Planta (1994) 194:492–497

Planta

Interaction between fatty-acid and starch synthesis in isolated amyloplasts from cauliflower floral buds

Torsten Möhlmann, Renate Scheibe, H. Ekkehard Neuhaus

Pflanzenphysiologie, Fachbereich Biologie/Chemie, Universität Osnabrück, Barbarastrasse 11, D-49069 Osnabrück, Germany

Received: 2 November 1993 / Accepted: 12 February 1994

Abstract. The interaction of fatty-acid synthesis with starch synthesis has been studied in intact amyloplasts isolated from floral buds of cauliflower (*Brassica oleracea* L.). These amyloplasts perform acetate-dependent fatty-acid synthesis at maximum rates only at high external ATP concentrations. Neither pyruvate nor malate inhibit acetate-dependent fatty-acid synthesis. In contrast, acetate is inhibitory to the low pyruvate-dependent fatty-acid synthesis. These observations indicate that neither pyruvate nor malate are used as natural precursors of fatty-acid synthesis. In contrast to fatty-acid synthesis, the rate of glucose-6-phosphate-dependent starch synthesis is already saturated in the presence of much lower ATP concentrations. Rising rates of starch synthesis influence negatively the process of acetate-dependent fatty-acid synthesis. This inhibition appears to occur under both limiting and saturating concentrations of external ATP, indicating that the rate of ATP uptake is limiting when both biochemical pathways are active. The rate of starch synthesis is modulated specifically by the concentration of 3-phosphoglycerate in the incubation medium. This observation leads to the conclusion that the activity of ADP-glucose pyrophosphorylase is of primary importance for the control of both, starch and fatty-acid synthesis. Using the modified approach of Kacser and Burns (1973; Symp. Soc. Exp. Biol. **27**, 65-104) we have quantified the contribution of the rate of starch synthesis to the control of the metabolic flux through fatty-acid synthesis.

Key words: Amyloplast (bud) – *Brassica* – Control coefficient – Fatty-acid synthesis – Starch synthesis

Introduction

Isolated cauliflower-bud amyloplasts represent an excellent system to study the metabolism of heterotrophic plastids. The purification of these heterotrophic plastids is comparatively easy and the amyloplasts possess the physiological competence for acetate-dependent fatty-acid synthesis (Journet and Douce 1985) and for glucose-6-phosphate (Glc6P)-dependent starch synthesis (Neuhaus et al. 1993a). Both metabolic pathways are driven by the uptake of several compounds from the medium into the amyloplastic stroma. For example, acetate-dependent fatty-acid synthesis depends upon the import of acetate, CoA, and ATP (Journet and Douce 1985) whereas Glc6P-dependent starch synthesis depends upon the uptake of Glc6P, 3-phosphoglyceric acid (PGA) and ATP (Neuhaus et al. 1993a). Because both metabolic pathways require ATP in order to drive the conversion of the specific precursors, a possible interaction between these metabolic pathways can be assumed. The fact that starch synthesis and lipid metabolism influence each other is demonstrated by analysis of the effect of a reduced capacity for starch synthesis in pea endosperm upon the lipid content of this tissue. Bettey and Smith (1990) observed that wrinkled-seeded pea embryos, which are characterized by a mutation of the *r*-locus leading to a lower starch content, exhibit significantly increased levels of both storage and structural lipids.

Fatty-acid synthesis is strictly localized in the plastidic compartment (Stumpf 1980). The ability to synthesize fatty acids in isolated heterotrophic plastids has been shown several times. Kleinig and Liedvogel (1980) demonstrated an acetate-dependent fatty-acid synthesis in isolated chromoplasts from the daffodil. Isolated plastids from castor-bean endosperm were shown to be able to incorporate [^{14}C]acetate into lipids (Miernyk and Dennis 1983). Pea-root amyloplasts exhibit high rates of acetate-dependent fatty-acid synthesis if they are energized by external ATP and if they are supplied with reducing power via dihydroxyacetone phosphate (DHAP) in combination with oxaloacetate (Kleppinger-Sparace et al. 1992).

