

Changes in Photosynthetic Capacity and Photosynthetic Protein Pattern during Tomato Fruit Ripening¹

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ABSTRACT

Levels of polypeptides participating in the photosynthetic light and dark reactions have been measured during fruit ripening in tomato. Photosynthetic proteins were identified by Western blot analysis with heterologous antibodies. The concentrations of proteins of photosystem (PS) I (14 kilodaltons), of PSII (47-kilodalton reaction center protein, 32-kilodalton 'Q_B binding' protein and light harvesting complex proteins), of the photosynthetic electron transport chain (ferredoxin-NADP-oxidoreductase and plastocyanin), and of the stroma (ribulose-1,5-bisphosphate carboxylase) decrease during the ripening process. The 32-kilodalton protein and plastocyanin were detectable in pericarp protein preparations of ripe tomato fruits. Absorbance difference spectrophotometry provided information on the relative concentrations of PSII and PSI reaction centers in leaf and green fruit tissue of tomato. These results indicate that green fruit pericarp of tomato is photosynthetically active. Photosynthetic activity decreases during chloroplast/chromoplast differentiation. This is consistent with changes that occur at the transcript level of photosynthesis-specific proteins during the differentiation process.

branes are different in the plastids from leaf and fruit tissue (15). Detailed information is currently available on the structural and functional organization of photosynthetic membranes and on the component stoichiometry in thylakoid membranes of chloroplasts (1), while chloroplasts from other green plant tissues, such as stems, fruits during development, and some flower petals have been less well characterized.

We have therefore investigated the changes that occur at the organelle level during the chloroplast/chromoplast transition in tomato. In our previous work we have shown that most of the mRNAs for PSI and PSII, as well as stromal peptides, have decreased to nondetectable levels in chromoplasts (25). In this report we correlate the changes of mRNA levels with alterations that occur at the photosynthesis and polypeptide level. We present evidence that chloroplasts in green tomato fruits are photosynthetically active since substantial levels of the large subunit of ribulose 1,5-bisP carboxylase, the reaction center proteins of PSI and PSII, the light harvesting complex proteins of PSII, plastocyanin and Fd-NADP-oxidoreductase are detected in green fruit pericarp.

MATERIALS AND METHODS

Plant Material and Protein Isolation. Tomato plants (*Lycopersicon esculentum*, cherry line, VFNT LA 1221) were grown under greenhouse conditions. Fruits were harvested at different stages: mature green fruit (35–40 d after pollination); intermediate ripe fruit (approximately 45 d after pollination); and fully ripe fruit (approximately 50 d after pollination).

Freshly isolated tomato pericarp (100 g) and 100 ml TE-buffer (50 mM Tris (pH 7.5), 5 mM EDTA, 20 μ M phenylmethylsulfonyl fluoride) were homogenized for 15 s in a Waring Blendor equipped with extra razor blades, as described elsewhere (25). The homogenate was passed through four layers of Miracloth (Calbiochem). Soluble and membrane protein extracts were prepared as described elsewhere (19). Proteins were concentrated by 70% ammonium sulfate precipitations, resuspended in a small volume of TE-buffer, dialyzed against TE-buffer and TE-buffer containing 50% glycerol. All steps were carried out at 4°C. Protein concentrations were determined using the Bradford method (Bio-Rad Laboratories, reaction kit). Protein samples were stored at –20°C. Prior to electrophoresis, samples were treated with 1% SDS (membrane fractions were treated with 1% Triton X-100 in addition), and diluted with H₂O (1:2).

SDS-PAGE and Protein Transfer to Nitrocellulose Filters. SDS polyacrylamide gel (12.5% and gradient 9–19%) electrophoresis was performed as described by Laemmli (13). Ten and 20 μ g of the protein suspensions, isolated from leaf or fruit tissue, were electrophoresed for 5 h at 200 V (constant) and for 15 min at 250 V. Proteins were either stained with silver (22) or electro-

