INCORPORATION OF UDP-GALACTOSE-^{14}C BY SPINACH CHLOROPLASTS

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SUMMARY

The incorporation of UDP-galactose-^{14}C into lipids of spinach chloroplasts as affected by different experimental conditions was studied. It was found that the use of a pyrophosphate buffer and the addition of albumine during isolation and incubation of chloroplasts enhance the incorporation of UDP-galactose-^{14}C compared with other buffers or no addition of albumine. Furthermore, the ability of chloroplasts to incorporate UDP-galactose decreased with the age of the chloroplasts. The rate of UDP-galactose incorporation into lipids of the chloroplasts appears to depend on the condition of the chloroplasts.

1. INTRODUCTION

The galactolipids monogalactosyl diglyceride and digalactosyl diglyceride were isolated for the first time by the group of CARTER (1956, 1961a, 1961b) and have since been found by several authors in photosynthetic plant tissues as well as in photosynthetic bacteria. The lipid fraction of the chloroplasts consists mainly of galactosyl diglycerides (BENSON, WINTERMANS & WISER 1959). A possible function of the galactolipids has been proposed by ROSENBERG (1967) and by CHANG & LUNDIN (1965). Together, the findings in the literature seem to indicate that galactolipids are important for the photosynthetic apparatus of the plants. Most investigators deal with the enzymatic break-down of galactolipids (SASTRY & KATES 1964, HELMSING 1967, 1969). However, little research has been carried out on the biosynthesis of galactolipids in higher plants. NEUFELD & HALL (1964) reported the incorporation of galactose from UDP-galactose into lipids by isolated chloroplasts. The presence of enzymes, necessary for galactolipid biosynthesis, in etiolated spinach tissue has been reported by ONGUN & MUDD (1968), who also described the kinetics of galactolipid formation in isolated chloroplasts and the nature of the acceptor for galactosyl units.

This paper deals with the effect of different buffers and the addition of albumine during the isolation and incubation of the chloroplasts, and the duration of the incubation on the incorporation of UDP-galactose into the lipids of spinach chloroplasts.

2. MATERIALS AND METHODS

Chloroplasts, obtained from spinach plants grown in the greenhouse, were prepared according to the method described by WINTERMANS, HELMSING,

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POLMAN, VAN GISBERGEN & COLLARD (1969). UDP-galactose-^{14}C, specific activity 280 mC/mmole, uniformly labelled in the galactose moiety (New England Nuclear), UDP-galactose-^{12}C (Sigma), and 1,2 diolein containing less than 0.5% of the 1,3-isomer (Supelco) were used in the experiments.

The incubations took place in a waterbath at 30° under continuous shaking. The incubation mixtures consisted of 1 ml chloroplast suspension, 20 µl UDP-galactose-^{12}C (5 mg/ml ethanol 35%), 10 µl UDP-galactose-^{14}C (about 0.1 µC). The reaction was stopped by adding 2 ml methanol, 2 ml chloroform and 0.6 ml water. After mixing thoroughly the incubation mixtures were centrifuged at 2500 × g for 4 min. Samples of 0.2–0.6 ml of the upper as well as the lower phase were brought into scintillation vials and evaporated to dryness. Subsequently the residues were dissolved in 10 ml of Brays scintillation liquid and the radioactivity determined in a Philips scintillation analyzer. Incorporation of UDP-galactose-^{14}C is expressed as total cpm lower phase / total cpm lower phase plus upper phase times 100%. The chlorophyll content was determined according to WINTERMANS & DE MOTS (1965) in 96% ethanol and the absorbance of each sample was read on a Spectronic 505 (Bausch & Lomb) spectrophotometer.

3. RESULTS AND DISCUSSION

In preliminary experiments it was established that the pH optimum for UDP-galactose incorporation was 7.5 using pyrophosphate buffers.

In the following experiment two different buffers were used during isolation of the chloroplasts, namely, 0.01 M phosphate plus 0.5 M sucrose, pH 7.4, and 0.01 M pyrophosphate plus 0.5 M mannite, pH 7.5. Subsequently, the chloroplast suspensions were incubated in the same buffers for one hour following the above described procedures. In the case of phosphate buffer 4.8% (2107 cpm) of the total radioactivity/10 µg chlorophyll was found in the chloroform phase versus 17.2% (7280 cpm) when the pyrophosphate buffer was used. On account of this result the pyrophosphate buffer was used in the following experiments for the isolation of the chloroplasts, unless mentioned otherwise.

