°C, day/night temperatures and illuminated over 12 h/day. The plants were cultured for 2 weeks following planting in vermiculite with a nutrition solution containing 9 mM KNO_3/6 mM $Ca(NO_3)_2$/3 mM KH_2PO_4/

135 μM Fe-EDTA/63.9 μM H_3BO_3/13.7 μM $MnCl_2$/1.15 μM $ZnSO_4$/0.48 μM $CuSO_4$/0.17 μM Na_2MoO_4. After 2 weeks, the plants were transferred to the nutrition medium without Cu and grown for 2 or 4 weeks corresponding to mild and severe Cu deficiency, respectively.

Chloroplasts preparation. Leaves were homogenized at 0°C in a medium containing 0.4 M D-sorbitol; 10 mM NaCl; 5 mM $MgCl_2$; 2 mM EDTA; 1 mM $MnCl_2$; 2 mM ascorbate, 0.4% bovine serum albumin and 50 mM Mes (pH 6.5) [22]. The brei was filtered through four layers of nylon cloth, while chloroplasts were sedimented by centrifugation at 3000 × *g* for 5 min. The chloroplasts were washed once in the isolation medium and resuspended in a medium containing 0.4 M D-sorbitol/10 mM NaCl/5 mM $MgCl_2$/2 mM EDTA/1 mM $MnCl_2$/0.4% bovine serum albumin/50 mM Hepes (pH 7.5).

Measurement of photosynthetic activity. The rate of photosynthetic oxygen evolution and uptake was measured using a Clark-type electrode (Rank Browths., Cambridge, UK) in a temperature-controlled cuvette under saturating white light [23]. The assay medium contained 0.1 M D-sorbitol/4 mM $MgCl_2$/20 mM NaCl/10 mM K_2HPO_4/2 mM EDTA/50 mM Hepes (pH 7.5) and chloroplasts equivalent to 50 μg chlorophyll per sample [22].

Different parts of the electron-transport chain were studied by addition of various electron donors and acceptors: 5 mM $K_3Fe(CN)_6$ or 0.1 mM methyl viologen together with 2 mM NaN_3 were used for whole-chain electron transport. PS II electron transport was measured by using 0.25 mM pBQ. For the PS I reaction, electron transport was monitored by using either 40 μM DCIP and 2 mM ascorbate or 125 mM duroquinol, depending on the system used. Duroquinone was reduced to duroquinol by $NaBH_4$ according to Izawa [24]. The rate of uncoupled electron flow was measured by adding 10 mM methylamine to the samples.

Thermoluminescence was measured in an apparatus similar to that described by Tatake et al. [25]. 0.3 ml aliquots of chloroplast suspension containing 50 μg chlorophylls were excited by white light during a period of continuous cooling from +20°C to −80°C for 90 s or by a series of xenon flashes (1 Hz) at +2°C. After flash excitation, the samples were quickly cooled down to −30°C. TL measurements were performed at a heating rate of 20°C/min.

Electron-spin resonance studies. 200 μl aliquots containing 0.4 mg chlorophyll were labelled by the addition of 2 μl ethanolic solution of 16-(4′,4′-dimethoxyloxazolidine *N*-oxyl) stearic acid, 16-SASL (corresponding to 20 μg label) and vigorously mixed with a Vortex mixer for 5 min. The membrane suspension was transferred into a 100 μl capillary and pelleted down in a bench centrifuge. ESR spectra were recorded with a JEOL JES-PE-1X spectrometer using 100 kHz modulation technique. During measurements the sample capillary was thermostatically controlled in a nitrogen gas flow system. At the depth of C-16 carbon atoms, no significant signal reduction was observed and, therefore, the rotational correlation time was calculated from the uncorrected peak-to-peak amplitudes and widths as described by Schreier et al. [26].

