Light-dependent changes in redox status of the plastidic acetyl-CoA carboxylase and its regulatory component

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Plastidic acetyl-CoA carboxylase (ACCase; EC 6.4.1.2), which catalyses the synthesis of malonyl-CoA and is the regulatory enzyme of fatty acid synthesis, is activated by light, presumably under redox regulation. To obtain evidence of redox regulation *in vivo*, the activity of ACCase was examined in pea chloroplasts isolated from plants kept in darkness (dark-ACCase) or after exposure to light for 1 h (light-ACCase) in the presence or absence of a thiol-reducing agent, dithiothreitol (DTT). The protein level was similar for light-ACCase and dark-ACCase, but the activity of light-ACCase in the absence of DTT was approx. 3-fold that of dark-ACCase. The light-ACCase and dark-ACCase were activated approx. 2-fold and 6-fold by DTT respectively, indicating that light-ACCase was in a much more reduced, active form than the dark-ACCase. This is the first demonstration of the light-dependent reduction of ACCase *in*

vivo. Measurement of the activities of ACCase, carboxyltransferase and biotin carboxylase in the presence and absence of DTT, and the thiol-oxidizing agent, 5,5'-dithiobis-(2-nitrobenzoic) acid, revealed that the carboxyltransferase reaction, but not the biotin carboxylase reaction, was redox-regulated. The cysteine residue(s) responsible for redox regulation probably reside on the carboxyltransferase component. Measurement of the pH dependence of biotin carboxylase and carboxyltransferase activities in the ACCase suggested that both components affect the activity of ACCase *in vivo* at a physiological pH range. These results suggest that the activation of ACCase by light is caused partly by the pH-dependent activation of two components and by the reductive activation of carboxyltransferase.

Key words: biotin carboxylase, carboxyltransferase, pea.

INTRODUCTION

Fatty acid synthesis in chloroplasts is modulated by light. The first committed step is the synthesis of malonyl-CoA, which is catalysed by acetyl-CoA carboxylase (ACCase). The plastidic ACCase activity in isolated chloroplasts is also modulated by light, and the light-dependent activation of fatty acid synthesis is caused partly by the activation of ACCase [1]. Chloroplasts have a light-signal transduction pathway via a redox cascade [2], which is used to regulate enzymes of the reductive pentose phosphate cycle. In the light, electrons from photosystem I are shuttled through the electron transport chain to ferredoxin and are transferred to thioredoxins by ferredoxin–thioredoxin reductase, and then to target enzymes, reducing disulphide bonds in the enzymes and changing their catalytic activities. The reduced (dithiol) form of the enzymes can be reoxidized by O₂ produced concomitantly. If so, the target enzyme could be turning over continuously between its reduced and oxidized forms in light. In the dark, however, any such cycling would stop, and the enzyme would be mainly in its oxidized form. We have previously shown that the plastidic ACCase partly purified from pea leaves is activated *in vitro* by dithiothreitol (DTT), a known reductant of disulphide bonds, and reduced thioredoxin, which is a transducer of redox potential during illumination [3]. We proposed that a redox cascade is involved in the light/dark modulation of ACCase, like that of the enzymes in the reductive pentose phosphate cycle, which can thus modulate fatty acid synthesis. To examine whether the plastidic ACCase in leaves in light is indeed in an active reduced form and whether that of leaves in the dark is in an inactive oxidized form, we compared the redox status of the plastidic ACCase extracted from leaves kept under different light conditions in this study.

Plastidic ACCase is a multienzyme complex [4–6] composed of biotin carboxyl carrier protein (BCCP), biotin carboxylase, and a carboxyltransferase complex made up of two pairs of α and β subunits. This enzyme catalyses two different half-reactions, biotin carboxylase (step 1) and carboxyltransferase reactions (step 2), to form malonyl-CoA:

To determine the reaction regulated by redox, we examined the effects of thiol-reducing (DTT) and thiol-oxidizing [5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)] reagents on the two reactions (steps 1 and 2) and compared their effects with those on ACCase activity.

MATERIALS AND METHODS

Reagents

DTNB and DTT were purchased from Sigma.

Plant materials

Pea seedlings (*Pisum sativum* cv. Alaska) were grown in a 16 h light/8 h dark cycle. Plants (8 days old) were kept in darkness for 12 h and then exposed to 1 or 3 h of white light of 60 $\mu\text{E}/\text{s}$ per m² (light-adapted plants) or darkness (dark-adapted plants).