The ripening process of tomato fruits is characterized by a number of major changes, such as the loss of Chl, the accumulation of lycopene, fruit softening, and alterations in the metabolism of organic acids and monosaccharides (6, 12). During the early stages of fruit ripening, tomato chloroplasts differentiate into chromoplasts. In general, such differentiation of organelles result in plastids of various shapes and functions (31). The chloroplast/chromoplast transition in tomato fruits is accompanied by major ultrastructural changes such as the breakdown of thylakoid membranes (9, 10, 20, 29). The most evident changes are the degradation of Chl and increasing synthesis and accumulation of lycopene (4, 27). Plastids in green tomato fruits contain high levels of starch and therefore resemble amyloplasts more closely than chloroplasts (29). It has been reported, however that thylakoid membrane systems are present and that green fruit chloroplasts are able to incorporate CO₂ (5, 16). However, CO₂ fixation activity, the relative quantities of various lipids, and the total protein and Chl concentrations in the thylakoid mem-

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phoretically transferred to nitrocellulose filter (Western blotting) as described by Towbin *et al.* (32). Proteins were detected by double immunoprecipitation using specific antibodies and FITC (fluoresceinisothiocyanate) or peroxidase conjugated goat anti-rabbit antibodies. The substrate for the horseradish peroxidase was 4-chloro-1-naphthol (Bio-Rad Laboratories, reaction assay).

Determination of Ribulose 1,5-bisP Carboxylase Activity. The activity of ribulose 1,5-bisP carboxylase was determined in protein preparations from leaf and fruit pericarp tissues by incorporation of ^{14}C -labeled bicarbonate into ribulose 1,5-bisP to form nonvolatile glycerate-3-P molecules (23).

Spectroscopic Measurements. Chloroplast absorbance difference measurements were performed with a laboratory-constructed modulated split-beam difference spectrophotometer (17, 19). For the purpose of these measurements chloroplasts were isolated from tomato leaf and fruit tissue as described by Melis and Anderson (19). The isolation buffer for tomato leaves also contained 0.2% ascorbate, 0.1% BSA, and 0.1% PVP. Concentrations of PSI and PSII reaction centers were determined from quantitative measurements of Q, the primary quinone electron acceptor of PSII, and P700, the reaction center protein of PSI (17, 19). Chl concentrations and Chl *a/b* ratios were determined from 80% (v/v) acetone extracts using the following calculation formulas: Chl *a*: $12.7(A_{663\text{nm}} - 2.69(A_{645\text{nm}}))$ and Chl *b*: $22.9(A_{645\text{nm}}) - 4.68(A_{663\text{nm}})$.

Photosynthetic Electron Transport Measurements. The electron transport capacity in leaf and fruit tissue was determined by 'flash induced fluorescence' measurements (21). Freshly harvested leaves and fruits were illuminated with saturating light flashes on a background of constant illumination. The flash induced changes in the fluorescence signal were electronically isolated and measured for a series of different constant light intensities from 50 to 250 $\mu\text{E}/\text{m}^2 \cdot \text{s}$. Under these conditions, flash induced fluorescence is proportional to PSII electron transport in the illuminated volume of tissue.

RESULTS

Stoichiometry of Thylakoid Membrane Components in Leaf and Fruit Pericarp Tissue. We have compared the relative concentrations of several photosynthetic components in tomato leaf and fruit pericarp tissue to investigate the photosynthetic capacities in the two functionally different tissues.

The concentrations of P700 (reaction center protein of PSI) and Q⁴ (primary quinone electron acceptor of PSII) were determined from the absorbance changes at 700 nm and 320 nm, respectively. The absorbance difference at 320 nm was corrected for the particle flattening effect (19). In tomato leaf chloroplasts the ratio of total Chl content to P700 was estimated to be 600 and the total Chl to Q ratio was 440. As a result, the PSII/PSI reaction center ratio (Q/P700) in tomato leaf tissue was 1.36 (Table I). It appears therefore that tomato leaf chloroplasts contain fewer PSI than PSII reaction centers. In leaves of a variety of higher plants the Q/P700 ratios are approximately 1.5, although seasonal changes may occur (19).

The quantitative measurements of PSII and PSI reaction center concentrations were extended to plastids from green and red fruit pericarp. The Chl/P700 and Chl/Q ratios of green fruit tissue were 410 and 250, respectively, which results in a PSII/PSI reaction center ratio of 1.64 (Table I). No absorbance change due to the photooxidation of P700 or photoreduction of Q was detectable in pericarp tissue from red tomato fruits. These results are supported by our measurements of the photosynthetic elec-

tron transport activity in tomato leaves and fruits of different ripening stages (Table I). Compared to leaf tissue, pericarp tissue in green tomato fruit carries out 41.4% photosynthetic electron transport. In orange fruit this value decreases to 13%, while in ripe tomato fruit no photosynthetic electron transport is detectable.