Extraction and analysis of lipids. Lipids were extracted according to Folch et al. [27] following the inactivation of phospholipase D by boiling the probes in isopropyl alcohol for 3 min. The extract, recovered in chloroform containing 0.05% butylated hydroxytoluene, was quantitatively spotted onto Silicagel 60 plates (E. Merck, Darmstadt, F.R.G.) to separate the polar lipids in acetone/benzene/water (91 : 30 : 8, v/v) as solvent. The plates were then sprayed with 0.5% 8-anilinonaphthalene sulphonic acid in methanol and viewed under UV light. Authentic standards obtained from the Serdary Research Laboratories (London, Ontario, Canada) and from Sigma were used for identification. For quantitation, a known amount of heptadecanoic acid was added to each spot before removing them into methylation ampullae. Transmethylation took place in the presence of 5% hydrochloric acid in methanol under inert atmosphere at 80°C. A JEOL 20K gas chromatograph equipped with dual flame ionization detector and connected to a Packard 603 type integrator served to resolve the fatty acid methyl esters. SP 2330 on 100–120 mesh Chromosorb V AW (Supelco, Bellefonte, CA) filled in 2 mm long stainless steel columns (3 mm, i.d.) was used as stationary phase. The runs were made in dupli-

cate. The standard error was less than 2% in the case of the major fatty acids and around 5% in the case of the minor ones.

Two-dimensional gel electrophoresis of thylakoids. The isolated chloroplasts were disrupted by osmotic shock in 25 mM Tris-HCl buffer (pH 7.5) containing 25 mM NaCl/5 mM $MgCl_2$/0.06 M mercaptoethanol and centrifuged at 18 000 × *g* for 10 min. The sediment was washed once with Tris-HCl buffer and twice with water.

The two-dimensional gel electrophoresis was carried out using O'Farrell's technique [28] with several modifications [29,30] including Nonidet P-40 (first dimension) and LiDS (second dimension) solubilization instead of Triton X-100 and SDS, respectively, and reverse electrofocusing. In the first dimension, proteins were separated by electrofocusing on the rod gels (2 mm inner diameter, length 75 mm) with following regime: at 50 V for 30 min; 100 V for 1 h; 200 V for 1 h; 400 V for 14 h and 800 V for 1 h without prefocusing. 0.2% ethylenediamine was used instead of 0.02 M NaOH as the lower reservoir buffer. Aliquots of the sample containing about 80–120 μg of proteins were layered on the rod gels. In the second dimension DMAPD was used instead of TEMED and 10–20% gradient gel instead of homogeneous one. The parameters of the gel were: thickness, 1.5 mm; width, 130 mm and length, 85 mm for separating gel and 20 mm length for the stacking gel. After electrophoresis, the slabs were stepwise washed with 50% methanol; 25% ethanol/10% acetic acid; 50% methanol/0.1% glutaraldehyde and four times with water. Silver staining procedure was used for visualization of the protein spots as described by Wray et al. [31].

Results

Fig. 1 shows the Arrhenius plots of the photosynthetic electron-transport activity, measured from $H_2O \rightarrow$ methyl viologen, in control and Cu deficient chloroplasts of spinach. As seen, the control exhibits the breakpoint at around +5°C, while in Cu-deficient chloroplasts the breakpoint is shifted up to around +13°C. The whole electron-transport activity in deficient chlrooplasts was significantly lower (by 50–65%) than that of the control in the entire temperature range measured.

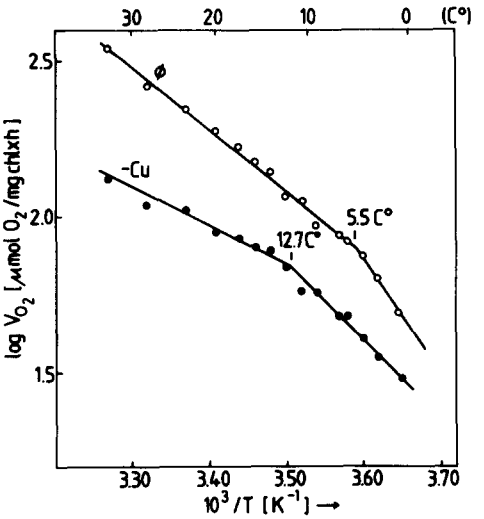

Fig. 1. Arrhenius plots of the photosynthetic electron-transport activity in control (○) and Cu-deficient chloroplasts (●). Whole electron transport was measured from $H_2O \rightarrow$ methyl viologen at various temperatures. The rate of electron transport was measured in the presence of 10 mM methylamine to obtain the maximal activity. Other measuring conditions as given in Materials and Methods.