Isolation of chloroplasts and extraction of ACCase

All procedures for the isolation of chloroplasts and the preparation of the stroma fraction from dark-adapted plants were performed in a darkroom under a dim-green safety light, and

Abbreviations used: ACCase, acetyl-CoA carboxylase; BCCP, biotin carboxyl carrier protein; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); DTT, dithiothreitol.

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those from the light-adapted plants under light. Intact chloroplasts were isolated by using a Percoll gradient [7] and were ruptured in a lysis buffer [50 mM Tricine/KOH (pH 8)/1 mM EDTA/1 mM PMSF/1 mM benzamidine/5 mM ϵ -amino-n-hexanoic acid] as described previously [4]. In brief, lysis buffer was added to adjust the chlorophyll concentration to 1 mg/ml. The lysed chloroplasts were centrifuged for 10 min at 55000 g. To the supernatant fraction (stroma fraction) was added an equal volume of satd. $(\text{NH}_4)_2\text{SO}_4$ solution (pH 7.5); it was then stirred for 10 min and stored for 1 h at 4 °C. ACCase activity was stable during this procedure. The 50%-satd. $(\text{NH}_4)_2\text{SO}_4$ suspension was divided into several portions (0.5 ml each) and centrifuged. The supernatant was removed and the precipitate was stored at -80 °C until use. This precipitate was stable at least for 6 months. When this fraction was analysed on a gel-filtration column, the activity of the plastidic ACCase (molecular mass 700 kDa), but not that of cytosolic ACCase (molecular mass 500 kDa), was detected, and this fraction was not contaminated with the cytosolic ACCase. We omitted thiol reagents such as mercaptoethanol and DTT to avoid the reduction of ACCase *in vitro* during preparation from the lysis buffer, and added EDTA to avoid the possible oxidation of protein by air caused by metal ions. The procedure took approx. 15 min from homogenization of the leaves to lysis of the chloroplasts; throughout, a rapid oxidation of ACCase by air was not detected.

Partial purification of plastidic ACCase

The $(\text{NH}_4)_2\text{SO}_4$ precipitate of the stroma fraction obtained from chloroplasts isolated from 200 g of leaves of irradiated plants was dissolved in 2 ml of lysis buffer and applied to a Sephacryl S-300 column (1.6 cm $\times$ 60 cm; Pharmacia) as described before [4]. All solutions used contained 5–10 mM 2-mercaptoethanol. Fractions with ACCase activity were precipitated with $(\text{NH}_4)_2\text{SO}_4$ (50% satn.). The $(\text{NH}_4)_2\text{SO}_4$ precipitates were recovered by brief centrifugation and stored at -80 °C.

Measurement of ACCase activity

The $(\text{NH}_4)_2\text{SO}_4$ precipitates stored at -80 °C were dissolved in 100 μ l of lysis buffer and desalted by centrifugation in a gel-filtration column (Biospin 30; Bio-Rad). Small molecules with a molecular mass of less than approx. 40000 Da were removed by gel filtration. Chloroplast thioredoxins (molecular mass approx. 12000 Da) were removed at this gel-filtration step. When fully oxidized ACCase was necessary, the enzyme was incubated with 0.01 mM DTNB, and excess oxidizing reagent was removed by centrifugation in a gel-filtration column (Biospin 6). These ACCase fractions were used immediately for activity measurement. ACCase activity was measured as described elsewhere [4]. In brief, 4 μ l of protein solution containing 5–7 μ g of protein was added to a solution containing 50 mM Tricine/KOH, pH 8, 1 mM ATP, 5 mM MgCl_2 , 50 mM KCl, 1.2 mM acetyl-CoA and 3.4 mM $\text{NaH}^{14}\text{CO}_3$ (14.8 kBq) in a final volume of 20 μ l. Incubation was at 30 °C for 20 min; the reaction was stopped by the addition of 5 μ l of 6 M HCl. Then 25 μ l of the solution was applied to Whatman 3MM paper (1 cm $\times$ 1 cm) and dried. The radioactivity was measured in a scintillation counter. Where the enzyme was preincubated with thiol-reducing or thiol-oxidizing reagents, a total of 10 μ l of a mixture of the enzyme and each reagent containing 100 mM Tricine/KOH (pH 8) was incubated at 30 °C for 10 min. The reaction was started by the addition of ATP/ MgCl_2 /KCl/acetyl-CoA/ $\text{NaH}^{14}\text{CO}_3$. The final concentrations were the same as those described above.

