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Measurements of Starch Breakdown as Estimates of Glycolysis during Thermogenesis by the Spadix of *Arum maculatum* L.

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Abstract. The rates of starch breakdown at thermogenesis by clubs of the spadices of *Arum maculatum* L. were measured in individual clubs still attached to the plants. The values obtained were used as estimates of the rate of glycolysis at thermogenesis. Such rates were shown to exceed the maximum catalytic activities of phosphofructokinase (E.C. 2.7.1.11.), aldolase (E.C. 4.1.2.7.), and glyceraldehydephosphate dehydrogenase (E.C. 1.2.1.12.) in developing clubs. The marked increases in the activities of the above enzymes that occur during the development of the club have been shown to be a prerequisite for the attainment of the high rate of glycolysis found at thermogenesis, and thus to be an example of coarse control of glycolysis.

Key words: *Arum maculatum* – Glycolysis – Spadix – Starch breakdown – Thermogenesis.

Introduction

The aim of the work in this paper was to provide a further test for our view that coarse control contributes to the regulation of glycolysis in the club of the spadix of *Arum maculatum* (ap Rees et al., 1976). During flowering such clubs pass through a recognizable sequence of developmental stages which culminates in a rapid and extensive rise in respiration that is responsible for a period of heat production during pollination (Meeuse, 1975). We have called these stages, successively, α, β, γ, pre-thermogenesis, and thermogenesis. At the α and β stages, both glycolysis and the pentose phosphate pathway make appreciable contributions to carbohydrate oxidation. Subsequent development involves a shift towards an almost complete dependence upon glycolysis, and there is a massive increase in glycolytic activity at thermogenesis. Development also involves marked and specific increases in the maximum catalytic activities of key glycolytic enzymes (ap Rees et al., 1976). In our previous work we estimated the glycolytic flux at thermogenesis from measurements of the CO_2 production of excised clubs. We compared these estimates with the activities of phosphofructokinase and aldolase during club development, and concluded that the results provide strong evidence that the glycolytic flux at thermogenesis exceeds the maximum catalytic activities of the enzymes at α and β stages. Thus we argued that the increases in the maximum catalytic activities of the glycolytic enzymes are a prerequisite for the rapid rise in glycolysis at thermogenesis, and are an example of coarse control of glycolysis.

In the experiments referred to above our estimation of glycolysis from measurements of the CO_2 production of isolated clubs suffers from two disadvantages. First, excision of the club may have altered the rate of carbohydrate oxidation. Second, it is difficult to be entirely sure of the relationship between the rate of CO_2 production and the rate at which glucose-6-phosphate enters glycolysis. In this paper we report measurements of the rate of starch breakdown at thermogenesis in clubs still attached to plants, and present these measurements as more accurate estimates of the rate of glycolysis than our previous figures. There are two main reasons why we believe that there must be a close correspondence between the rates of starch breakdown and glycolysis at thermogenesis. First, the extremely rapid fall in starch at thermogenesis is not accompanied by any significant accumulation of reducing sugars or sucrose (Lance, 1972). Second, there is substantial evidence that carbohydrate is oxidized almost exclusively via glycolysis at thermogenesis (ap Rees et al., 1976).

Materials and Methods

Plants

All data refer to the swollen club-shaped portion, the club, of the appendix of the spadix of *Arum maculatum* L. All plants were taken from local natural sites. The characteristics of the different

stages of the development of the spadix, the way in which we recognized the stages from α to pre-thermogenesis, and the procedure used for the collection of the clubs assayed for glyceraldehydephosphate dehydrogenase, were as described previously (ap Rees et al., 1976). For all the other experiments, we dug up complete plants, at late γ stage, with the minimum disturbance of their roots, transferred them with their attendant soil into flower pots (diameter, 25 cm), and put them in a growth room at 14 or 15° C, with a photoperiod of 14 h, for 20 to 30 h before starting the experiment.