It should be noted that the activity of PS II (measured from $H_2O \rightarrow$ pBQ) and PS I (measured from DCIP → methyl viologen) at +25°C was only decreased by 15–20%, respectively. This is in agreement with the earlier observation obtained with sugar beet chloroplasts at mild Cu deficiency [20].

Since the breakpoint in the Arrhenius plots of the electron-spin labelling and that of the electron-transport activity are considered as two different probes of the liquid-crystal/gel phase transition of the membrane [32–36], we assumed that similar shift of the breakpoint can be observed in both measurements. For measuring the bulk fluidity of the chloroplast membrane, the C-16 positional isomer of spin-labelled fatty acid (16-SASL) was chosen because this probe selectively monitors the hydrocarbon core [37]. The Arrhenius plots of the rotational correlation time data of Cu-deficient and control samples show a break at +6°C and +12°C, respectively (Fig. 2). The shift of discontinuity obtained with ESR probe is indeed in good agreement with the shift observed in the electron-transport activity (cf. Fig. 1 and Fig. 2).

It is generally accepted that the change of the

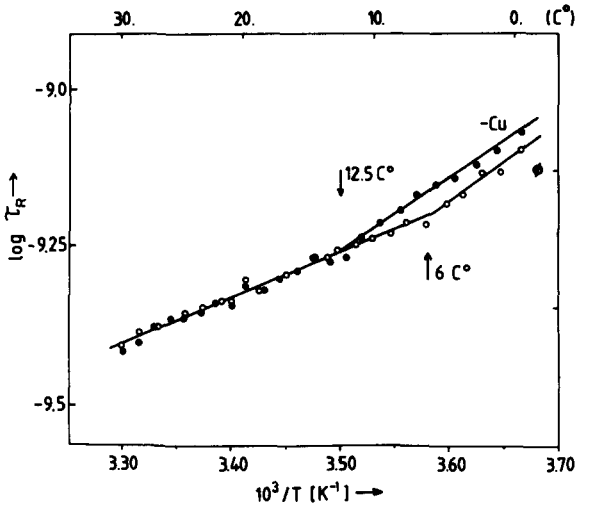

Fig. 2. Arrhenius plots of the rotational correlation time of C-16 spin-labelled fatty acids (16-SASL) in control (○) and Cu-deficient chloroplasts (●). Measuring conditions are described in Materials and Methods.

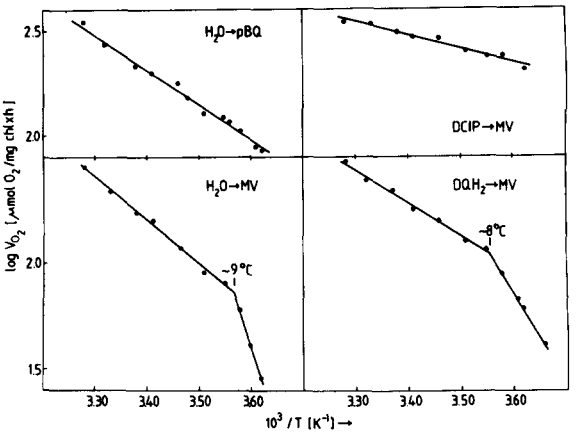

Fig. 3. A comparison of temperature dependence of the activity of various part of the photosynthetic electron-transport chain measured in control chloroplasts in the presence of 10 mM methylamine. Measuring conditions are as given in Materials and Methods. MV, methyl viologen.