Additional information for photosynthetic membrane components of chloroplasts from different tissues were derived from Chl *a/b* ratios and ratios of Chl/g total protein (Table I). The Chl *a/b* ratio in chloroplasts from leaves and green fruit pericarp are very similar (2.89 and 2.56, respectively) and also do not differ significantly from ratios of other higher plants (18, 19). However, the amount of Chl/g total protein is approximately 8-fold higher in leaves than in green fruit. The F_v/F_o ratios (Table I) (15) also indicate a smaller antenna size in chloroplasts of tomato pericarp than of leaf tissue. This decrease is significant, since the size of the light harvesting antenna provides a measure for the overall electron transport capacity of both photosystems. From our results we conclude that the photosynthetic capacity expressed on a Chl basis in green fruit pericarp is lower than in leaf tissue.

Changes in the Protein Patterns during Fruit Ripening. We reported earlier that mRNAs for photosynthetic polypeptides disappear during fruit ripening (25). We have extended this work to analyze changes in polypeptide levels for respective mRNAs. To confirm that the measured decrease of photosynthetic activity during chloroplast/chromoplast differentiation is concomitant with the turnover and disappearance of photosynthetic polypeptides, we isolated total protein from pericarp tissue and measured the levels of photosynthetic polypeptides with specific antibodies (Table II). Total extracts of membrane and stromal protein fractions were separated on SDS polyacrylamide gels (Fig. 1). The comparison of the protein profiles shows that the concentrations of a number of mostly unidentified proteins of the soluble and membrane fractions vary significantly during the ripening process. The amounts of proteins with relative molecular masses of 57, 44, 40.5, and 35 kD increase, while those of 54, 27, 17, and 14 kD decrease when soluble protein fractions of green and red fruit preparations are compared (Fig. 1A). The levels of other proteins, *i.e.* polypeptides with molecular masses of 28.5 and 15 kD do not change significantly in green, orange and red fruit pericarp. In the membrane fractions (Fig. 1B) of red fruit pericarp, proteins with a molecular masses of 29, 28, 18.5, 17.5, 16, 11, and 10 kD are present in significantly lower amounts than in green fruit pericarp, while other proteins increase during fruit ripening (*e.g.* 44, 35, 16, and 15 kD protein). The 35 kD protein appears to have the maximum of its concentration in intermediate stage tomato fruits. We have used specific antibodies (Table II) to follow the level of proteins characteristic for PSI and PSII, the electron transport chain, and the stroma in differentiating chloroplasts during tomato fruit ripening.

PSI and PSII. Proteins of both photosystem I and II have been identified in different ripening stages of tomato with specific heterologous antibodies (Table II, Fig. 2). The protein levels of PSI '14 kD protein' was identified in membrane fractions of the green fruit, soluble fraction of tomato leaves, and to a smaller extent in the soluble fraction of mature green fruits, but not in red tomato fruit preparations (Fig. 2A). Based on this observation it is most likely that this protein is degraded during the ripening process. The antibody against the '14 kD protein' cross-reacts with two proteins of molecular masses of 16.5 and 17 kD in the membrane fraction of the green fruit tissue. The lower band may be the result of proteolytic degradation. The increase in apparent molecular mass of the 17 kD protein in the soluble fraction of green fruit pericarp is most likely a consequence of electrophoresis conditions, but we cannot exclude the possibility that a precursor of the '14 kD protein' is detected in the soluble fraction.

⁴ Abbreviations: Q, primary quinone electron acceptor of photosystem II; P700 reaction center of photosystem I; F_v , variable fluorescence yield; F_o , nonvariable fluorescence yield; LHC, chlorophyll *a/b* light harvesting complex.

Table I. Determination of Photosynthetic Components during Tomato Fruit Ripening

Photosynthetic components and/or activities of leaf and fruit pericarp tissues from *L. esculentum* cultivar VFNT LA 1221 of different ripening stages (mature green fruit, 35 to 40 d after pollination; intermediate ripe fruits, 45 d after pollination; ripe fruits, 50 d after pollination) were compared. All data are mean values from 2 or 3 experiments.

