THE DIRECT OXIDATION PATHWAY IN PLANT RESPIRATION[1, 2]

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An important development in biochemistry has been the discovery of enzymes which can attack glucose-6-phosphate oxidatively to produce pentose phosphate and CO_2. The diversion of glucose-6-phosphate by such a pathway has been variously called the hexose monophosphate shunt and the oxidative pathway and the names of several eminent research workers are linked with the elucidation of the reactions involved (reviewed by Horecker (9)). Recent work on a great variety of tissues, including higher plants (1, 2, 7, 8) has demonstrated that such systems are widespread. In view of this accumulating evidence, some workers have been led to consider that this metabolic "byway" may have importance in addition to its being a probable route to the pentoses in vivo, and the further elucidation of the reactions subsequent to pentose formation have lent color to this view. In particular, the recognition of the importance of sedoheptulose phosphate and its breakdown products has made possible the formulation of a cyclic sequence of events whose operation might of itself account for the total oxidation of glucose (9) and which would, at the least, produce metabolites which are also intermediates in the classical Embden-Meyerhof-Parnas (E.M.P.) glycolytic sequence. Although the details of the reactions remain to be clarified, the recent findings, to which experiments on higher plant tissues have contributed (1, 2, 7, 8), render the operation of the direct oxidation pathway in normal respiration quite plausible. There is ample evidence, of course, for the operation of the E.M.P. sequence of reactions in plant materials (11); at the present time, however, no evidence concerning the relative importance of the two alternatives in plant respiration has been offered. The present investigation was carried out to obtain evidence bearing on this point.

Use was made of the fact that the method of glucose breakdown will determine the relative rates of release of its constituent carbon atoms. (For a discussion of the application of this principle see Bloom, Stetten, and Stetten (5) and Bloom and Stetten (6)). If the glucose molecule were broken down by glycolysis which includes equilibration at the triose level, two pyruvate molecules would be produced in which carbon atom 1 (C-1) and C-6 of the glucose would appear as the methyl carbons of the acid, C-2 and C-5 as the carbonyl carbons, and C-3 and C-4 as the carboxyl carbons. Carbons 1, 2, and 3 of the original glucose would, therefore, be indistinguishable respectively from 6, 5, and 4, and in the subsequent oxidative breakdown of the pyruvate, each member of a pair would appear in the respiratory CO_2 at the same rate as its partner. (Of the pairs, (C-3 and C-4) would be expected to appear first in the CO_2 followed by (C-2 and C-5) and then (C-1 and C-6).) Thus, if comparable samples of tissue were respiring on glucose-1-C^{14} and glucose-6-C^{14} respectively, the contribution of C^{14} to the CO_2 given off would be the same in each case. If, on the contrary, a glucose molecule was metabolized by way of the oxidative pathway, the CO_2 from the glucose-1-C^{14} would be expected to be initially higher in C^{14} than that from the glucose-6-C^{14} since C-1 of the glucose molecule is the first to be converted to CO_2. Provided that no assimilation of carbon residues containing different amounts of C-1 and C-6 occurred, the total yield of $C^{14}O_2$ from the two glucose samples would be the same when oxidation was complete regardless of the path of breakdown. In the present experiments, it should be stressed, the time intervals were such that only a few percent of the supplied glucose was respired and the yields of $C^{14}O_2$ are, therefore, regarded as initial as distinct from total. In these experiments, then, initial yields of $C^{14}O_2$ from 2 glucose samples were compared, and the ratio, % radiochemical yield from glucose 6-C^{14}/% radiochemical yield from glucose 1-C^{14} evaluated. Clearly, a ratio of near unity would indicate that schemes other than E.M.P. were playing a very minor part in glucose breakdown, whereas a ratio of less than unity would implicate the participation of the direct oxidation pathway.

MATERIALS AND METHODS

PLANT MATERIAL: Root tips from corn were prepared as described earlier (3) and 1 gm fresh weight was used as a sample.