membrane fluidity might be a result of the altered lipid/protein ratio [38] or induced by the decrease of the double bonds of fatty acyl constituents of the lipids in the membrane [39]. Table I shows the distribution of the individual lipid classes and the lipid/protein ratio in both control and Cu-deficient chloroplasts. It is obvious from Table I that the lipid/protein ratio was unchanged, albeit that the distribution of the individual lipids was altered by Cu deficiency. Interestingly, the amounts of phospho- and sulpholipids were preferentially increased, but the galactolipids were decreased in the Cu-deficient chloroplasts. The fatty acid com-

TABLE I

EFFECT OF Cu DEFICIENCY ON THE LIPID COMPOSITION OF SPINACH CHLOROPLASTS

Percent distribution	Control chloroplasts	Cu-deficient chloroplasts
Phosphatidylcholine (PC)	6.0	6.7
Phosphatidylglycerol (PG)	10.7	11.7
Phosphatidylinositol (PI)	7.3	11.9
Sulpholipids (SL)	7.2	9.9
Monogalactosyldiacylglycerol (MGDG)	51.6	42.9
Digalactosyldiacylglycerol (DGDG)	23.6	16.7
Total polar lipids (μg/mg Chl)	975.4	906.2
Lipid/protein ratio	0.095	0.105

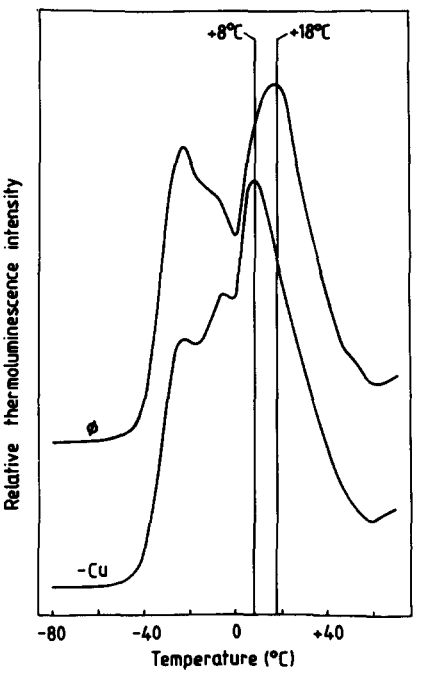

Fig. 4. The glow curve of control and Cu-deficient chloroplasts. Measuring conditions as described in Materials and Methods.

TABLE II

RELATIVE PROPORTION OF VARIOUS FATTY ACIDS IN CONTROL AND Cu-DEFICIENT CHLOROPLASTS

Fatty acids	PC		PG		SL		MGDG		DGDG	
	control	− Cu	control	− Cu	control	− Cu	control	− Cu	control	− Cu
16 : 0	25.3	31.0	21.5	24.3	42.6	51.5	6.5	1.7	18.4	14.3
16 : 1	tr	tr	28.6	32.7	tr	5.6	tr	tr	tr	tr
18 : 0	tr	3.3	2.6	1.9	5.3	4.2	3.4	1.0	2.5	1.1
18 : 1	19.3	12.2	3.7	2.8	6.1	3.5	24.0	31.5	8.1	6.8
18 : 2	29.8	18.4	7.9	5.5	8.1	10.8	.23	1.6	5.1	3.5
18 : 3	25.5	35.1	35.7	32.8	37.7	34.8	63.8	64.2	66.0	74.3
$\frac{\text{sat}}{\text{unsat}}$	0.34	0.52	0.32	0.36	0.92	1.02	0.11	0.03	0.26	0.18

position of the various lipid classes is shown in Table II. The saturated/unsaturated ratio of both MGDG and DGDG was dramatically decreased; however, this ratio was increased in both phospho- and sulpholipids.

