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The effect of hypoxia on the control of carbohydrate metabolism in ripening bananas

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Abstract. The aim of this work was to determine the effects of hypoxia on the major fluxes of carbohydrate metabolism in climacteric fruit of banana (*Musa cavendishii* Lamb ex Paxton). Hands of bananas, untreated with ethylene, were allowed to ripen in air at 21°C in the dark. When the climacteric began, fruit were transferred to 15 or 10% oxygen and were analysed once the climacteric peak had been reached 8–12 h later. The rates of starch breakdown, sucrose, glucose and fructose accumulation, and CO_2 production were determined, as were the contents of hexose monophosphates, adenylates and pyruvate. In addition, the detailed distribution of label was determined after supplying [U-^{14}C]-, [1-^{14}C]-, [3,4-^{14}C]- and [6-^{14}C]glucose, and [U-^{14}C]glycerol to cores of tissue under hypoxia. The data were used to estimate the major fluxes of carbohydrate metabolism. There was a reduction in the rate of respiration. The ATP/ADP ratio was unaffected but there was a significant increase in the content of AMP. In 15% oxygen only minor changes in fluxes were observed. In 10% oxygen starch breakdown was reduced and starch synthesis was not detected. The rate of sucrose synthesis decreased, as did the rate of re-entry of hexose sugars into the hexose monophosphate pool. There was a large increase in both the glycolytic flux and in the flux from triose phosphates to hexose monophosphates. It is argued that the increase in these fluxes is due to activation of pyrophosphate: fructose-6-phosphate 1-phosphotransferase, and that this enzyme has an important role in hypoxia. The results are discussed in relation to our understanding of the control of carbohydrate metabolism in hypoxia.

Keywords: Carbohydrate metabolism (fluxes) – Hypoxia – *Musa* (fruit ripening) – Respiration – Starch breakdown – Sucrose synthesis

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Abbreviations: Glc6P = glucose-6-phosphate; Glc1P = glucose-1-phosphate; Fru6P = fructose-6-phosphate; PP_i = inorganic pyrophosphate

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Introduction

Our aim is to demonstrate how perturbation with low oxygen tension can be used to study the control of carbohydrate metabolism quantitatively.

We have argued that 'top-down' metabolic control analysis provides a powerful means to study the control of plant metabolism quantitatively (ap Rees and Hill 1994; Hill and ap Rees 1995). In order to use this approach it is necessary to measure flux, and to be able to perturb that flux; specifically, for a branched pathway, such as carbohydrate metabolism, we need to be able to perturb fluxes by two independent methods. This allows the calculation of relative elasticity coefficients, and from them flux control coefficients (Brown et al. 1990). We have measured the fluxes of carbohydrate metabolism in ripening banana fruit (Hill and ap Rees 1994), and have described one method for perturbing fluxes in this system, namely the supply of exogeneous glucose to cores of fruit (Hill and ap Rees 1995). In the present paper, we show that low oxygen tension can be used as a second, independent, method to perturb fluxes.

Hypoxia and anoxia have frequently been used as a means for studying the control of plant metabolism (Barker et al. 1967; Effer and Ranson 1967; Kobr and Beevers 1971; Faiz-ur-Rhaman et al. 1974; Dixon and ap Rees 1980). This stems largely from the belief that the Pasteur effect (i.e. the anoxia-induced increase in the glycolytic flux) is widespread (Barker et al. 1967; Faiz-ur-Rhaman et al. 1974), and that the treatment affords a means of altering the rate of glycolysis. However, there is appreciable evidence that the Pasteur effect is by no means universal in plant tissues (Effer and Ranson 1967; Smith and ap Rees 1979; Leshuk and Saltveit 1991). Be that as it may, hypoxia still represents a specific means of perturbing metabolism as a whole. For example, exposure of roots to atmospheres containing less than 6% oxygen leads to fermentation and a general cessation of polymer synthesis (Jenkin and ap Rees 1986; ap Rees et al. 1987). These effects are likely to be mediated through a reduced flux through cytochrome oxidase and a concomitant reduction in the flux through the citric-acid cycle. These changes are likely to affect the major fluxes of

carbohydrate metabolism. Therefore, we decided to see if this is so for banana fruit.