Abbreviations: ADPGlc-PPase = ADPglucose pyrophosphorylase; Glc6P = glucose-6-phosphate; PGA = 3-phosphoglyceric acid

Correspondence to: H.E. Neuhaus; FAX: 49(541) 9692870; e-mail: neuhaus@sfbbio1.biologie.uni-osnabrueck.de

Recently, Smith et al. (1992) demonstrated that plastids isolated from castor-bean endosperm are able to use pyruvate or malate instead of acetate as a source to drive fatty-acid synthesis. This use is strictly dependent on the presence of an active pyruvate dehydrogenase. The presence of this enzyme in isolated cauliflower-bud amyloplasts has already been demonstrated (Journet and Douce 1985).

Because isolated cauliflower-bud amyloplasts are able to drive ATP-stimulated fatty-acid and starch synthesis, and because they possess a pyruvate dehydrogenase we tried to answer the questions: (i) Do isolated cauliflower-bud amyloplasts use pyruvate or malate at significant rates to drive fatty-acid synthesis; (ii) what are the ATP optima for fatty-acid and starch synthesis, respectively; (iii) is there an interaction between the two metabolic pathways; and (iv) is it possible to quantify the contribution of the rate of Glc6P-dependent starch synthesis to the control of the rate of acetate-dependent fatty-acid synthesis?

Materials and methods

Isolation of cauliflower-bud amyloplasts. Cauliflower (*Brassica oleracea* L. cv. botrytis, cult. Prince de Bretagne) curds (floral buds) were purchased from the local market. Cauliflower-bud amyloplasts were isolated according to the method of Journet and Douce (1985), modified as described in Neuhaus et al. (1993b). The final plastid preparation was resuspended in an incubation medium consisting of 15 mM Hepes-KOH (pH 7.2), 2 mM $MgCl_2$, 2 mM EDTA, 350 mM sorbitol. The total protein concentration was measured colorimetrically using a detergent-insensitive assay: a bicinchoninic acid-copper II sulfate solution (Sigma, Deisenhofen, Germany) used according to the supplier's instructions.

Glucose-6-phosphate-dependent starch synthesis was assessed as given in Neuhaus et al. (1993a). The standard medium consisted of the incubation medium as given above and contained in addition 5 mM [1-^{14}C]Glc6P (0.74 MBq·mmol^{-1}), and various concentrations of both ATP and PGA. Starch synthesis was carried out for 30 min and was stopped by heat-inactivation. Removal of unincorporated radioactivity and digestion of radioactively labeled starch was as described earlier (Neuhaus et al. 1993a).

Acetate- or pyruvate-dependent fatty-acid synthesis was carried out by a method similar to that of Journet and Douce (1985). The standard medium ("fatty-acid synthesis medium") consisted of the incubation medium, containing the following compounds in addition: 0.5 mM [^{14}C]sodium acetate (Amersham-Buchler, Braunschweig, Germany) or 0.5 mM [2-^{14}C]sodium pyruvate (NEN, Dreieich, Germany; 14.8 MBq·mmol^{-1} each), 3 µM CoA, 0.5 mM $NADH_2$, 0.3 mM P_i, 1 mM dithiothreitol (DTT), 1 mM $MnCl_2$, ATP and PGA at the given concentrations. The incubation was carried out in a 2-ml Eppendorf-reaction vessel which contained 150 µl of the incubation medium and the additional compounds at twofold concentrations. The reactions were started by the addition of 150 µl amyloplast suspension containing 400 µg of plastid protein. Termination of the reaction and extraction of the products of fatty-acid synthesis were as given in Mudd and DeZacks (1981), modified for the small reaction volumes.