Methods

Club temperature was measured by keeping the plant in a growth room at 14 or 15° C and making a continuous recording of the difference between the club and the air temperatures with a copper-constantan thermocouple inserted into the club. The details of this method of measuring temperature have been described by Rackham (1975). For comparison of club temperature and CO_2 production the potted plant was kept at 15° C and the temperature of the club was monitored as described above. Once thermogenesis started, the spathe was removed and the club, still attached to the plant, was sealed into a plastic chamber (volume, 30 ml) which was connected to an infrared gas analyser. Balance air with a CO_2 concentration of 0.03% (v/v) was passed through the chamber at a rate of 0.3 l min^{-1}, and gain of CO_2 was measured. During the measurement of CO_2 production, the temperature of the club was recorded by a thermocouple that was sealed into the side of the plastic chamber and inserted into the club.

For measurements of starch breakdown, plants at late γ stage were kept at 14 or 15° C until measurements of club temperature showed that thermogenesis was well under way. Then the spathe was removed and a vertical, thin, representative slice (fresh weight 20 to 100 mg) of the club was carefully excised. The wound in the club was quickly sealed with vaseline warmed to the same temperature as the club, and the measurements of club temperature were continued until the peak of thermogenesis had passed. Then two further slices, similar to the one removed as the initial sample, were taken from the club in quick succession. The starch content of the slices was measured by the following procedure which was developed by P.V. Bulpin (personal communication). As soon as a slice was removed from the club it was killed in 25 ml boiling 80% aqueous ethanol, and then extracted with three further 25 ml volumes of boiling 80% aqueous ethanol. The insoluble residue was homogenized, suspended in 40 ml water, and the resulting suspension was boiled for 20 min before being autoclaved for 160 min at 120° C at 104 kN m^2. Next, 5.0-ml portions of the autoclaved suspension were incubated for 2 h at 37° C with 5.0 ml 0.2 M acetate buffer, pH 4.8, that contained 14 units of amylo-α-1,4-α-1,6-glucosidase (Boehringer) and 3.1 units of pig pancreas α-amylase (Sigma Chemical Co.). Finally, samples of the above incubation mixture were assayed for glucose by the method involving glucose oxidase as described by Kilburn and Taylor (1969) except that the buffer was that described by Lloyd and Whelan (1969).

Glyceraldehydephosphate dehydrogenase was assayed as described by Pollock and ap Rees (1975) except that the pH was pH 7.8, the concentration of sodium arsenate 7.5 mM, and the amount of aldolase per assay was 0.8 units. The methods used to prepare the extracts and to measure their protein content have been described (ap Rees et al., 1976).

Results and Discussion

The very high starch content of the club drops extremely rapidly during thermogenesis. However, the extent and timing of thermogenesis vary considerably with the club. These facts made it impracticable for us to measure the rate of starch breakdown by comparing the starch content of different clubs at different stages of thermogenesis. Thus we developed a procedure whereby the starch content of an individual club could be determined by removing, at the onset of thermogenesis, a thin vertical slice of the club in a way that did not prevent the rest of the club, still attached to the plant, from passing through the subsequent stages of thermogenesis. The rate of starch breakdown was obtained by comparing the starch content of the initial slice with those of similar slices taken later in thermogenesis.