Measurement of biotin carboxylase activity

Biotin carboxylase activity was measured as described elsewhere [8], with a slight modification. In brief, approx. 5–7 μ g of the ACCase fraction in a total of 100 μ l of reaction mixture [100 mM Tricine/KOH (pH 8)/1 mM ATP/4 mM MgCl_2 /50 mM biotin/1.5 mM $\text{NaH}^{14}\text{CO}_3$ (32.6 kBq)/24 μ g of BSA/5 μ l of ethanol] was incubated at 30 °C for 20 min; the reaction was terminated by transfer of a 90 μ l aliquot to a vial containing 0.5 ml of ice-cold water and one drop of octan-1-ol. The solution in the vial was bubbled with CO_2 for 30 min at 0–2 °C to remove the excess $\text{H}^{14}\text{CO}_3^-$. Then 5 μ l of 1 M NaOH and 1 ml of scintillator were added. After mixing, the radioactivity was measured.

Measurement of carboxyltransferase activity

Carboxyltransferase activity was measured by the reverse reaction method as described elsewhere [8], with a slight modification. The carboxy group transfer from [2- ^{14}C]malonyl-CoA to biotin methyl ester was measured. The resultant [^{14}C]acetyl-CoA was evaporated and the residual [2- ^{14}C]malonyl-CoA was determined. Approx. 12.5–17.5 μ g of the ACCase fraction in a total of 50 μ l of reaction mixture [100 mM Tricine/KOH (pH 8)/10 mM biotin methyl ester/100 μ M malonyl-CoA (0.64 kBq)/37.5 μ g of BSA] was incubated at 30 °C. After 0 and 15 min of incubation, 20 μ l of the mixture was transferred to a tube containing 5 μ l of 6 M HCl to terminate the reaction. The mixture was heated to dryness at 98 °C for 20 min, dissolved in 25 μ l of water and applied to Whatman 3MM paper (1 cm $\times$ 1 cm). For the determination of radioactivity, the difference between radioactivity at zero time and after 15 min of incubation was designated carboxyltransferase activity.

Detection of components of plastidic ACCase

Crude chloroplast proteins were separated by SDS/PAGE [10% (w/v) gel] and transferred to a nitrocellulose membrane. The blots were probed with anti-(carboxyltransferase β) IgG (1 μ g/ml) and then with goat anti-rabbit IgG conjugated to peroxidase (Bio-Rad) diluted 1:3000 [4]; the blots were developed with an immunostaining system (Konica) or an enhanced chemiluminescence kit (Amersham Corp.). The protein containing biotin on the nitrocellulose membrane was probed with streptavidin conjugated with horseradish peroxidase (Sigma).

RESULTS

Redox status of ACCase in dark-adapted and light-adapted plants

The ACCase activities in the crude extracts of chloroplasts isolated from plants kept in darkness and in those exposed to light for 1 h were designated dark-ACCase and light-ACCase respectively. SDS/PAGE revealed no difference in the total protein concentration or in the protein components between the plants kept in darkness and those exposed to light for 1 h (results not shown). The BCCP levels in the light-ACCase and dark-ACCase were similar (Figure 1a). The carboxyltransferase β levels in the light-ACCase and dark-ACCase were similar (Figure 1b). Thus the ACCase protein levels were similar in the light-ACCase and dark-ACCase.

We examined the relationship between the concentration of the extract and the ACCase reaction. DTT-reduced ACCase activities in both extracts increased linearly in the range 1–4 μ l of extract per 20 μ l assay mixture (results not shown). We therefore used 4 μ l of crude extract in the following experiments. To

Figure 1 Effects of light on two components of ACCase in crude extracts

The crude proteins (7.5 μ g) in the chloroplast extracts obtained from the plants kept in darkness (D) and those exposed to 1 h of light (L) as described in the Materials and methods section were separated by SDS/PAGE [10% (w/v) gel]. The proteins were probed with streptavidin conjugated with horseradish peroxidase, which reacts with biotinyl proteins such as BCCP detected as a 38 kDa protein (a), or with anti-(carboxyltransferase β) IgG, resulting in a band at 87 kDa (b). The positions of molecular mass markers are indicated (in kDa) at the left.