Termination of fatty-acid synthesis and extraction of the reaction products. The reactions were stopped by the addition of 1 ml chloroform/methanol/acetic acid (50/100/5, by vol.) and, after adding another 330 µl of chloroform plus 330 µl double-distilled H_2O, mixed vigorously. After centrifugation for 2 min in an Eppendorf (Hamburg, Germany) centrifuge (16000·g), the supernatant was removed and discarded, whereas 160 µl of methanol plus 500 µl double-distilled H_2O were added to the remaining lower phase. Vigorous shaking was followed by separation of the phases by centrifugation as given above. This extraction was performed twice. The remaining radioactivity in the lower phase was quantified after transfer of the whole lower phase into a 20-ml scintillation vessel, followed by evaporation of the solvent in an air stream, and addition of 18 ml scintillation cocktail (Hydroluma®, Baker-Chemicals, Groß-Gerau, Germany). Scintillation counting was performed in a Tricarb 2500 scintillation counter (Packard, Darmstadt, Germany). Using this extraction protocol, more than 99.8% of the unincorporated radioactively labeled acetate could be removed, as determined by extraction of control samples which lacked plastid protein. The efficiency of this method for extraction of hydrophobic compounds was checked by the extraction of control samples which were supplied with ^{14}C-labeled palmitate. More than 88% of the added ^{14}C-labeled palmitate was recovered.

Results

Precursor dependency of fatty-acid synthesis in isolated cauliflower-bud amyloplasts. Both biochemical pathways, Glc6P-dependent starch synthesis and acetate-dependent fatty-acid synthesis are dependent upon the presence of ATP. The Glc6P-dependent starch synthesis requires ATP for the generation of the nucleotide sugar ADP-glucose (ADPGlc), whereas fatty-acid synthesis requires ATP to supply acetyl-CoA synthetase and the subsequent carboxylation of acetyl-CoA. Therefore, we ana-

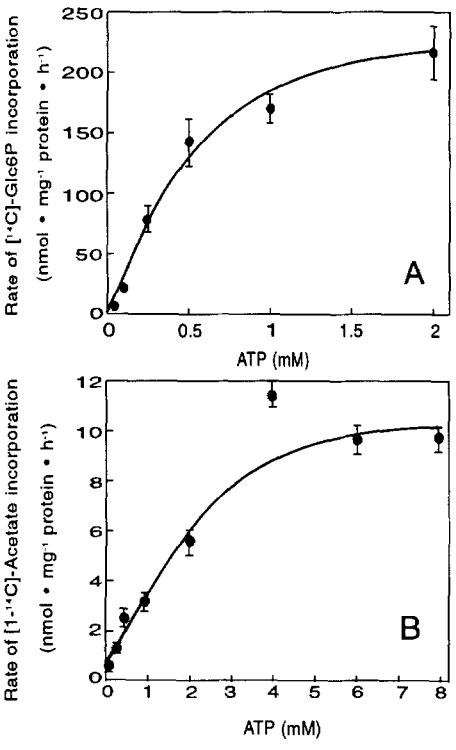

Fig. 1 A, B. Effect of rising ATP concentrations upon Glc6P-dependent starch synthesis and acetate-dependent fatty-acid synthesis in amyloplasts isolated from cauliflower buds. **A** Glc6P-dependent starch synthesis was performed in incubation medium containing in addition [1-^{14}C]Glc6P (5 mM), PGA (1 mM) and varying concentrations of ATP. **B** Fatty-acid synthesis was performed in "fatty-acid synthesis medium" containing varying concentrations of ATP. Data are the means (±SE) of three independent experiments

Table 1. Precursor dependency of fatty-acid synthesis in isolated cauliflower-bud amyloplasts. Incubations were performed in "fatty-acid synthesis medium" containing radioactively labeled acetate or pyruvate (0.5 mM each) and the unlabeled compounds at the indicated concentrations. Data are the means (±SE) of three independent experiments

Labeled substrate	Unlabeled compound	Rate of fatty-acid synthesis
		[nmol C2 unit · (mg protein)$^{-1}$ · h^{-1}]
Acetate	–	12.4 ±1.40
	Malate (1 mM)	13.6 ±2.10
	Pyruvate (1 mM)	12.7 ±1.80
Pyruvate	–	1.11±0.06
	Acetate (0.5 mM)	0.79±0.09

lyzed how rising concentrations of external ATP influence both biochemical pathways. Figure 1 demonstrates that the rate of Glc6P-dependent starch synthesis is saturated at an ATP concentration of around 1 mM. In contrast, acetate-dependent fatty-acid synthesis reaches its maximum rates only at significantly higher concentrations of ATP. Under the chosen conditions a concentration of 4 mM ATP was sufficient to saturate fatty-acid synthesis.