Our experimental approach was to allow banana fruit to ripen in air then, when the rate of CO_2 production had begun to rise we transferred the fruit to atmospheres containing either 10 or 15% oxygen in nitrogen for the 8–12 h prior to the climacteric. We allowed fruit to ripen in air prior to transfer to low-oxygen environments because low-oxygen tension inhibits the onset of ripening (Kanellis et al. 1989). We chose 10% as the minimum oxygen concentration because we did not wish to cause significant fermentation. The latter would change the nature of the pathways, as well as the fluxes, and unnecessarily complicate the analysis of control. In our previous work with roots we found that the external concentration of oxygen at which substantial fermentation began was 6%. As there is evidence that the internal concentration of oxygen in climacteric banana fruit is considerably lower than ambient (Banks 1983), we used 10% in the present work. During the climacteric we determined the effects of low oxygen tension on the following fluxes: starch to hexose monophosphates and vice versa; sucrose synthesis and breakdown; conversion of carbohydrate to CO_2; relative activities of glycolysis and the oxidative pentose-phosphate pathway; recycling between triose phosphates and hexose monophosphates; and the flux from triose phosphates to protein and amino acids. The fluxes were measured as described previously (Hill and ap Rees 1994, 1995) We also made measurements of the contents of the following key intermediates: glucose-1-phosphate (Glc1P), glucose-6-phosphate (Glc6P), fructose-6-phosphate (Fru6P), pyruvate, ATP, ADP, AMP.

Materials and methods

Materials. Substrates, enzymes and co-factors were from Boehringer, Lewes, Sussex, UK. Radiochemicals were from Amersham International, Amersham, Bucks, UK, except that [3,4-^{14}C] glucose was from NEN Products, Boston, Mass, USA. Compressed gases were obtained from BOC, Guilford, Surrey, UK. The 10% oxygen in nitrogen was obtained pre-mixed; 15% oxygen was generated by mixing CO_2-free compressed air and oxygen-free nitrogen, using a critical-orifice gas blender (Model No. GM-602; Analytical Development Co., Hoddeston, Herts, UK). Hands of green bananas (*Musa cavendishii* Lamb ex Paxton) that had not been treated with ethylene were obtained from a local wholesaler. For each experiment a hand of bananas was put in the dark in an incubator (volume, 0.125 m^2) at 21°C, and allowed to ripen without further treatment. The rates of CO_2 production of six fruit were measured, by infra-red gas analysis, and the starch and sugar contents were determined, exactly as described previously (Hill and ap Rees 1994). When the respiratory rise had begun three fruit were transferred to a sealed chamber (volume, 0.006 m^2) through which either 10% or 15% oxygen were passed at a flow rate of 50 $ml \cdot min^{-1}$. Three control fruit were placed in a similar chamber through which CO_2-free compressed air was passed. Several other fruit from the same hand were also transferred to the low-oxygen environment for use in the ^{14}C-feeding experiments. At the climacteric peak (8–12 h later) fruit were sampled for metabolite measurements and ^{14}C-feeding experiments.

Measurement of metabolites. Fruit were sampled very rapidly. The time between opening the chamber and freeze-clamping was no more than 1 min. Cores of tissue (6 mm diameter × 25 mm length) were removed from the fruit and peeled. Samples of pulp (2–3 g) were freeze-clamped and then rapidly divided into two weighed and equivalent portions. Known amounts of the metabolites to be measured were added to one portion, and both were stored under liquid nitrogen until required. Metabolites were extracted and assayed exactly as described in Hill and ap Rees (1995).

