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ESTIMATION OF THE ACTIVITY OF THE OXIDATIVE PENTOSE PHOSPHATE PATHWAY IN PEA CHLOROPLASTS

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Key Word Index—*Pisum sativum*; Leguminosae; pea chloroplasts; oxidative pentose phosphate pathway; measurement; glucose-[^{14}C].

Abstract—The fraction of glucose 6-phosphate metabolism in isolated intact chloroplasts of *Pisum sativum* in the dark that occurs via the oxidative pentose phosphate pathway has been estimated from the distribution of ^{14}C from specifically labelled glucose-[^{14}C] supplied to the chloroplasts.

INTRODUCTION

Studies of the enzymic capacities [1], of the products of starch breakdown, and of the metabolism of glucose-[^{14}C] by intact functional chloroplasts [2] provide the following qualitative picture of the way in which these chloroplasts oxidize hexose phosphates in the dark. Hexose 6-phosphates are converted to CO_2, triose phosphates and 3-phosphoglycerate, which are exported to the cytoplasm, and may be regarded as the products of carbohydrate oxidation by the chloroplasts. The above conversion occurs, in part, via the initial reactions of glycolysis, the steps from fructose 6-phosphate to 3-phosphoglycerate, and, in part, via the oxidative pentose phosphate pathway. Glycolysis does not proceed beyond 3-phosphoglycerate because the chloroplasts do not contain phosphoglyceromutase. The oxidative pentose phosphate pathway operates with extensive recycling. The aim of the present paper is to use our previously published data [2] to obtain a quantitative estimate of the contribution of the oxidative pentose phosphate pathway to the total metabolism of glucose 6-phosphate (G-6-P) in the dark in isolated chloroplasts from pea shoots. Such an estimate is important in respect of our understanding of chloroplasts *per se*, in the development of methods for measurement of flux in sugar metabolism, and as a basis for quantitative studies of the control of carbohydrate metabolism in chloroplasts. As carbohydrate oxidation by chloroplasts is simpler than that by intact cells, many of the difficulties associated with quantitative evaluation of the pathways in cells [3] are absent. Most pertinent to our arguments is the substantial evidence that CO_2 production by our chloroplast preparations was due entirely to the oxidative pentose phosphate pathway [1, 2].

We define total metabolism of G-6-P as the sum of the activities of the oxidative pentose phosphate pathway, glycolysis, and any non-triose phosphate pathways. We regard the latter as any pathway that converts G-6-P into compounds without involving the formation of triose phosphates or any other rearrangement of the hexose carbon skeleton. As we have demonstrated extensive recycling in the oxidative pentose phosphate pathway in chloroplasts we represent this pathway as

$$1\ \text{G-6-P} \rightarrow 3\ CO_2 + 1\ \text{triose phosphate} \qquad (1)$$

and we define activity of the pathway as the conversion of G-6-P to smaller units. These are the definitions of Katz and Wood [4] and they differ from the more commonly held view of the pathway as

$$3\ \text{G-6-P} \rightarrow 3\ CO_2 + 1\ \text{triose phosphate} + 2\ \text{fructose 6-phosphate}. \qquad (2)$$

In assessing the contribution of the oxidative pentose phosphate pathway to the total metabolism of G-6-P, it is important to appreciate the difference between the above definitions. By equation 2 30% metabolism via the pentose phosphate pathway means that for every 100 mol of G-6-P metabolized, 30 are converted to 6-phosphogluconate and thence to pentose phosphate. However, with recycling as in equation 1, the pathway will regenerate 20 mol of hexose 6-phosphate and only 10 mol of hexose phosphate will have been converted to smaller units, CO_2 and triose phosphate. Thus 30% pentose phosphate pathway by equation 2 is only 12.5% $[\frac{30}{3}/(\frac{30}{3}+70)]$ by equation 1 [4].

RESULTS AND DISCUSSION

We estimate the fraction of the total metabolism of G-6-P that proceeded through the oxidative pentose phosphate pathway in the dark in chloroplasts, isolated from pea shoots, in two ways.

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Method 1

When equation 1 holds, CO_2 production by the pathway will equal 50% of the G-6-P metabolized by the pathway. In our isolated chloroplasts the pathway was the sole source of CO_2, thus production of $^{14}CO_2$ from hexose-[^{14}C] is a measure of the pathway. We incubated pea chloroplasts in glucose-[U-^{14}C] in the light, then we removed the labelled glucose and incubated the chloroplasts in the dark [2]. Starch, labelled in the light, was rapidly broken down in the dark. The products of this breakdown were metabolized by glycolysis and the oxidative pentose phosphate pathway and accumulated as phosphorylated intermediates of these pathways and CO_2. We suggest that the ^{14}C recovered in these intermediates plus that in CO_2 represents G-6-P metabolized, and that the activity of the pentose phosphate pathway is given by:

$$\frac{(^{14}\text{C released as } ^{14}CO_2)\times 2}{(^{14}\text{C released as } ^{14}CO_2)+(^{14}\text{C in phosphorylated products of starch})}$$

In our studies of starch breakdown by isolated chloroplasts we have provided evidence that the acidic components of the water-soluble fraction of the chloroplast suspensions represent the phosphorylated products of starch breakdown. Thus we use the increase in label in these acidic components and in CO_2 during starch breakdown (ref. [2], table 1) to calculate the activity of the pentose phosphate pathway according to the above equation. In two separate experiments we obtained estimates of 22 and 36% of the total G-6-P metabolized.