Journet and Douce (1985) demonstrated that cauliflower-bud amyloplasts are able to convert acetate into fatty acids at rates similar to those observed by us. Isolated plastids from developing castor-bean endosperm are able to use malate or pyruvate for fatty-acid synthesis (Smith et al. 1992). These authors have demonstrated that the addition of pyruvate or malate reduces the rate of incorporation of radioactively labeled acetate into fatty acids. Therefore, we used a similar approach to evaluate whether these compounds can drive fatty-acid synthesis. Table 1 shows that the rate of acetate-dependent fatty-acid synthesis was nearly unchanged by the simultaneous addition of pyruvate or malate. Using radioactively labeled pyruvate (2-^{14}C) it was demonstrated that this compound leads to a rate of fatty-acid synthesis which was less than 9% of the comparable acetate-dependent fatty-acid synthesis. This low pyruvate-induced fatty-acid synthesis is further decreased in the presence of acetate (Table 1). These results indicate that acetate serves as the major substrate for fatty-acid synthesis in isolated cauliflower-bud amyloplasts.

Interaction between starch synthesis and fatty-acid synthesis. Both biochemical pathways require exogenously supplied ATP for the conversion of the specific precursors to the endproducts starch and fatty acids. In order to evaluate a possible influence of starch synthesis upon fatty-acid synthesis, we performed experiments in which the rate of starch synthesis was specifically increased by raising the PGA levels. Figure 2A clearly demonstrates that rising levels of PGA activate Glc6P-dependent starch synthesis under limiting ATP concentrations. Starch synthesis is significantly increased even by submillimolar concentrations of PGA. The reason for this stimulation is probably due to an activation of ADPGlc pyrophosphorylase (ADPGlc-PPase; see Neuhaus et al. 1993a). Figure 2B further demonstrates that rising rates of starch synthesis correlate with a significant decrease of acetate-dependent fatty-acid synthesis.

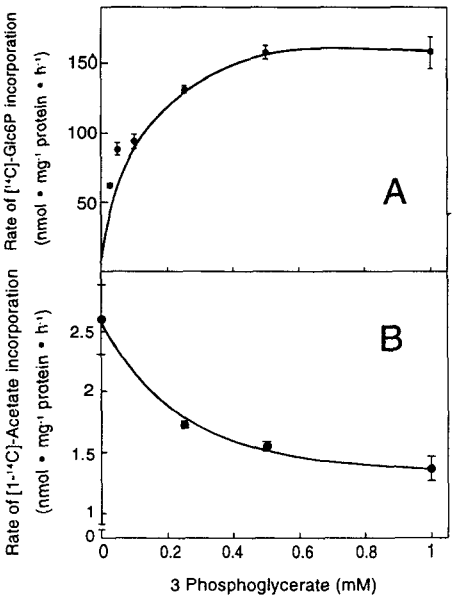

Fig. 2A, B. Effect of PGA upon Glc6P-dependent starch synthesis and acetate-dependent fatty-acid synthesis under limiting ATP in amyloplasts isolated from cauliflower buds. **A** Glc6P-dependent starch synthesis was performed in incubation medium containing in addition: [1-^{14}C]Glc6P (5 mM), ATP (0.5 mM), and varying concentrations of PGA. **B** Fatty-acid synthesis was performed in "fatty-acid synthesis medium" containing in addition Glc6P (5 mM), ATP (0.5 mM), and varying concentrations of PGA. Data are the means (±SE) of three independent experiments

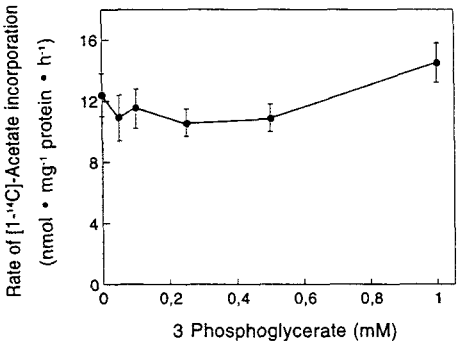

Fig. 3. Effect of rising PGA concentrations upon the rate of acetate-dependent fatty-acid synthesis in the absence of Glc6P. Fatty-acid synthesis was performed in "fatty-acid synthesis medium" containing ATP (4 mM) and the given concentrations of PGA. Data are the means (±SE) of three independent experiments