Figure 2 Effects of thiol-reducing and thiol-oxidizing reagents on the ACCase activities in the crude chloroplast extracts in the light and darkness

ACCcase activities in the crude extracts obtained from the plants kept in darkness (dark-ACCcase) and those exposed to light for 1 h (light-ACCcase) were measured as described in the Materials and methods section. The effects of DTT, H₂O₂ or DTNB on light-ACCcase and dark-ACCcase are shown. The extracts were preincubated with H₂O (control) (○), 4 mM DTT (■), 2 mM H₂O₂ (△) or 0.02 mM DTNB (□) for 10 min at 30 °C before the reaction was started. Changes in activity with time are shown.

Figure 3 Light- and dark-dependent changes in redox status of ACCase

ACCcase activity in the crude chloroplast extracts obtained from the plant leaves exposed to light or darkness as indicated was measured in the presence or absence of 4 mM DTT. The original activity without DTT is shown relative to that in the presence of 4 mM DTT. Results show means of four determinations.

compare the effect of DTT on dark-ACCcase and light-ACCcase, we examined the reaction time courses (Figure 2). The enzyme fractions were preincubated with or without 4 mM DTT at 30 °C for 10 min before the reaction was started. The reaction of both ACCcases proceeded almost linearly in either solution with or without DTT. The activity of light-ACCcase in the absence of DTT (control) was higher than that of dark-ACCcase. The activity of light-ACCcase was stimulated 2-fold by DTT, and that of dark-ACCcase 6-fold. Therefore the effect of the reducing agent on light-ACCcase was weaker than that on dark-ACCcase. To confirm the presence of the reduced form of the enzyme in dark-ACCcase and light-ACCcase we added the thiol-oxidizing agent H₂O₂ or DTNB to the control fraction. The activity of dark-ACCcase was completely inhibited by H₂O₂ at 2 mM, but some activity of light-ACCcase was observed. The activity of both ACCcases was completely inhibited by DTNB at 0.02 mM. These results indicate that light-ACCcase contains a much more reduced active form than dark-ACCcase and that light activates ACCcase by reducing it *in vivo*.

Figure 3 shows the ACCcase activity in the crude extract of chloroplasts isolated from leaves exposed to light or darkness. The activity was measured in the presence or absence of DTT; the original (control) activity in the absence of DTT was calculated as a percentage of that in the presence of DTT. Before irradiation, the activity was approx. 15 % of the DTT-stimulated activity. After exposure to light for 1 or 3 h, the activity was 50–60 % of the DTT-stimulated activity (Figure 3a). This suggested that light reductively activated ACCcase *in vivo* within 1 h. During centrifugation for isolation of chloroplasts from light-adapted plants, the chloroplasts were not irradiated, so it is possible that some conversion of the reduced form of ACCcase to the oxidized form occurred in the dark. There might therefore have been more of the reduced form of ACCcase *in vivo* in light-adapted plants.

The plants exposed to light for 1 h were subsequently returned to darkness, and ACCcase was extracted from the plants kept in darkness for 1 or 3 h. The activities in the presence and absence of DTT were compared (Figure 3b). The activity after 1 h of irradiation was approx. 50 % of the DTT-stimulated activity but

Figure 4 Effects of DTT on ACCase, biotin carboxylase and carboxyltransferase activities

Partly purified enzyme obtained from plants exposed to light for 1 h was preincubated with DTT at the indicated concentrations at 30 °C for 10 min, and ACCase (●), biotin carboxylase (△) and carboxyltransferase (■) activities were measured. The activity at 0 mM DTT was 62 nmol/20 min per mg of protein for ACCase, 28 nmol/20 min per mg of protein for biotin carboxylase, and 177 nmol/15 min per mg of protein for carboxyltransferase. The activity is relative to that at 0 mM DTT. Results are the means of duplicate determinations that differed by less than 10%.

Figure 5 Effects of DTNB on ACCase, biotin carboxylase and carboxyltransferase activities

Partly purified enzyme obtained from light-adapted plants was preincubated with the indicated concentrations of DTNB at 30 °C for 10 min, and ACCase (●), biotin carboxylase (△) and carboxyltransferase (■) activities were measured. The activity at 0 mM DTNB was the same as in Figure 4. Activities are shown relative to that at 0 mM DTNB.

the activity 1 or 3 h in darkness was only 10–15 % of the DTT-stimulated activity. Therefore the activity of ACCase was lowered in darkness within 1 h, probably by oxidation of the active ACCase *in vivo*.

Effects of thiol-reducing and thiol-oxidizing agents on biotin carboxylase and carboxyltransferase activities

To determine the reaction involved in the redox modification of ACCase, we examined the effects of thiol-reducing and thiol-oxidizing agents on the biotin carboxylase and carboxyltransferase activities of ACCase by using partly purified enzyme from light-adapted plants (Figure 4). The ACCase was activated approx. 2.3-fold by the addition of 2 mM DTT, as expected.