In a subsequent experiment the following buffers were used during the incubation of the chloroplasts: 0.01 M pyrophosphate plus 0.5 M mannite, pH 7.5 (a), 0.05 M tris-HCl plus 0.35 M NaCl, pH 7.5 (b), and water (c). In this case the following amounts of incorporation of the UDP-galactose-^{14}C were observed: 17.6% (1579 cpm), 7.7% (1147 cpm) and 2.3% (471 cpm)/10 µg chlorophyl for (a), (b) and (c) respectively. This result suggests a correlation between the incorporation of UDP-galactose and the condition of the chloroplasts, since it has been found previously by Wintermans & Sassen (unpublished results) that a buffer consisting of tris-HCl plus 0.35 M NaCl caused broken outer membranes in 75–80% of the chloroplasts, whereas in a pyrophosphate buffer plus mannite nearly all the chloroplasts remain visually in a good morphological condition.

The influence of the addition of albumine to the pyrophosphate buffer during

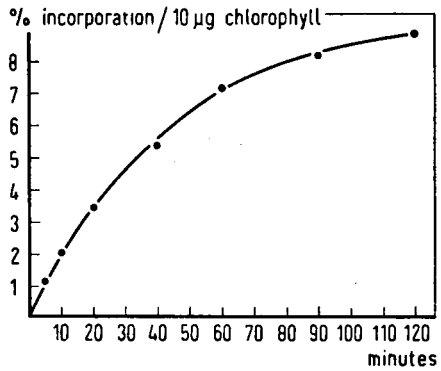

Fig. 1.
The incorporation of UDP-galactose-^{14}C by chloroplasts during incubation for different time intervals.

the isolation as well as during the incubation of chloroplasts on the incorporation of UDP-galactose-^{14}C was also investigated. Without the addition of albumine 12.8 % (5012 cpm) of the total radioactivity per 25 µg chlorophyll was retained by the chloroform phase. However, when albumine to 1 % was added as much as 23.8 % (9275 cpm) of the total radioactivity per 25 µg chlorophyll was found in the chloroform phase. Thus the addition of albumine enhances the incorporation of the label to as much as twice the incorporation without albumine. These results are similar to those reported by WASSERMANN & FLEISCHER (1968) and by WINTERMANS *et al.* (1969), who also found that the addition of albumine stabilizes the condition of the chloroplasts.

In all the above experiments the duration of the incubation was one hour. *Fig. 1* gives the results of different durations of incubation of the chloroplasts in pyrophosphate buffer. It is shown that the incorporation approaches a maximum level after 100–120 minutes.

In the above experiments the chloroplasts were incubated inmediately after preparation. *Fig. 2* gives the results of an experiment in which the chloroplasts were kept at 4° for different time intervals before the incubation took place. It

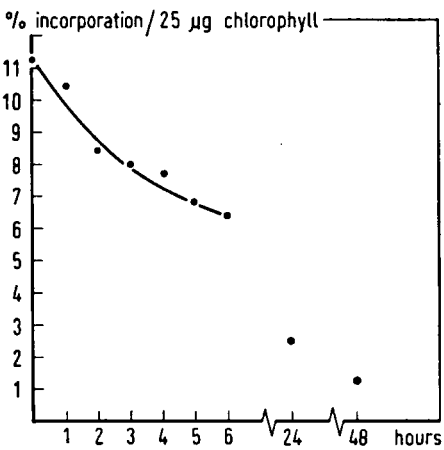

Fig. 2.
The incorporation of UDP-galactose-^{14}C by aging chloroplasts.

is obvious from the results that the ability of chloroplasts to incorporate UDP-galactose-^{14}C decreases with age of the chloroplasts.

Since all the experimental conditions described above in fact regulate the condition of the chloroplasts it follows that the incorporation of UDP-galactose is highly dependent on the condition of the chloroplasts.

Finally, two-dimensional thin layer chromatography was applied to the radioactive fraction present in the chloroform phase after incubation. In the first direction, chloroform-methanol-7N NH_4OH (65:30:4, v/v) was used as developing solvent followed by development in the second direction with a solvent consisting of chloroform-methanol-acetic acid-water (170:25:25:6, v/v). The radioactive spots were determined by means of autoradiography. Two spots were identified as mono- and digalactosyl diglyceride, respectively. A third spot tentatively indicated the presence of trigalactosyl diglyceride as was inferred from the data obtained by ONGUNN & MUDD (1968). All three spots showed, as expected, a positive reaction with the periodate-Schiff reagent.

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