The samples to which known amounts of metabolites had been added were used to estimate the recovery of the added compound. The amounts added were comparable to those found in the tissue. The recoveries obtained were as follows: Glc6P, 90 ± 1%; Glc1P, 94 ± 1%; Fru6P, 93 ± 1%; ATP, 91 ± 1%; ADP, 92 ± 1%; AMP, 90 ± 1%; pyruvate 92 ± 2% (means ± SE, $n = 12$ extracts, 2 samples from each of 6 different fruit).

Metabolism of labelled substrates. Cores of pulp (6 mm diameter × 25 mm length) were taken from fruit that had been incubated in 10 or 15% oxygen. Three cores (2–3 g) from the same fruit were rapidly transferred to 150-ml Erlenmeyer flasks, fitted with a centre well. The flasks were then flushed with either 10 or 15% oxygen for 5 min. To start the incubation, 3.0 ml of 10 mM 2-(N-morpholino)ethanesulphonic acid (Mes; pH 5.5) that contained either 0.3 mM [^{14}C]glucose or 0.3 mM [^{14}C]glycerol was added. The incubation media were equilibrated with either 10 or 15% oxygen prior to the addition. After addition of the medium the required gas mixture was flushed through the flasks for a further 30 s. The flasks were then sealed and incubated at 21°C. Between the 'pulse' and 'chase' phases of pulse-chase experiments the flasks were again flushed with either 10 or 15% oxygen. The chase medium was equilibrated with the required gas mixture, and, after, addition of the chase medium to the flasks, the flasks were flushed with gas for a further 30 s. The flasks were then sealed and the chase incubation carried out. The specific activities were (GBq · mol^{-1}): [U-^{14}C]-, [1-^{14}C] , [3,4-^{14}C]-, and [6-^{14}C]glucose, 74.0; [U-^{14}C]glycerol, 63.0. After incubation in the dark at 21°C for 4–6 h, tissue was killed, extracted and analysed exactly as described by Hill and ap Rees (1994). In order to compare both within and between experiments it was necessary to express the data as a percentage of the total ^{14}C metabolised by the sample in question. The recoveries of added ^{14}C during extraction and analysis were as follows: extraction, 98.3 ± 0.9%; ion-exchange chromatography, 99.9 ± 0.9%; paper chromatography, 97.2 ± 0.7% (means ± SE, $n = 24$).

Results

Characterisation of ripening. The timecourses of changes in starch content, sugar content and CO_2 production in air and in 15 or 10% oxygen are shown in Fig. 1. For each treatment the control fruit behaved similarly to those we described previously (Hill and ap Rees 1994). The control fruit showed considerable variation in both the timecourse of ripening and in the contents of starch and sugars. However, as we have demonstrated previously (Hill and ap Rees 1994), the net fluxes of starch breakdown and sugar accumulation in the control fruit at the climacteric were similar, both between fruit of the same hand and between hands (Fig. 1; Table 1). Incubation in 15% oxygen had little effect on the starch content of the fruit, or on the rate of CO_2 production. With this treatment there was a slight decrease in the sucrose content and slight increases in the glucose and fructose contents of the fruit. In contrast, treatment with 10% oxygen led to an appreciable drop in the rate of CO_2 production, a reduction in the sucrose content and an increase in the starch content of the fruit. There was a small increase in the glucose content of the fruit relative to the controls.