To locate the site of the Arrhenius break within

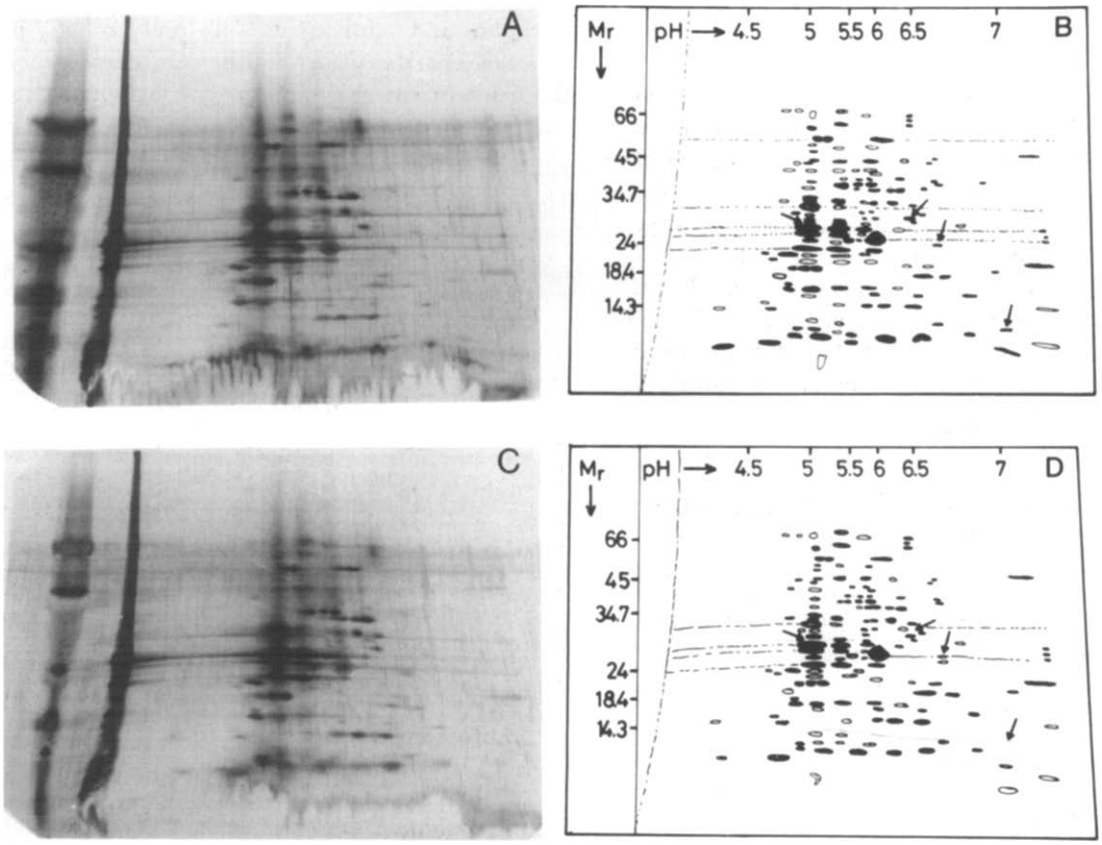

Fig. 5. Thylakoid membrane polypeptides of control (a, b) and Cu-deficient chloroplasts (c, d) separated by two-dimensional gel electrophoresis (isoelectric focusing for the first dimension and linear gradient LiDS-polyacrylamide gel for the second dimension). Figs. a and c show the original electrophoretograms, b and c illustrate the schematic maps.

the electron-transport chain, the various parts of electron transport of control chloroplasts were measured as a function of temperature (Fig. 3). The Arrhenius plots of the PS II activity, measured from $H_2O \rightarrow$ pBQ, and PS I activity, measured from DCIP $\rightarrow$ methyl viologen, do not show any breakpoints in the temperature range between 0°C and +32°C. The electron flow from DQH_2 $\rightarrow$ methyl viologen, however, shows a breakpoint at around +8°C similarly to that of whole electron transport measured from $H_2O \rightarrow$ methyl viologen (+9°C). This indicates that the section of the electron-transport chain responsible for the break in the Arrhenius plot is located between the electron donation site of DCIP and DQH_2.

In our experiments, thermoluminescence (TL) was chosen as a tool to characterize the Cu-deficiency induced decrease in the electron transport of PS II (Fig. 4). The control chloroplast exhibits the main band of the glow curve at +18°C with two additional bands at −25°C and −10°C. It was shown recently that the band at −10°C is not related to the photosynthetic activity of chloroplasts [40]. In Cu-deficient chloroplasts the main band appears at around +8°C and the negative band at −25°C markedly decreased. In flash-excited Cu-deficient chloroplasts, the band at +8°C exhibited quadruple oscillation with the maxima at 2nd, 6th and 10th flashes (data not shown). This is in good agreement with the results described previously [41].