To check whether the effect of rising rates of starch synthesis upon the rate of acetate-dependent fatty-acid synthesis is a specific rather than an unspecific inhibitory effect of PGA, we analyzed the effect of PGA upon fatty-acid synthesis in the absence of Glc6P. As demonstrated in Fig. 3, rising concentrations of PGA in the absence of Glc6P do not influence acetate-dependent fatty-acid synthesis.

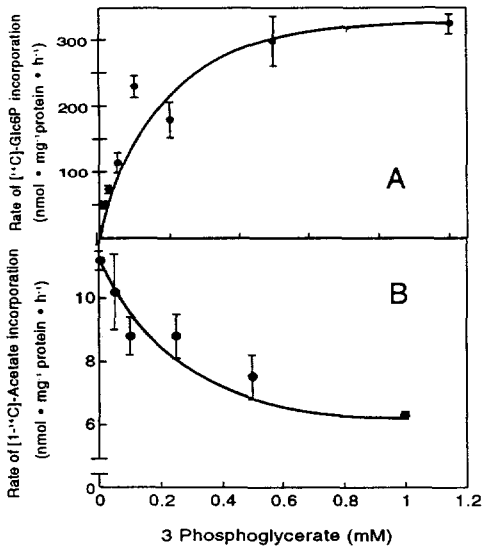

Fig. 4A, B. Effect of PGA upon Glc6P-dependent starch synthesis and acetate-dependent fatty-acid synthesis under saturating ATP. **A** Glc6P-dependent starch synthesis was performed in incubation medium containing in addition: [1-^{14}C]Glc6P (5 mM), ATP (4 mM), and varying concentrations of PGA. **B** Fatty-acid synthesis was performed in "fatty-acid synthesis medium" containing in addition Glc6P (5 mM), ATP (4 mM), and varying concentrations of PGA. Data are the means (±SE) of three independent experiments

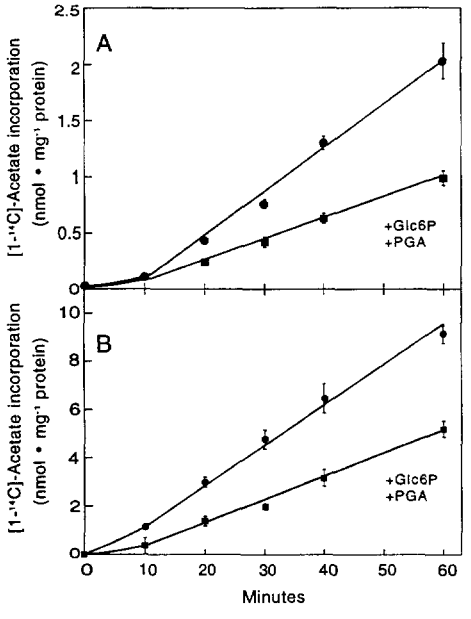

Fig. 5A, B. Time course of acetate-dependent fatty-acid synthesis in the absence or presence of stimulated Glc6P-dependent starch synthesis. Isolated cauliflower-bud amyloplasts were incubated for the given time periods. **A** Fatty-acid synthesis under limiting ATP concentrations was performed in "fatty-acid synthesis medium" containing ATP (0.5 mM) plus/minus Glc6P (5 mM) and PGA (1 mM). **B** Fatty-acid synthesis under saturating ATP concentrations was performed in "fatty-acid synthesis medium" containing ATP (4 mM) plus/minus Glc6P (5 mM) and PGA (1 mM). Data are the means (±SE) of three independent experiments