	Leaf	Green Fruit	Intermediate Fruit	Red Fruit
Ribulose 1,5-bisP carboxylase nmol/min · mg protein	9.32	3.25	0.20	0.07
%	100	34.9	2.2	0.8
Photosynthetic electron transport capacity (area basis), %	100	41.4 ^a	13.0	ND ^b
Chl <i>a/b</i> , mol/mol	2.89	2.56		
Chl/protein, g/g	0.44	0.06		
Chl/P700, mol/mol	600	410		ND
Chl/Q ^c , mol/mol	440	250		ND
Q/P700, mol/mol	1.36	1.64		
<i>F_v</i> / <i>F_o</i>	1.33	0.8		

^a Average from dark and light green tissue. ^b ND, not detectable. ^c Spectrophotometric measurements of Q: assay contained Triton X-100 at a final concentration of 0.1%.

Table II. Heterologous Antibodies

Antibodies Raised against	Species	Localization	Molecular Mass	Reference
			<i>kD</i>	
Fd-NADP-oxidoreductase	Spinach	Electron transport chain	37	(7)
Plastocyanin	Spinach	Electron transport chain	10	(7)
14 kD protein	Spinach	PSI	14	R. Malkin N. H. Chua
Light harvesting complex protein	Tomato	PSII	25–26	N. Hoffmann A. Cashmore
32 kD Q _B -binding protein	Maize	PSII	32	(24)
Reaction center apo-protein	Spinach	PSII	47	(14)

Proteins of PSII were also identified with heterologous antibodies (Table II; Fig. 2, B–D). The 32 kD antibody cross-reacts with a 33.5 kD protein in leaf and fruit tissue of all ripening stages. The strongest response is obtained in protein preparations from leaf and green fruit (membrane fraction) pericarp tissues. The level decreases during the ripening process, but the protein is detectable in orange tomato fruits and trace amounts are found in the membrane fraction of red fruits. The apparent change in mol wt of the 32,000 protein in extracts from green fruit pericarp (soluble fraction) is most likely a consequence of the protein composition in this mol wt range and electrophoresis conditions, but we cannot exclude that the antibody detects high levels of the 32,000 precursor protein. The antibody of the light harvesting complex proteins of PSII (LHCII) identifies proteins of 27 and 28 kD in the membrane fractions of green fruit pericarp. These proteins disappear simultaneously during the chloroplast/chromoplast conversion and are not detected in soluble fractions of fruit pericarp. The antibody of the reaction center protein of PSII shows a signal with a 48 kD protein in green tomato fruits and leaf extracts, but is not detectable in intermediate and fully ripe fruits. An additional lower mol wt protein of 44,000 is apparent in leaves and membrane fractions of green fruit which might be due to partial proteolytic degradation. A protein with a mol wt of 46,000 that shows increasing protein levels during ripening is

most likely due to cross-reaction with the polygalacturonase. This protein is present in high concentrations in red tomato fruits (6) (Fig. 1).

Electron Transport Chain. As described above, we detect a loss of photosynthetic activity in red fruit tissue. Consequently, we extended our work to measure the level of components of the electron transport chain, Fd-NADP-oxidoreductase and plastocyanin, during the ripening process (Fig. 3, A and B). The Fd-NADP-oxidoreductase-specific antibody cross reacts with a protein of 35 kD in protein extracts of tomato leaves and green fruit (membrane fraction). The concentration of the Fd-NADP-oxidoreductase decreases to non-detectable levels in red fruit protein extracts during ripening. The mol wt of the tomato Fd-NADP-oxidoreductase is similar to the size of the spinach polypeptide (37,000). Plastocyanin (apparent mol wt of 12,000) was found in soluble protein fractions from green, intermediate, and red fruits. The amount of plastocyanin in green and red fruit does not change significantly in these soluble fractions. However, the concentration in the membrane fractions decreases during ripening, and no plastocyanin is detectable in the red fruit membrane protein fraction. It is possible that plastocyanin has been solubilized either during the preparation procedure or as a consequence of the membrane degradation during the chloroplast/chromoplast conversion.