The effect of the Cu deficiency on the polypeptide composition of thylakoids was inspected with two-dimensional gel electrophoresis because this technique gives a much higher resolution than the one-dimensional electrophoresis [28] and with silver-staining results in about a 100-fold increase in sensitivity [31].

Fig. 5 shows the two-dimensional electrophoresis of the control (a, b) and Cu-deficient chloroplast (c, d). In all experiments, two apoproteins (p*K* 5.15 and 5.4) of the 29 kDa polypeptide as well as 12.6 kDa polypeptide (p*K* 6.95) were completely absent or dramatically reduced in the Cu-deficient chloroplasts. Some other small changes can also be observed on the presented electrophoretograms, for example, the apoproteins (p*K* 5.4) of the 42.5 and 22 kDa polypeptides, but these changes are not significant. It is interesting, however, that in all experiments two new spots appeared at 31.5 and 25.5 kDa with the p*K* value of 6.3 and 6.7, respectively. These might be considered as a premature form of the polypeptides eliminated by Cu deficiency.

Discussion

In the present work we have demonstrated that Cu deficiency affected the photosynthetic electron-transport activity of spinach chloroplasts in a similar way as previously found in sugar beet [20]. In Cu-deficient chloroplasts, the discontinuity of the Arrhenius plot of both electron transport and ESR spin labelling is shifted toward the higher temperatures with a similar extent which might indicate the occurrence of the liquid-crystal/solid phase transition of the membrane at higher temperature [34,42]. The change of the phase transition temperature in the membrane is generally attributed to two different phenomena: (a) changes in the fluidity characteristics of thylakoids can result by the alteration of the lipid/protein ratio within the membrane [38], and/or (b) by the length and degree of saturation of alkyl chains in fatty acids of constituent lipids [29,43]. The lipid/protein ratio was found to be unaltered by Cu deficiency despite an increase in sulpho- and phospholipids and a reduction in the galactolipids (Table I). The lipid analysis data show that Cu deficiency indeed increased the saturation of both phospho- and sulpholipids. On the other hand, the unsaturation level in fatty acids of both MGDG and DGDG shows an increase in Cu-deficient chloroplasts instead of the expected decrease. Since the polar lipid fraction of the membrane consists of approx. 70% of galactolipids (cf. Table I and Ref. 36), it is unlikely that the entire fluidity of the membrane would be changed by Cu deficiency. It is more reasonable to assume that the decreased unsaturation of phospho- and sulpholipid fraction might influence the local microenvironment of certain components of the electron-transport chain. This also indicates that the ESR spin labelling monitors mainly not the change of the entire fluidity of the membrane, but rather the specific labelling of certain individual lipid classes. This type of phase separation of the spin labelling was indicated previously [44].

Measuring the temperature dependence of the activity of various parts of the electron-transport chain, we found no discontinuity in the Arrhenius plots either in PS II, measured from $H_2O \rightarrow$ pBQ or in PS I measured from DCIP → methyl viologen. Since pBQ accepts electron from Q_B [24] and DCIP donates electron in the cytochrome *f*/PC region [24] it is obvious that the section of electron transport responsible for the Arrhenius break is located between the donation and acceptance of these molecules. As it is expected, the $DQH_2 \rightarrow$ methyl viologen reaction indeed exhibits the discontinuity of the Arrhenius plot. DQH_2 is known to donate electrons to the plastoquinone [45] thus it might be concluded that the mobility and the accessibility of the plastoquinone have been changed by Cu deficiency. The inhibition of the accessibility of the plastoquinone in PS II can be excluded, since the $H_2O \rightarrow$ pBQ is quite intact even when the $DQH_2 \rightarrow$ methyl viologen reaction is inhibited. To explain the discontinuity to the Arrhenius plot we assume that phospholipids (in which the unsaturation was only decreased by Cu deficiency) specifically ensure the mobility of the plastoquinone within the bilayer or they play a specific role in the binding of plastoquinone to the cytochrome *b/f* complex. This assumption seems to be supported by the observation of Chain who found that the addition of phosphatidylcholine stimulated the oxidoreductase activity of isolated cytochrome *b/f* complex [46]. This means that prior to the Cu-deficiency-induced plastocyanin depletion, slight decrease in the Cu content of the chloroplast (no more than 20–25% [20]) affects the electron transfer in the region of the plastoquinone and cytochrome *b/f* complex by modulating the lipid environments of these molecules.