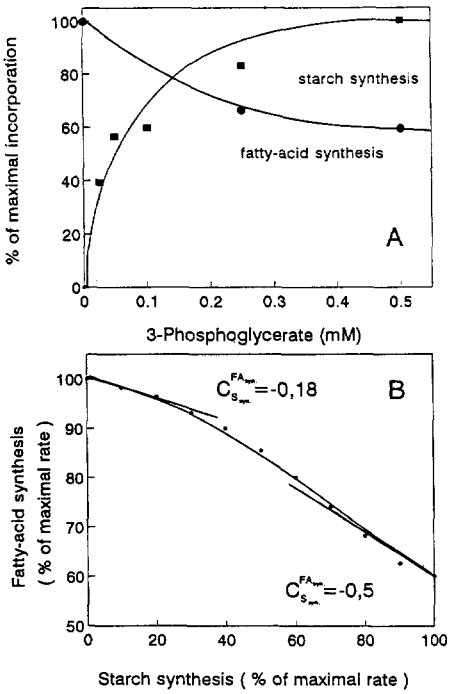

Fig. 6A, B. Relative changes of Glc6P-dependent starch synthesis and acetate-dependent fatty-acid synthesis and calculation of the control coefficient of starch synthesis for fatty-acid synthesis. **A** Data are calculated from Fig. 2. **B** The data of **A** are replotted as relative changes of starch synthesis (δss/ss) and relative changes of fatty-acid synthesis (δfas/fas). The tangent represents the control coefficient C of Glc6P-dependent starch synthesis over the flux through fatty-acid synthesis according to the equation: C=(δss/ss)/(δfas/fas)

Because we observed an inhibition of acetate-dependent fatty-acid synthesis by rising rates of starch synthesis under limiting ATP concentrations, we were interested to analyze the effect of rising rates of starch synthesis upon fatty-acid synthesis under high ATP concentrations. Figure 4 shows that even under conditions of saturating ATP (4 mM) an increase of starch synthesis (Fig. 4A) leads to a decrease of acetate-dependent fatty-acid synthesis (Fig. 4B).

To examine whether the decreased rate of acetate-dependent fatty-acid synthesis is due to the absolute availability of ATP rather than to the ATP concentration inside the amyloplast stroma, we analyzed the time course of the effect of starch synthesis upon the rate of fatty-acid synthesis. Figure 5 demonstrates that under both conditions, at limiting and saturating ATP levels, the rate of acetate-dependent fatty-acid synthesis is significantly lowered in the presence of simultaneous starch synthesis. At limiting ATP concentrations the rate of acetate-dependent fatty-acid synthesis is decreased by about 55% (Fig. 5A) by the simultaneous starch synthesis, whereas a 32% decrease was observed at saturating ATP concentrations (Fig. 5B).

Quantitation of the influence of starch synthesis upon fatty-acid synthesis. According to the results presented so far, there is a clear influence of Glc6P-dependent starch synthesis upon acetate-dependent fatty-acid synthesis. To

further analyze this interaction we plotted the relative changes of PGA-stimulated Glc6P-dependent starch synthesis and of an inhibited acetate-dependent fatty-acid synthesis against the PGA concentration. As revealed in Fig. 6A, starch synthesis has already started to increase at submillimolar concentrations of PGA. This increase leads to a significant decrease of fatty-acid synthesis.

From the curves in Fig. 6A we replotted the relative changes of the rate of fatty-acid synthesis against the relative changes of starch synthesis. This kind of plot allows the calculation of the correlation between the increase of the flux in starch synthesis and the decrease of the metabolic flux in the pathway of acetate-dependent fatty-acid synthesis. Because an increase of the rate of starch synthesis correlates with a decrease of the rate of fatty-acid synthesis, a negative correlation is observed. It is evident from this plot that the relative influence of starch synthesis upon the rate of fatty-acid synthesis is increased as starch synthesis gets closer to the maximal rate. An increase of the PGA concentration from 0 to 0.2 mM leads to a dramatic stimulation of the rate of starch synthesis, and is accompanied by a significant inhibition of the rate of fatty-acid synthesis. Under conditions where the rate of starch synthesis is close to its maximum (e.g. 90%) a small further increase of the rate of starch synthesis leads to a comparably more pronounced decrease of the rate of fatty-acid synthesis. This result explains the increase of the control coefficient of starch synthesis for fatty-acid synthesis.