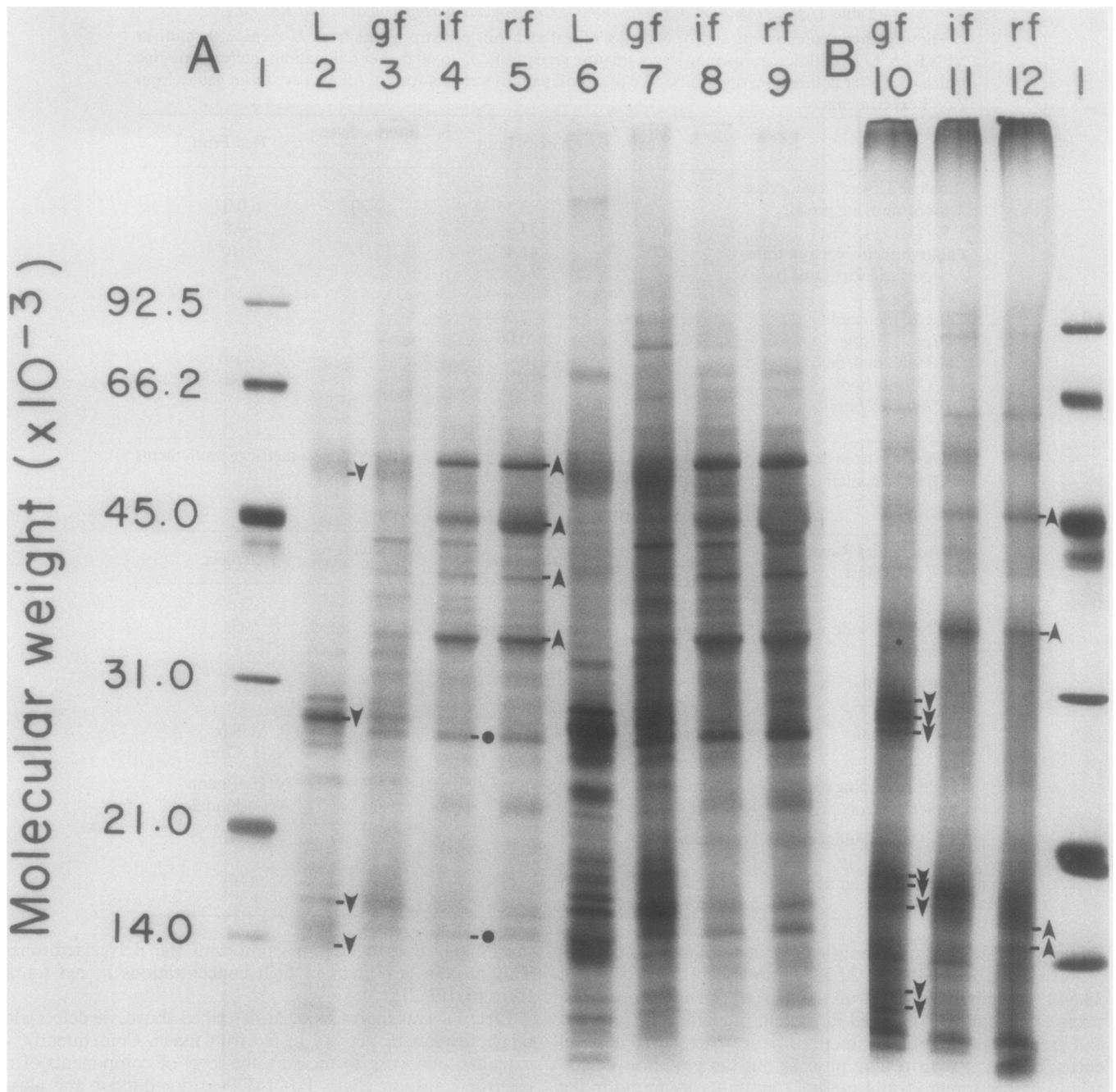


FIG. 1. Separation of protein extracts of tomato leaves and fruits of different ripening stages on SDS polyacrylamide gradient gels (9–19%). A, Soluble protein fractions from leaves (lane 2 and 6), mature green fruits (lane 3 and 7), intermediate ripe fruits (lane 4 and 8), and ripe fruits (lane 5 and 9) were separated. Lanes 2 to 5 and lanes 6 to 9 contain 10 μ g and 20 μ g of total protein extracts, respectively. The apparent molecular masses of polypeptides with increasing amounts during ripening are: 57, 44, 40.5, 35, and 14 kD (\blacktriangle), polypeptides with decreasing amounts are: 54, 27, 17, and 14 kD (\blacktriangledown). Polypeptides with molecular masses 28.5 and 15 kD show little changes (\bullet). B, Proteins of membrane fractions (10 μ g total protein each lane) from mature green fruits (lane 10), intermediate ripe fruits (lane 11) and ripe fruits (lane 12) were separated. The molecular masses of polypeptides with increasing amounts during ripening are: 44, 35, 16, and 15 kD (\blacktriangle), those polypeptides with decreasing amounts are: 29, 28, 27, 18.5, 17.5, 16, 11, and 10 kD (\blacktriangledown).