Our first experiments were designed to show that the removal of a thin slice of the club did not seriously disturb the normal course of thermogenesis. We determined the latter by making continuous measurements of the club temperature of intact plants as they progressed from late γ stage to the end of thermogenesis. These measurements were made under conditions very similar to the natural environment of the plants, and the insertion of the small thermocouple into the club was the only way in which the plants were disturbed. We found that the rate at which the club temperature rose, the peak temperature attained, and the time for which the peak was maintained, varied with the plant. However, the data shown in Figure 1a may be taken as typical. These results emphasize both the rapidity with which thermogenesis develops, and also the ability of the plant to raise the club temperature some 10° C above ambient temperature. Next, we showed that the temperature of the club correlated extremely closely with its rate of respiration. We demonstrated this point by making continuous and simultaneous measurements of the temperature and CO_2 production of the same club whilst it was still attached to the plant. Results similar to those shown in Figure 1c were obtained for four different plants. The uneven rise in club temperature in these experiments was probably due to the fact that the club had to be enclosed in order to measure its CO_2 production. None the less this uneveness emphasizes the extremely close realtionship between club temperature and respiration. We conclude from these experiments that measurements of club temperature may be used to pinpoint the timing of the changes in the rate of glycolysis during thermogenesis. Thus for our measurements of starch breakdown we used club temperature to determine when to sample the club, and to show that the removal of the initial slice did not have any serious effect on the normal course of thermogenesis. Typical results are shown in Figure 1b.

Our estimates of the rate of starch breakdown

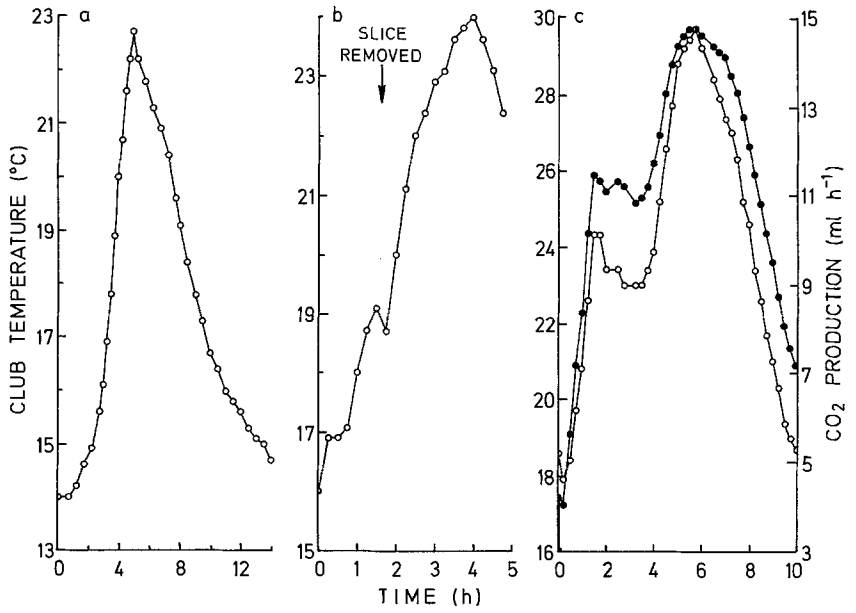

Fig. 1. Temperature (○—○) of club of spadix of *Arum maculatum* during thermogenesis;
a intact,
b after removal of slice for measurement of starch,
c in relation to CO_2 production (●—●)

during thermogenesis are given in Table 1. The starch content of the initial samples taken from different clubs varied according to the precise point in the development of thermogenesis at which we took the sample. In general the values obtained for these initial samples are comparable to those reported for clubs at pre-thermogenesis by Lance (1972). As the two final samples from any one club were taken almost simultaneously, the agreement between the values obtained for these samples is an indication of the reliability of our sampling technique. The data indicate that this was adequate. We attribute the variation in the rates of starch breakdown in different clubs to differences between clubs. Such variation is to be expected in view of the differences which we found in the extent and timing of thermogenesis, and the differences in peak rates of respiration noted by Lance (1972). The rates of starch breakdown are likely to be minimal for the periods examined and it is probable that higher rates occurred for short times at the peak of thermogenesis. Thus it is permissible to take the average of the two highest rates in Table 1 as a measure of the minimum rate of glycolysis at thermogenesis. The value obtained is about double that which we got from measurements of the CO_2 production of excised clubs (ap Rees et al., 1976). It is likely that this discrepancy is mainly due to the effects of excising the club. It is of interest to note that our estimates of glycolysis obtained from measurements of starch breakdown show that the rate in the club of *Arum* spadix significantly exceeds that in many actively metabolizing mammalian tissues, including tetanized muscle (Scrutton and Utter, 1968).