Peas (var. Alaska) were grown on gravel in the greenhouse and the other plant materials (see table), with the exception of carrot, were from stock greenhouse plants. Carrots were purchased locally. The procedure in each case was to cut the experimental material into sections or pieces about 1 mm in thickness. These were washed in several changes of water, dried lightly and weighed out into replicate samples of 1 to 2 gm fresh weight for transfer to Warburg vessels.

MANOMETRIC EXPERIMENTS: In most of these experiments four equal samples of tissue were placed in Warburg vessels of 100 ml capacity. The large vessels permitted the use of a relatively large volume of tissue and a small liquid volume (2.5 to 4.0 ml). The liquid contained 1 ml of 0.03 M phosphate buffer at pH 5.0, plant tissue and water. To two of the four vessels 20 to 40 micromoles of glucose-1-C^{14} of known activity were added while the others received the same amount of glucose-6-C^{14}. The glucose was

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added immediately prior to attaching the vessels. The vessels were shaken at 25° C for a period of 4 to 5 hours during which the light was excluded in the case of green tissues. The respired CO_2 was absorbed in 0.2 ml 20 % KOH in the center well. The values for CO_2 determination from the four samples, which include errors in sampling, pipetting, transfer and conversion to $BaCO_3$ showed that the technique was adequate; the spread between four such values was usually considerably less than 10 %.

Measurements of Radioactivity: At the end of the experiments the KOH was removed and the absorbed CO_2 was converted to $BaCO_3$ by a method described by Steele and Sfortunato (10). The radioactivity of the $BaCO_3$ was measured with a methane flow proportional counter.

The glucose-1-C^{14} was kindly supplied by Dr. H. Isbell of the National Bureau of Standards. The glucose-6-C^{14} was kindly given to us by Dr. J. C. Sowden, Washington University of St. Louis.

Results and Discussion

From the primary data the radiochemical yield in millimicrocuries from each glucose sample was evaluated and this was then expressed as a percentage of the total radioactivity supplied in the original glucose. These values showed that, under the experimental conditions, only a small fraction of the glucose had been respired (usually less than 10 % of the C-1 carbon having appeared). This permitted the evaluation of initial C_6/C_1 ratios as shown in table I, which includes values obtained from a variety of plant tissues.

It is significant that the value of the C_6/C_1 ratio for excised corn root tips was near unity in each of three experiments, since this is a value which indicates that the oxidative pathway, if it does operate in the normal respiration, plays a very minor role (3). A point of major importance is that in contrast to the corn root tips, most of the tissues tested gave values of the ratio which were considerably less than unity and even in some cases less than 0.5. In these tissues, then, although the actual rates of CO_2 output on the 2 kinds of glucose were the same, the ratio shows that in the early stages of glucose utilization, C-1 was making a significantly greater contribution to the CO_2 than C-6. This is what would be expected if a portion of the glucose followed the oxidative pathway.

Indeed, if we adopt the procedure outlined by Bloom and Stetten (6) and Bloom et al (5) we can arrive, with similar reservations, at a limiting estimate of the amount of glucose which was actually being respired by pathways other than E.M.P. Clearly if glucose catabolism occurs simultaneously by: (a) the E.M.P. pathway, with equal contributions to the CO_2 coming from C_1 and C_6 of the glucose, and (b) the oxidative pathway, in which a rela-