Stromal Protein. In addition to the photosynthetic membrane proteins, we have identified the ribulose 1,5-bisP carboxylase with heterologous antibodies in tomato leaf and fruit tissue and have analyzed the steady-state levels during the ripening process (26). The concentration of the large subunit decreases in intermediate stage tomato fruits and is not detectable in protein preparations of ripe fruits. This result is consistent with the decrease of ribulose 1,5-bisP carboxylase activity in different ripening stages (Table I). A protein extract of mature green fruit

pericarp fixes 3.25 nmol of $^{14}\text{CO}_2$ per mg total protein per min; in intermediate and ripe fruits the ability decreases to 0.2 and 0.075 nmol/mg·min, respectively. Oxygen fixation activity in leaf extracts is approximately 3-fold higher than in green fruit pericarp tissue. The decrease of carboxylase activity during fruit ripening parallels the inactivation of other photosynthetic processes during plastid differentiation, and the decrease of chloroplast and nuclear mRNAs for the large and small subunit polypeptides, respectively (27).

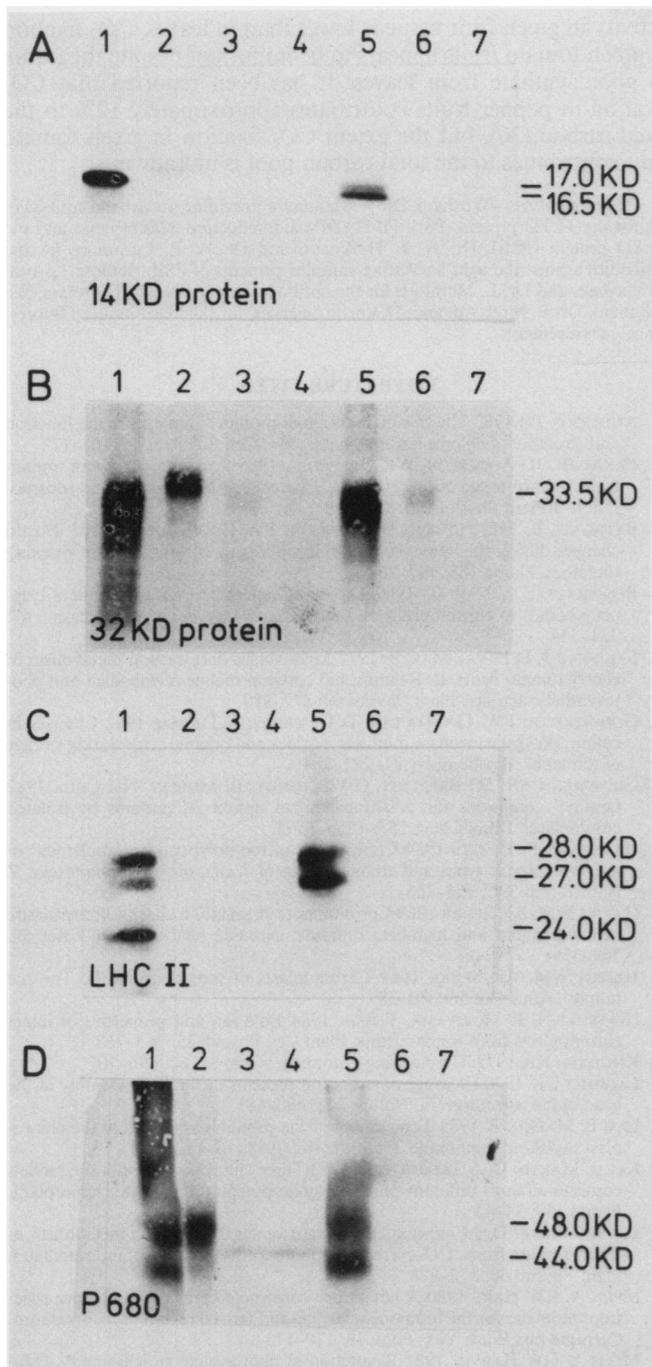


FIG. 2. Identification of proteins of PSI and PSII in leaf and pericarp tissue of different ripening stages of tomato fruits by 'Western blot' analysis. Twenty μ g protein extracts of leaves (lane 1), mature green fruits (lanes 2 and 5), intermediate ripe fruits (lanes 3 and 6), and ripe fruits (lanes 4 and 7) were separated on SDS polyacrylamide gels. Proteins of soluble (lanes 1-4) and crude membrane (lanes 5-7) fractions were analyzed with heterologous antibodies. A, The 17 kD protein was identified in tomato extracts with specific heterologous antibodies of the '14 kD protein' of PSI. Protein fractions were separated on 9-19% gradient gels. B, Identification of the 32 kD Q_B -binding protein in tomato extracts with specific heterologous antibodies. Proteins were separated on a SDS polyacrylamide gel (9-19%). C, Two light harvesting complex (LHCII) proteins with mol wt of 27 and 28 kD were identified in tomato leaf and green fruit (membrane fraction) preparations with homologous antibodies. Protein fractions were separated on 12.5% SDS polyacrylamide gels. D, A 48 kD reaction center protein of PSII was detected with specific

