MALTOSE PHOSPHATE IN ISOLATED SPINACH CHLOROPLASTS

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1. Introduction

Although it is well documented that maltose is formed by the action of amylase on starch [1], recent findings show that it is also synthesised from α-glucose 1-phosphate and that it serves as an intermediate in sugar metabolism. The latter is indicated firstly by the labelling kinetics of maltose during photosynthesis in $^{14}CO_2$, which resemble those of sugar phosphates but not those of reserve material [2], and secondly by the unequal labelling of the two glucosyl moieties after short term photosynthesis of leaves and isolated chloroplasts in $^{14}CO_2$ [3].

Direct evidence for the de novo synthesis of maltose was obtained from in vitro experiments with enzyme preparations from spinach leaves. It could be demonstrated that maltose is formed when α-glucose 1-phosphate is supplied as the glucosyl donor [4]. In order to investigate whether maltose phosphate, which might be an intermediate in maltose biosynthesis, is formed in chloroplasts [4], isolated intact chloroplasts were prepared according to Beck et al. [5] and $^{14}CO_2$-fixation was carried out for 8 min in a rotating flask at an illumination of 55 000 lux. The incubation mixture, which was maintained at 20°C and flushed with N_2, contained 22 ml HEPES buffer [6] pH 7.6, chloroplasts equivalent to 3 mg of chlorophyll and 17.2 μM $NaH^{14}CO_3$ (spec. act. 7.55 μCi/μmole). The reaction was terminated by the addition of an equal vol of boiling methanol.

After having been concentrated to about 4 ml, the extract was subjected to column chromatography on a Dowex 1 × 8 formate column (100–200 mesh, 200 × 0.5 cm) and fractions of 3 ml were collected. Elution was performed with 45 ml of water (15 fractions) followed by the gradient of formic acid plus formate described as system I by Heldt and Klingenberg [7].

The fractions containing the sugarmonophosphates (fraction numbers 60–80) were clearly separated from those containing the neutral substances (fractions 1–20), glycolic acid (fractions 53–57), 3-phosphoglycerate (fractions 115–130), and the sugar diphosphates (fractions 135–145 and fractions 155–165). Free maltose was eluted together with the neutral compounds.

After removal of formic acid and formate (cation exchange followed by evaporation), the monophosphate fraction was dephosphorylated with acid phosphatase (Boehringer, Mannheim) and the incubation mixture was subjected to two dimensional paper chromatography using the following solvents: (I) 88% phenol–water–acetic acid–1 M Na_2EDTA (840 : 160 : 10 : 1), (II) 1-butanol–pyridine–acetic acid–water (60 : 40 : 3 : 30). The autoradiogram of the chromatogram showed a spot coinciding with maltose. The substance co-chromatographed with maltose also in a third solvent system: (III) 1-butanol–water (15 : 1) and propionic acid–water (352 : 448) (equal vols mixed immediately before use). For further identification the compound was reduced with sodium borohydride [8], hydrolysed with 1 N HCl at 100°C and co-chromatographed in solvent (I) with authentic glucose and sorbitol.

The two resulting radioactive spots coincided with glucose and sorbitol, as was to be expected if the original compound had been maltose.

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The results show that radioactive maltose monophosphate is synthesised in isolated spinach chloroplasts during photosynthesis in $^{14}CO_2$. It remains to be determined, however, if maltose monophosphate is indeed an intermediate in the synthesis of maltose from glucose phosphates.

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