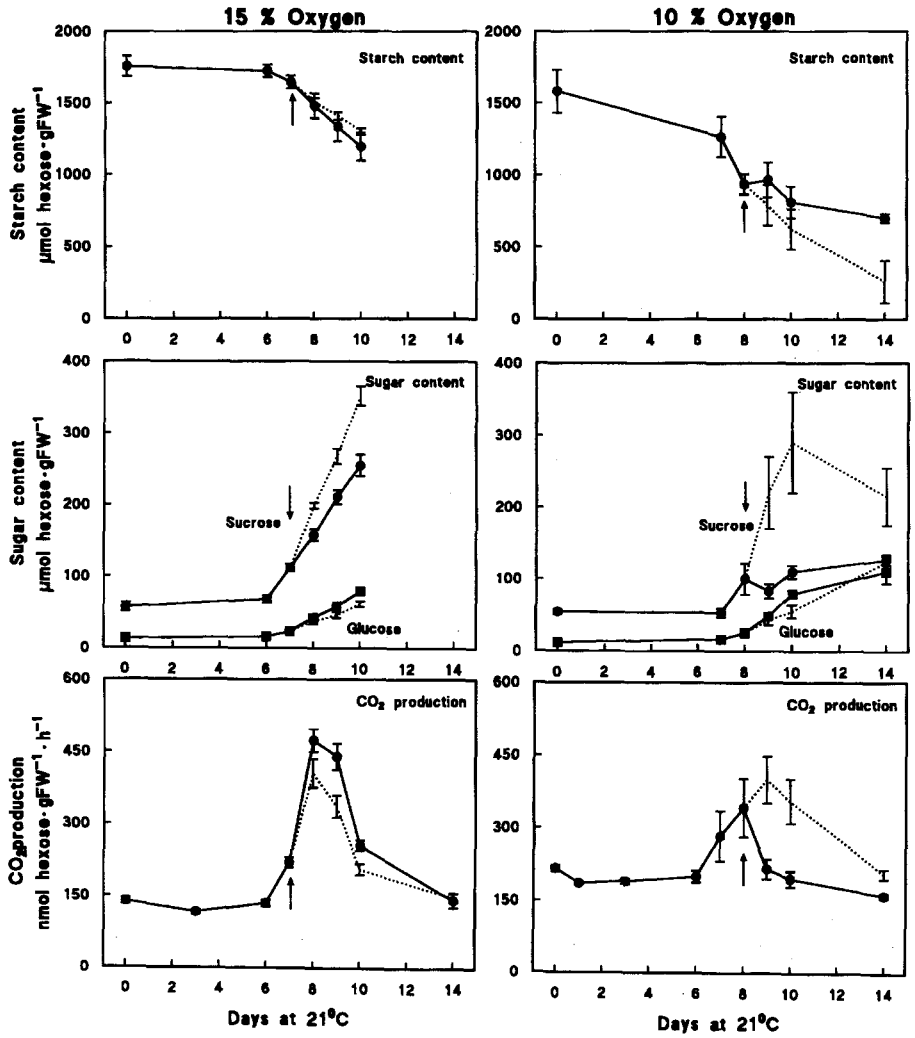


Fig. 1. The effect of oxygen concentration on the carbohydrate content of, and CO_2 production by, banana fruit. Six fruit from the same hand were ripened in air, then at the point indicated by the *arrow*, three of them were transferred to either 15 or 10% oxygen. The values for the three control fruit are shown as dotted lines. Values are the means ± SE of 6 fruit prior to oxygen treatment and 3 fruit therafter. The values for fructose content are not shown, but were not significantly different from those for glucose.

Net fluxes and metabolite contents. The net rates of starch breakdown, and sucrose, glucose and fructose accumulation during the climacteric (day 8 and day 9 for 15 and 10% oxygen, respectively) were calculated from Fig. 1. These rates, together with the rates of CO_2 production, are shown in Table 1. The fluxes in the controls were not significantly different from those described in our previous work (Hill and ap Rees 1994, 1995). After incubation in 15% oxygen there was a significant increase in the rates of glucose and fructose accumulation, but the other fluxes were unaltered. Treatment with 10% oxygen led to a significant reduction in the rates of CO_2 production and sucrose accumulation, and a significant increase in the rates of glucose and fructose accumulation. There was also a substantial fall in the rate of starch breakdown. This was not significant when compared to the control fruit, but was significantly different from the rate of starch breakdown in 15% oxygen. As discussed in Hill and ap Rees (1995), we wish to compare fluxes in hypoxia with those previously reported for air (Hill and ap Rees 1994). Therefore, in the subsequent calculations, we have used the normalised values reported in parentheses in Table 1 and the net flux values reported in Hill and ap Rees (1994) to estimate the effect that low oxygen tension would have had on the fruit studied previously.