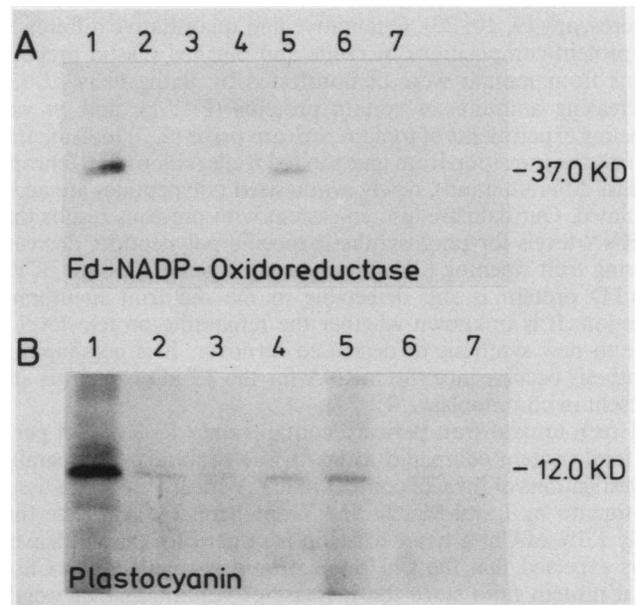


FIG. 3. Identification of proteins of the photosynthetic electron transport chain in different ripening stages of tomato fruits. For designation of lanes see legend of Figure 2. A, The Fd-NADP-oxidoreductase of 37 kD was identified in leaf and green fruit (membrane fraction) preparations of tomato with heterologous antibodies. Proteins were separated on a 12.5% SDS polyacrylamide gel. B, Detection of plastocyanin (12 kD) in tomato extracts with specific heterologous antibodies. Proteins were separated on 9 to 19% SDS polyacrylamide gels.

DISCUSSION

Photosynthesis in green plants is facilitated by two photoreactions. The trapping of excitation energy by the reaction center of PSII causes the reduction of the primary quinone electron acceptor Q. Excitation of PSI reaction center causes the oxidation of P700. It has been shown, that the stoichiometry of the two photosystems (PSII/PSI ratio) varies over a wide range in photosynthetic membranes of leaves in different as well as in the same species, depending on the environmental conditions (18, 19). In our experiments we have determined the Chl/P700, the Chl/Q and the Q/P700 ratios of two different tissues (leaves and fruit pericarp) of tomato plants. The PSII/PSI reaction center ratio of the green fruit pericarp is similar to that measured in leaves, indicating a functional similarity in the two tissues. Since no absorbance change due to photooxidation of P700 or photo-reduction of Q was detectable in pericarp tissue from red fruits, we conclude that these photosynthetic components are missing and therefore no photosynthetic electron flow occurs in chloroplasts of ripe tomatoes. Repeated illuminations to the leaf and green fruit pericarp sample resulted in inactivation of Q. Since it cannot be excluded that such inactivation of Q also occurs during the preparation of the sample, the actual *in vivo* Q/P700 ratio for tomato leaves might be higher than 1.36. Such inactivation was not observed in previous measurements with pea, spinach, and maize chloroplasts.