Table 1. Starch breakdown during thermogenesis by spadix of *Arum maculatum*. In each experiment a plant at late γ stage was transferred to 14° C. When the club temperature reached 17 to 18° C, a slice (fresh weight 10 to 90 mg) was cut vertically from the club and taken for assay of starch. Club temperature was monitored until the peak passed and then two further slices were removed in rapid succession and assayed

Experiment	Starch content (mg g^{-1} fresh weight)		Interval between initial and final samples (min)	Rate of starch breakdown (μmol hexose produced g^{-1} fresh weight min^{-1})
	Initial sample	Final samples		
1	215	178; 168	34.5	7.63
2	303	110; 86	208	6.09
3	191	104; 96	150	3.77
4	221	96; 94	264	2.94

In order to test our view that the development of this high rate of glycolysis at thermogenesis is dependent upon increases in the maximum catalytic activities of glycolytic enzymes that occur during club development, we compare the latter with the former (Table 2). We think that the results are best expressed per club because the number of cells in a club changes much less than either fresh weight or protein content during development (ap Rees et al., 1976). None the less the other bases are used in order to show that our arguments do not depend upon the way in which the rates are expressed. The data for phosphofructokinase (E.C. 2.7.1.11.), aldolase (E.C. 4.1.2.7.), fresh weight, and protein are derived from previously described experiments (ap Rees et al., 1976). We have supplemented these results by measurements of the activities of another glycolytic enzyme, glyceraldehy-

Table 2. Comparison of the rate of starch breakdown at thermogenesis with the activities of glycolytic enzymes during development of the club of the spadix of *Arum maculatum*. The rate of starch breakdown is as μmol hexose produced and is the average of the two highest values from Table 1. Enzyme activities are μmol substrate consumed and are means ±S.E. of estimates made on at least five different clubs. Data for phosphofructokinase and aldolase are derived from experiments reported by ap Rees et al. (1976)

Measurement	Stage of development	Rate as μmol min^{-1} per		
		Club	Fresh weight of club (g)	Protein content of club (mg)
Starch breakdown	Thermogenesis	5.2	6.9	0.32
Phosphofructokinase	α	0.21±0.02	1.15±0.09	0.04±0.003
	β	2.10±0.23	4.10±0.70	0.16±0.02
	γ	11.06±1.66	11.52±1.85	0.30±0.03
Aldolase	α	0.68±0.04	3.74±0.16	0.14±0.004
	β	4.56±1.07	6.08±0.97	0.21±0.04
	γ	13.63±1.75	11.45±1.23	0.34±0.04
Glyceraldehydephosphate dehydrogenase	α	1.05±0.14	5.71±0.73	0.20±0.02
	β	8.89±0.42	11.98±0.42	0.41±0.03
	Pre-thermogenesis	100.8 ±9	77.4±13.2	3.96±1.01

dephosphate dehydrogenase (E.C. 1.2.1.12.). We obtained evidence, of the type described previously (ap Rees et al., 1976), that our estimates of glyceraldehydephosphate dehydrogenase reflect the maximum catalytic activities of the clubs. It is clear that the activity of this enzyme shows the same striking increase during club development as has been shown for phosphofructokinase and aldolase. When the maximum catalytic activities of the three glycolytic enzymes in the developing clubs are compared with the rate of starch breakdown, it can be seen that the maximum activities of the enzymes at both α and β stage are not high enough to catalyse the minimum rate of starch breakdown at thermogenesis. This discrepancy is enhanced by the fact that the enzyme activities were measured at 25 °C whilst the club temperatures in the experiments reported in Table 1 ranged from 17 to 23° C. We conclude that the high rates of glycolysis at thermogenesis are dependent upon the increases in the maximum catalytic activities of the glycolytic enzymes that occur during club development. This confirms our view that the changes in enzyme activity are an example of coarse control of glycolysis.

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