Method 2

Conversion of G-6-P to fructose 6-phosphate via the complete sequence of the oxidative pentose phosphate pathway results in a specific rearrangement of the first three carbons of the hexose molecule. The extent of this randomization is proportional to the fraction of total metabolized G-6-P that proceeds via the pentose phosphate pathway and may be used as a measure of the pathway [4]. Wood and Katz [5], and Katz and Wood [4] have calculated the relationship between the degree of this randomization and the percentage of metabolized G-6-P entering the pentose phosphate pathway, as defined by equation 1. They calculated, for a tissue supplied with glucose-[1-^{14}C], the relationship between the sp. act. of C-1 of G-6-P and the activity of the pentose phosphate pathway. They made a similar calculation in respect of tissue supplied with glucose-[2-^{14}C]. Table 1 summarizes the results of these calculations and allows derivation of the relationship between the fraction of G-6-P metabolized via the pentose phosphate pathway and the ratio

$$\frac{\text{sp. act. of C-1 of G-6-P after supplying glucose-[1-}^{14}\text{C]}}{\text{sp. act. of C-1 of G-6-P after supplying glucose-[2-}^{14}\text{C]}}.$$

If the pentose phosphate pathway is the only source of CO_2 in isolated chloroplasts, then $^{14}CO_2$ production after supplying glucose-[^{14}C] should reflect the sp. act. of C-1 of G-6-P and the above ratio reduces to

$$\frac{^{14}CO_2 \text{ production from glucose-[1-}^{14}\text{C]}}{^{14}CO_2 \text{ production from glucose-[2-}^{14}\text{C]}}.$$

Table 1. Relationship between activity of oxidative pentose phosphate pathway and sp. act. of C-1 of G-6-P after metabolism of glucose-[1-^{14}C] and -[2-^{14}C]

Percentage of G-6-P metabolized via pathway	Sp. act. of C-1 of G-6-P after metabolism of	
	Glucose-[1-^{14}C]	Glucose-[2-^{14}C]
0	100	0
10	83	14
20	72	21
40	56	28
50	50	30
60	46	31
80	39	32
90	36	33
100	33	33

The relationship between this ratio and the activity of the pentose phosphate pathway is given in Fig. 1.

Chloroplasts isolated from pea shoots readily released $^{14}CO_2$ from glucose-[1-^{14}C], and also from glucose-[2-^{14}C] (ref. [2], fig. 2). The rates of release were linear over the period 5–60 min from the addition of the glucose-[^{14}C]. The mean value ± S.E. of the ratio of the yield of $^{14}CO_2$ from glucose-[1-^{14}C] to that from glucose-[2-^{14}C] in 7 separate experiments was 4.11 ± 0.13. From Fig. 1 this gives an estimate of the pentose phosphate pathway equal to 16.1 ± 0.6% of the total metabolism of G-6-P. In comparable experiments we determined the effects on $^{14}CO_2$ production of omitting orthophosphate from, and of adding 2-phosphoglycollate to, the medium in which the chloroplasts were suspended. Lack of orthophosphate was expected to reduce the activity of the phosphate translocator, increase triose phosphate in the chloroplast and thereby increase the amount recycled through the pentose phosphate pathway. 2-Phosphoglycollate inhibits chloroplast phosphofructokinase [6] and was expected to increase the relative activity of the pentose phosphate pathway through inhibition of

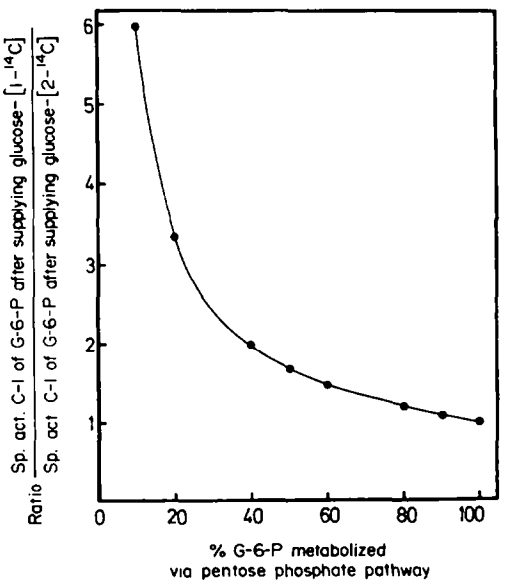

Fig. 1. Relationship between labelling of C-1 of G-6-P and the activity of the oxidative pentose phosphate pathway.

glycolysis. Our estimates (mean ± S.E. with number of experiments given in parenthesis) for the activity of the pathway in the absence of orthophosphate, and in the presence of 3 mM 2-phosphoglycollate, were, respectively, 24.7 ± 2.9% (3) and 25.1 ± 2.4% (4). Both predictions were borne out. We suggest that this simple approach has particular value in the study of the control of carbohydrate breakdown by chloroplasts.

Our two methods of estimating the pentose phosphate pathway give comparable values and suggest that 15–30% of G-6-P metabolism in isolated pea chloroplasts in the dark proceeds via the pathway. It can be argued that our estimates do not take into account the fact that some of the triose phosphate formed in the pentose phosphate pathway is recycled via that pathway. The relative yields of $^{14}CO_2$ from glucose-[1-^{14}C] and -[6-^{14}C] suggest that such recycling was small in relation to the activity of the pathway and would have had very little effect on the ratio of glucose C-1/C-2 in the respired CO_2. For this reason, and those given by Katz and Wood [4], we suggest that recycling of triose phosphate would not greatly affect our estimates. As explained in the introduction, our estimate of 15–30% may be converted to the more conventional assessment of the pathway to show that of every 100 mol of G-6-P metabolized by the chloroplast in the dark, 35–56 would enter the oxidative pentose phosphate pathway. The remainder are metabolized via glycolysis and any non-triose pathways operating in the chloroplasts. This suggests that the oxidative pentose phosphate pathway is more active, relative to glycolysis, in chloroplasts in the dark than in the leaf as a whole [7] or in plant tissues in general [3].

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