Discussion

Smith et al. (1992) demonstrated that plastids isolated from castor-bean endosperm can use malate or pyruvate as exogenous substrates for fatty-acid synthesis. Table 1 clearly demonstrates that this is not the case for fatty-acid synthesis in isolated cauliflower-bud amyloplasts, because the additional presence of malate or pyruvate did not lower the incorporation of acetate into newly synthesized fatty acids. Furthermore, specifically labeled pyruvate serves only as an inefficient substrate for fatty-acid synthesis (Table 1). Journet and Douce (1985) found appreciable amounts of pyruvate dehydrogenase activity in isolated plastids from cauliflower buds. Therefore, two possible reasons for the inability to convert pyruvate or malate into fatty acids can be envisioned: firstly, isolated amyloplasts might be unable to import pyruvate or malate, and secondly, it is possible that pyruvate dehydrogenase is not active under the chosen conditions. If the latter is true, the inability to convert malate into fatty acids is explainable. The presence of malic enzyme, which decarboxylates malate to pyruvate has been unequivocally demonstrated for heterotrophic plastids of various sources (El-Shohra and ap Rees 1991).

Both, acetate-driven fatty-acid synthesis and Glc6P-induced starch synthesis are dependent upon ATP uptake into the amyloplast stroma. Figure 1 demonstrates that rising levels of external ATP primarily favour Glc6P-dependent starch synthesis. At an ATP level which saturates Glc6P-dependent starch synthesis, acetate-dependent fatty-acid synthesis only exhibits around 30% of the maximal rate. This result implies that the process of starch synthesis has an important function during development of cauliflower-bud tissue. Starch represents a storage product which can be synthesized at high rates and which is also remobilized by isolated amyloplasts at significant rates (data not shown). Therefore, starch acts as a temporary carbon store in this tissue when there is an excess supply of carbon compounds. When these carbohydrates are required for anabolic processes, a rapid mobilization is induced by hydrolysis and a subsequent phosphorylation of the products (data not shown).

As demonstrated in Figs. 2 and 4, an increase of the rate of starch synthesis leads to a decrease of acetate-dependent fatty-acid synthesis. This correlation occurs under both limiting and saturating ATP concentrations and, as revealed in Fig. 5, the inhibited rate of acetate-dependent fatty-acid synthesis is strictly linear with time. This result leads to the conclusion that ATP uptake could be the limiting factor for the rate of acetate-dependent fatty-acid synthesis. If the available amount of stromal ATP is lowered by a second ATP-consuming reaction (e.g. Glc6P-dependent starch synthesis), the rate of acetate-dependent fatty-acid synthesis is reduced, indicating that the ATP translocator does not equilibrate ATP concentrations between the external medium and the amyloplastic stroma. Neuhaus et al. (1993a) have previously demonstrated that the V_{max} of the ATP uptake into isolated cauliflower-bud amyloplasts is only about 30% higher than the corresponding Glc6P-dependent starch synthesis. This result explains why a simultaneously ATP-consuming Glc6P-dependent starch synthesis must lead to an inhibition of acetate-dependent fatty-acid synthesis.

As revealed in Figs. 2 and 4, the PGA-stimulated starch synthesis induces an inhibition of acetate-dependent fatty-acid synthesis. Obviously, the activity of the PGA-stimulated ADPGlc-PPase is responsible for a fine control of both starch synthesis and acetate-dependent fatty-acid synthesis.

As demonstrated above, the rate of starch synthesis clearly influences the rate of acetate-dependent fatty-acid synthesis. Furthermore, by modifying the approach used by Kacser and Burns (1973), we have obtained information about the quantitative influence of starch synthesis upon fatty-acid synthesis.These authors quantified the control of a specific enzyme over the flux through a metabolic pathway by correlating the specific decrease of an enzyme activity with the corresponding changes in the metabolic pathway in which the enzyme is involved. This approach led to the concept of a "control coefficient" for a specific enzyme. Because changes in one metabolic pathway can influence fluxes through other metabolic pathways, this concept was extended by Sauro et al. (1987). Neuhaus and Stitt (1990) applied this approach to plant biochemistry for the quantitation of the effect of an inhibited starch synthesis upon the carbon flux into sucrose synthesis in green tissues.