Phospholipids were also found to be effective in stimulating the electron-transport activity of isolated PS II particles [47]. The importance of phospholipids in the regulation of PS II function might be one of the explanations of the decreased activity of PS II induced by Cu deficiency. On the other hand it has been shown previously that Cu itself is associated with PS II. Anderson et al. found two atoms Cu per 200 chlorophyll molecules in the $10000 \times g$ fraction of fractionated chloroplasts [10]. In isolated PS II particles Goldfeld and Khalilov [11] and Droppa et al. [20] also found 1.8 and 1.7 atom Cu per reaction centers, respectively. The latter demonstrated that in Cu-deficient sugar beet chloroplasts the removal of Cu was associated with the loss of the 29 kDa and 13.5 kDa polypeptides. It was assumed that two polypeptides might be associated with the two atom Cu present in PS II. This indicates that Cu deficiency affects rather directly the PS II activity. In spinach chloroplasts we also found that the 29 kDa and the 12.6 kDa (which very probably corresponds to the 13.5 kDa polypeptide in Ref. 20), were eliminated by Cu deficiency. Green and Camm [48] have found that a 29 kDa chlorophyll *a/b*-containing polypeptide (CP29) was coextracted with the LHC II and sedimented with LHC II in a sucrose gradient. They have hypothesized that CP29 is an internal antenna in PS II [49]. Very recently, Sibbald and Green demonstrated that 75% of Cu found in PS II was associated with LHC II [50]. On the basis of these observations, we assume that the 29 kDa polypeptide eliminated with Cu deficiency corresponds to the CP29 of PS II. The relationship of the 12.6 kDa polypeptide to the others and its function in the membrane is not known at this time.

The decreased activity of PS II was reflected by TL of Cu-deficient chloroplasts. TL originates from the charge recombination between positively charged donors and negatively charged acceptors of PS II [51]. In control chloroplasts the main band of the glow curve at +18°C and the band at −25°C are results of the charge recombination of the $S_3Q_B^-$ and $S_3Q_A^-$ redox pairs, respectively [41,52]. Both bands are inhibited by adding PS II inhibitors and a new band appears at around +5°C [41]. The main band in Cu-deficient chloroplats apppears around +8°C indicating that the redox span between the positive and negative charges has been changed. The fact that the band at +8°C exhibits the same oscillation pattern by flash excitation as the control makes unlikely that the donation side of PS II is changed by Cu deficiency. On the other hand, a similar shift of the main band can be observed, when DCMU-type inhibitors are added to the chloroplast suspension [53]. These inhibitors block the electron transfer between the 'primary' acceptor Q_A and the 'secondary' acceptor Q_B [53] by lowering the redox potential of the Q_B component [54], or by block-

ing the plastoquinone binding the Q_B binding site [55]. In both cases, electrons are accumulated on Q_A only and as a result, the main band at +18°C is replaced with a new band at lower temperature. Thus we conclude that Cu deficiency inhibits the electron transfer between Q_A and Q_B by either blocking the Q_B binding site or changing the redox state of Q_B. This is consistent with the previous observation obtained by fluorescence induction measured on Cu-deficient sugar beet chloroplasts [20]. Comparing the results obtained by TL with the protein and lipid data we assume that Cu deficiency affects the PS II electron-transport chain by changing the redox state of Q_B via the alteration of the protein and/or lipid microenvironment of this compound.

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