Table I

Evaluation of C_6/C_1 * Ratio for Various Plant Materials

Tissue	Glucose	Mμ C added	Mμ C recovered	% recovered	C_6/C_1 = Max. fraction of E.M.P.
Corn root	1-C^{14}	32.0	1.18	3.7	0.91 (1.12, 0.95) **
	6-C^{14}	33.9	1.13	3.4	
Pea internode	1-C^{14}	16.0	0.40	2.5	0.48
	6-C^{14}	17.0	0.20	1.2	
Pea leaf	1-C^{14}	10.2	0.57	5.5	0.61
	6-C^{14}	8.5	0.29	3.4	
Sunflower leaf	1-C^{14}	13.4	2.42	18.1	0.75 (0.63) **
	6-C^{14}	10.2	1.38	13.5	
Carrot petiole	1-C^{14}	13.4	0.82	6.1	0.36
	6-C^{14}	13.6	0.30	2.2	
Carrot root discs	1-C^{14}	13.4	0.51	3.8	0.50
	6-C^{14}	13.6	0.26	1.9	
Bryophyllum leaf	1-C^{14}	13.4	0.18	1.3	0.54
	6-C^{14}	9.2	0.06	0.7	
Corn leaf	1-C^{14}	13.4	0.78	5.8	0.79
	6-C^{14}	9.2	0.42	4.6	
Pea root (3 week old system)	1-C^{14}	13.4	1.49	11.1	0.58
	6-C^{14}	9.2	0.59	6.4	
Sunflower stem	1-C^{14}	13.4	1.05	7.9	0.75
	6-C^{14}	10.2	0.60	5.9	
Parsley leaf	1-C^{14}	13.4	0.65	4.8	0.65
	6-C^{14}	10.2	0.32	3.1	
Parsley stem	1-C^{14}	13.4	1.00	7.5	0.64
	6-C^{14}	10.2	0.49	4.8	

* % radiochemical yield from glucose-6-C^{14}/% radiochemical yield from glucose-1-C^{14}. Equal amounts (usually 30 micromoles) of glucose were supplied to samples of tissue in Warburg vessels at 25° C, and the CO_2 produced in a 4 hour experiment analyzed as described in the text.

** The values in parentheses are from individual experiments carried out on separate occasions.

tively greater contribution is initially made by C_1, the *maximum* amount of the $C^{14}O_2$ from glucose-1-C^{14} which can have been produced by E.M.P. is an amount equal to the total $C^{14}O_2$ from glucose-6-C^{14}. Thus the *maximum* fraction of the $C^{14}O_2$ from glucose-1-C^{14} which could have arisen from E.M.P. and hence the maximum fraction of the respiration which could be occurring through this pathway is given directly by the C_6/C_1 ratio and the *minimum* contribution of the oxidative pathway can be derived directly from this figure. The figures in the last column of table I therefore show that in most of the species tested the contribution of the oxidative pathway was a very considerable one.

Two points about the values of the C_6/C_1 ratios less than one are pertinent. First, it is not suggested that the values are rigid, since, as it has been pointed out elsewhere (4) they would be expected to approach one as the glucose was depleted and C-6 made an increasingly greater contribution to the CO_2. Second, individual species will no doubt differ in the relative efficiencies with which they accomplish reactions in the oxidative pathway prior to and subsequent to C_1 release. Since it is the difference in the rates of these reactions which determines what the C_6/C_1 ratio will be, it is clearly possible that two plant species in which the fraction of glucose entering the oxidative pathway was the same might, nevertheless, yield different ratios and hence different values for the *maximum* contribution of the E.M.P. pathway.

The list of tissues tested is certainly too small to warrant detailed deductions, and the values would have been more reasonably compared if they all could have been obtained at the same stage in the disappearance of added glucose. Nevertheless, it seems reasonable to conclude that the oxidative pathway plays a considerable role in plant respiration and that in none of the tissues tested except young corn root tips was the Embden-Meyerhof-Parnas scheme the sole means of glucose breakdown in respiration.

Summary

Experiments have been carried out on a variety of plant tissues to find whether the oxidative pathway of glucose breakdown ("hexosemonophosphate shunt") contributes to their respiration. When initial yields of $C^{14}O_2$ from equal amounts of glucose-1-C^{14} and glucose-6-C^{14} were compared there was clear evidence that, in most of the tissues, some of the glucose was, in fact, broken down in reactions in which C-1 was split off at an earlier stage than C-6. It has been calculated from the data that in several of the tissues a maximum of about half of the glucose was broken down by the E.M.P. pathway. The fact that the initial yields of $C^{14}O_2$ from the two kinds of glucose were the same when corn root tips were used confirms an earlier finding that in this juvenile tissue the classical glycolytic route is the sole one; in the other tissues it is concluded that a substantial fraction of the glucose is respired via the direct oxidation pathway.

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