As demonstrated in Fig. 6A, even low concentrations of PGA strongly stimulate Glc6P-dependent starch synthesis. This increase leads to a significant reciprocal de-

crease of acetate-dependent fatty-acid synthesis. If the relative changes of starch synthesis (δss/ss) are divided by the relative changes of fatty-acid synhesis (δfas/fas), a negative value is obtained. In the range of 10–20% activation of starch synthesis, a control coefficient for the flux through fatty-acid synthesis of −0.18 was determined. If this value is compared with changing rates of starch synthesis around 90%, the control coefficient appears to be more than threefold higher (−0.50). This means that, despite much lower absolute changes, a significantly higher percentage of the control over acetate-dependent fatty-acid synthesis is now exhibited by the rate of starch synthesis. This observation is probably the result of a lowered ATP concentration in the amyloplast stroma when Glc6P-dependent starch synthesis runs close to its maximal rate.

Acknowledgements. This work was financially supported by the Deutsche Forschungsgemeinschaft, SFB 171-C16.

References

Bettey, M., Smith, A.M. (1990) Nature of the effect of the *r*-locus on the lipid content of embryos of peas (*Pisum sativum* L.). Planta **180**, 420–428

El-Shora, H., ap Rees, T. (1991) Intracellular location of $NADP^+$-linked malic enzyme in C_3 plants. Planta **185**, 362–367

Journet, E.-P., Douce, R. (1985) Enzymic capacities of purified cauliflower bud plastids for lipid synthesis and carbohydrate metabolism. Plant Physiol. **79**, 458–467

Kacser, H., Burns, J.A. (1973) The control of flux. Symp. Soc. Exp. Biol. **27**, 65–104

Kleinig, H., Liedvogel, B. (1980) Fatty acid synthesis by isolated chromoplasts from the daffodil. Energy source and distribution patterns of the acids. Planta **150**, 166–169

Kleppinger-Sparace, K.F., Stahl, R.J., Sparace, S.A. (1992) Energy requirements for fatty acid synthesis and glycerolipid biosynthesis from acetate by isolated pea root plastids. Plant Physiol. **98**, 723–727

Miernyk, J.A., Dennis, D.T. (1983) The incorporation of glycolytic intermediates into lipids by plastids isolated from developing endosperm of castor oil seeds (*Ricinus communis* L.). J. Exp. Bot. **34**, 712–718

Mudd, J.B., DeZacks, R. (1981) Synthesis of phosphatidylglycerol by chloroplasts from leaves of *Spinacia oleracea* L. (spinach). Arch. Biochem. Biophys. **209**, 584–591

Neuhaus, H.E., Stitt, M. (1990) Control analysis of photosynthate partitioning impact of reduced activity of ADP-glucose pyrophosphorylase or plastid phosphoglucomutase on the fluxes to starch and sucrose in *Arabidopsis thaliana* (L.) Heynh. Planta **182**, 445–454

Neuhaus, H.E., Henrichs, G., Scheibe, R. (1993a) Characterization of glucose-6-phosphate incorporation into starch by isolated intact cauliflower-bud plastids. Plant Physiol. **101**, 573–578

Neuhaus, H.E., Thom, E., Batz, O., Scheibe, R. (1993b) Purification of highly intact plastids from various heterotrophic plant tissues. Analysis of enzymic equipment and precursor dependency for starch biosynthesis. Biochem. J. **296**, 495–501

Sauro, H.M., Small, J.R., Fell, D.A. (1987) Metabolic control and its analysis. Extensions to the theory and matrix model. Eur. J. Biochem. **165**, 215–221

Smith, R.G., Gauthier, D.A., Dennis, D.T., Turpin, D.H. (1992) Malate- and pyruvate-dependent fatty acid synthesis in leucoplasts from developing castor endosperm. Plant Physiol. **98**, 1233–1238

Stumpf, P.K. (1980) Biosynthesis of saturated and unsaturated fatty acids. In: The biochemistry of plants, vol. **4**, pp. 177–199, Stumpf P.K., Conn E.E., eds., Academic Press, New York