Proteins of PSI and PSII are found in the mature green tomato fruit pericarp (Fig. 2). The concentrations of most proteins decrease to undetectable levels in red fruits. The breakdown or disappearance of these photosynthetic membrane proteins is therefore coincident with the thylakoid membrane degradation during chloroplast/chromoplast conversion observed by electron

heterologous antibodies in the soluble and membrane fraction of green tomato fruits. Proteins were separated on 12.5% SDS polyacrylamide gels.

microscopy (9, 10, 20). Qualitative and quantitative differences of protein compositions of crude and purified plastid preparations from tomato were demonstrated by Bathgate *et al.* (3). Increasing amounts of certain proteins (Fig. 1), and *in vivo* labeling experiments of tomato pericarp tissue (2, 3) indicate that during the transition from green to red fruits (chloroplast/chromoplast differentiation), newly synthesized polypeptides are accumulated. Our data are also consistent with previous results that mRNA levels for photosynthesis-specific polypeptides decrease during fruit ripening (25). Interestingly, a small amount of the 32 kD protein is still detectable in the red fruit membrane fraction. It is unknown whether the remaining protein level is due to new synthesis or decreased turnover. It is possible that synthesis occurs since the mRNA for the 32 kD protein is still present in chromoplasts (25, 27).

Green tomato fruit pericarp contains only 13% of Chl per g of total protein compared to leaf tissue (Table I). Comparable investigations of the Chl content in leaf, pericarp and pulp tissue of tomato by Laval-Martin and Tremolieres (15) indicate that only 1.3% of Chl/g tissue exists in fruit pericarp than in leaves. It is expected that the Chl/g tissue ratio is smaller than Chl/g total protein ratio since the increase of volume and/or weight during fruit development is mainly due to water uptake and only partly due to increasing amounts of proteins, cell walls or other structural components. Since the amount of Chl and the F_v/F_o ratio are indications of the antenna size, we assume that the overall light harvesting capacity is much lower in green fruit than in leaves. This conclusion is also supported by our measurements of the photosynthetic electron transfer ('flash induced fluorescence' measurements, Table I). According to our results, the green tomato fruit has photosynthetic capacity itself, while it seems highly unlikely that chromoplasts in pericarp tissue generate energy for metabolic reactions through photophosphorylation. It is not known what role photosynthesis of the green fruit plays in the physiology of the whole plant or, in particular, in the developing fruit. Fruits have been physiologically defined as a reserve tissue or a metabolic "sink" for photosynthetic products such as glucose or sucrose synthesized in leaves. From electron microscopy investigations it is known that chromoplasts do not contain photosynthetic membranes (9, 10, 20, 29), and therefore, may have to receive their energy from biochemical reactions other than photosynthetic phosphorylation. The presence of mRNA of two mitochondrial ATPase subunits in intermediate and red tomato fruits suggest oxidative phosphorylation as a possible energy source for the ripening process (B Piechulla, W Gruissem, unpublished data). It is interesting to note that plastocyanin is still present in red fruit protein preparations. It seems possible that an electron transport chain exists in chromoplasts, which could be used to channel electrons from other chemical reactions to provide energy in the form of NAD(P)H. Based on measurements in total cell extracts, CO₂ fixation activity is reduced 3-fold in green pericarp tissue as compared to leaves, and decreases to almost non-detectable levels in red fruit tissue (Table I). In leaf extracts of tomato cultivars *L. esculentum*, DUN A GRAY and MILL, similar CO₂ fixation activities to the VFNT LA 1221 have been determined (16). These results are consistent with previously published results of CO₂ fixation activities in crude extracts from chloroplasts and chromoplasts (11). In addition, Hansmann *et al.* (8) found reduced amounts of the large and small subunit of ribulose 1,5-bisP carboxylase in chromoplasts of daffodils. Since we detect very little mRNA of the large subunit of the carboxylase/oxygenase in red tomato fruit pericarp (25), it can be assumed that no or significantly reduced new synthesis of the enzyme occurs in this tissue and that the residual CO₂ fixation activity is most likely due to the stability of enzyme complex synthesized and assembled early during fruit development. Although ribulose 1,5-bisP carboxylase

activity in green fruit tissue is lower than in leaves, CO₂ fixation in green tomato fruits appears to be important despite the influx of photosynthate from leaves. It has been reported that CO₂ fixation in pepper fruits contributes approximately 12% to the fixed carbon (30), but the extent CO₂ fixation in green tomato fruit contributes to the total carbon pool is unknown.

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