Figure 6 Reversible activation of oxidized enzyme by DTT

Partly purified enzyme obtained from plants exposed to light for 1 h was oxidized with 0.01 mM DTNB. After removal of the excess oxidizing reagent, the oxidized ACCase was preincubated with the indicated concentrations of DTT, and then (a) ACCase and (b) carboxyltransferase activities were measured. For comparison, the activity of enzyme not treated with oxidant (Figure 4) is shown by a dotted line.

The biotin carboxylase activity in this preparation was not activated by DTT, whereas the carboxyltransferase activity was activated 1.8-fold. This suggests that the carboxyltransferase activity is redox-regulated, although the activation by DTT was less than that for ACCase activity.

The effects of a thiol-oxidizing agent, DTNB, on the ACCase, biotin carboxylase and carboxyltransferase activities were examined (Figure 5). DTNB inhibited the ACCase and carboxyltransferase activities but not the biotin carboxylase activity. The concentrations required for 50 % inhibition of ACCase and carboxyltransferase were 1 and 8 μM respectively. These results suggest that the thiol groups of ACCase that were oxidized by DTNB and correlated with redox regulation are localized in the carboxyltransferase component.

To confirm this assumption, we examined the activity of carboxyltransferase in oxidized ACCase after incubation with a reducing reagent, DTT. Partly purified ACCase was first incubated with 0.01 mM DTNB. After removal of the excess oxidizing reagent, the effects of DTT on both ACCase and carboxyltransferase activities were examined (Figure 6). The activity of oxidized ACCase was very low in the absence of DTT, but increased with increasing concentration of DTT added, and attained a maximum value equivalent to the activity of ACCase not treated with oxidants. These results indicate that the oxidation and reduction of ACCase *in vitro* are reversible. Similar

Figure 7 Effects of pH on biotin carboxylase and carboxyltransferase activities

Partly purified enzyme from light-adapted plants was preincubated with 5 mM DTT at 30 °C for 10 min at pH 8.0, and biotin carboxylase (Δ) and carboxyltransferase ($\blacksquare$) activities were measured at different pH values; 100 mM Mops/KOH buffer (pH 6.8–7.5) and 100 mM Tricine/KOH buffer (pH 7.6–8.5) were used. The pH profile of ACCase in a previous paper [3] is shown by a dotted line. The activity is the value relative to that at pH 8.0, which was set at unity. The activities at pH 8 were 28 nmol/20 min per mg of protein for biotin carboxylase and 177 nmol/15 min per mg of protein for carboxyltransferase.

results were obtained for carboxyltransferase, indicating that the oxidation and reduction of carboxyltransferase activity of ACCase *in vitro* are reversible like that of ACCase activity. These results strongly support the idea that the thiol groups responsible for the redox regulation of ACCase reside on the carboxyltransferase component.

Effects of pH on biotin carboxylase and carboxyltransferase activities

The effect of pH on the biotin carboxylase and carboxyltransferase activities of ACCase was examined in the pH range 6.8–8.5 after preincubation of the ACCase with 5 mM DTT (Figure 7). Both activities showed profiles that were similar to, but slightly different from, that of the ACCase. The optimal pH values of biotin carboxylase and carboxyltransferase were more basic (pH 8.5) than that of ACCase (pH 8.1). In the physiologically relevant pH range (7–8), the activity of carboxyltransferase increased more rapidly than that of biotin carboxylase, like that of the ACCase. These results showed that both the biotin carboxylase and carboxyltransferase components in the ACCase were sensitive to pH, and were responsible for the pH dependence of the ACCase.

DISCUSSION

A growing body of evidence shows that the reducing power generated by the light reaction of photosynthesis reduces thioredoxin, which in turn reduces target enzymes of various processes in the chloroplasts [9,10]. The co-ordination of various chloroplast processes in light and darkness is performed partly by reduction and oxidation by using the redox cascade of the ferredoxin/thioredoxin system for reduction, and probably O_2 for oxidation. Recently we have shown that ACCase, a regulatory enzyme of fatty acid synthesis, is reductively activated by reduced thioredoxin *in vitro* [3]. Fatty acid synthesis might be linked to light by a redox cascade, resulting in co-ordination with photo-

synthesis. However, *in vivo* results are required to support this proposition.

We can assess the proportion of the reduced and oxidized forms of ACCase from the sensitivity to thiol-reducing and thiol-oxidizing agents. We have shown here that the stroma of chloroplasts isolated from dark-adapted plants has a higher proportion of oxidized, inactive ACCase and a lower proportion of the reduced form than the stroma isolated from light-adapted plants. Because ACCase protein levels did not change during irradiation, and carboxyltransferase β polypeptide is not light-induced [4], this means that the enzyme is reduced by light and is reoxidized in the dark *in vivo*.

It is interesting to measure how rapidly the enzyme undergoes oxidation/reduction *in vivo*. To measure plastidic ACCase activity we must isolate intact chloroplasts because the ACCase directly solubilized from leaves is always inactivated [4], probably by some components from leaves. It takes approx. 15 min to isolate intact chloroplasts, and we cannot exclude the effect of a brief light or dark period on chloroplasts during this procedure. It is therefore difficult to examine the effect of a brief irradiation on ACCase.

The activity in light-adapted plants was 3-fold that in dark-adapted plants (Figure 3). These activities were measured at pH 8. However, the stroma pH changes from 8 in the light to 7 in the dark. The ACCase activity at pH 7 is less than one-fifth of that at pH 8 [3], and the activities *in vivo* in dark-adapted plants is probably less than one-fifteenth of that in light-adapted plants. This might be why the ACCase activity *in vivo* in light differs markedly from that in the dark.

We have shown that a thiol-oxidizing agent, DTNB, inhibits ACCase activity (Figure 3) and that the oxidation is reversible (Figure 6). However, this agent is not present in the cell. One of the oxidizing agents found in the stroma is H_2O_2 , which oxidizes the thiol groups of reductively activated enzymes of the Calvin cycle [11]; this also inhibited ACCase. The concentration of H_2O_2 required for 90% inhibition of ACCase activity was similar to that for the enzymes of the reductive pentose phosphate cycle. H_2O_2 might oxidize ACCase *in vivo*, like the enzymes of the reductive pentose phosphate cycle.

ACCase catalyses two different half-reactions, the biotin carboxylase and carboxyltransferase reactions, to form malonyl-CoA. Our results showed that the carboxyltransferase reaction, but not the biotin carboxylase reaction, was redox-regulated. The profile of activation of carboxyltransferase by DTT and the profile of inhibition by DTNB are not the same as those of ACCase (Figures 4 and 5). These differences are probably caused by the assay method used here. Carboxyltransferase activity was measured in the reverse direction by using transcarboxylation from malonyl-CoA to free biotin methyl ester [8], and might differ from the activity in the true reaction indicated in step 2. Various factors might affect the responsiveness of carboxyltransferase to thiol-reducing and thiol-oxidizing reagents. The reversibility of the oxidation and the reduction of both ACCase and carboxyltransferase (Figure 6) suggests strongly that thiol groups of carboxyltransferase are responsible for the redox status of ACCase.

Recently, the biotin carboxylase from *Arabidopsis* was expressed in *Escherichia coli* and the activity was measured in the absence of DTT [12]. In that study the recombinant enzyme was suggested not to be redox-regulated.

In a previous paper [3] we showed that the activation of fatty acid synthesis in chloroplasts by light is caused partly by activation of the regulatory enzyme, ACCase. We proposed that the enzyme is probably stimulated by the transition of the stroma from pH 7 to pH 8 on illumination and by thiol-reducing reagents

produced by light. The ACCase activity *in vitro* at pH 8.0 was approx. 5-fold that at pH 7.0 [3]. The pH dependence of the biotin carboxylase and carboxyltransferase components in the ACCase showed that their activities at pH 8.0 were 5-fold and 7-fold that at pH 7.0 respectively (Figure 7), and both components affect the pH dependence of the ACCase. These results suggest that the activation of the ACCase *in vivo* by light arises from the activation of both components at elevated pH and from the activation of carboxyltransferase by thiol-reducing reagents. When the enzyme from dark-adapted plants was assayed, the activity was stimulated 6-fold by DTT (Figure 6). If these factors are taken together, the ACCase *in vivo* is probably stimulated approx. 6–30-fold by light, which results in the activation of fatty acid synthesis.

The carboxyltransferase α [13] and β subunits [14] in pea have two and eleven cysteine residues respectively; 2 and 5 cysteine residues of the α and β subunits respectively are conserved in higher plants. One or more of these cysteine residues are potential regulatory residues. Further studies are needed to identify the regulatory cysteine residues.

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