The effect of low oxygen tension on the contents of some key intermediates is shown in Table 2. The metabolite contents in control fruit were similar to those reported previously (Hill and ap Rees 1995). Control fruit were taken from the same hand as the treated fruit, and were sampled at the same time. The variation between fruit of the same hand was low (Table 2), and similar contents were obtained from control fruit from different hands (data not shown). Consequently, the data for all control fruit were pooled. The content of hexose phosphates, particularly Glc6P was reduced upon incubation in 15 and 10% oxygen. There was no significant change in the ATP/ADP ratio, but an increase in the AMP content led to a reduction in the adenylate energy charge (AEC) in both 15 and 10% oxygen. There was evidence to suggest that the pyruvate content increased on exposure to 15% oxygen, but we were unable to demonstrate a significant change in the content of this metabolite after exposure to 10% oxygen.

Turnover of starch and sugars. To measure the rate of starch synthesis and the rate of fructose entry into the

Table 1. Effect of oxygen concentration on the net fluxes of carbohydrate metabolism in climacteric banana fruit. Banana fruit were allowed to ripen in air, then transferred to an atmosphere containing 10 or 15% oxygen as the climateric rise in respiration began. Starch, sucrose, glucose and fructose contents were measured at the intervals indicated in Fig. 1, in order to calculate the net fluxes. Production of CO_2 was measured by infra-red gas analysis. Values are the mean ± SE of the number of samples shown. The values given in parentheses are those in hypoxia relative to those in air. NS = not significant

Description of flux	Estimate of net flux ($\mu mol \cdot gFW^{-1} \cdot h^{-1}$) during incubation in:		
	Air (n = 6) I	15% oxygen (n = 3) II	10% oxygen (n = 3) III
Starch breakdown	5.1 ± 0.7	6.2 ± 0.3 (1.21)	3.8 ± 0.1 (0.74)
Accumulation of:			
Sucrose	3.1 ± 0.4	2.2 ± 0.1 (0.71)	0.99 ± 0.08 (0.32)
Glucose	0.52 ± 0.05	0.73 ± 0.04 (1.40)	1.21 + 0.12 (2.33)
Fructose	0.48 ± 0.06	0.68 ± 0.05 (1.42)	1.21 ± 0.07 (2.52)
CO_2 production	0.42 ± 0.03	0.47 ± 0.02 (1.12)	0.21 ± 0.02 (0.50)
	Fisher's *P* values I vs III	I vs III	II vs III
Starch breakdown	NS	NS	< 0.01
Acumulation of:			
Sucrose	NS	< 0.01	< 0.01
Glucose	< 0.05	< 0.001	< 0.05
Fructose	< 0.05	< 0.001	< 0.05
CO_2 production	NS	< 0.01	< 0.01

hexose monophosphate pool we supplied [U-^{14}C]glucose to cores of climacteric fruit for 4-h in the presence of 15 or 10% oxygen. We determined the detailed distribution of label after this 4-h pulse and also after a subsequent 4-h chase in unlabelled glucose. Starch synthesis is indicated by incorporation of label into starch during the pulse, whilst loss of label from fructose during the chase is indicative of conversion of fructose to hexose monophosphates. The results of this analysis are shown in Table 3. At both oxygen concentrations the overall distribution of label was similar to that obtained in air (Hill and ap Rees 1994). Similar to our previous work (Hill and ap Rees 1994, 1995), there was incorporation of ^{14}C into oligosaccharides (Table 3). This incorporation was greater during incubation in 10% oxygen. We argue that this pool is in equilibrium with the major pathways of carbohydrate metabolism, but does not represent a major flux. Our reason is that, in both our earlier work (Hill and ap Rees, 1994) and in the present paper (Fig. 1), all of the carbon released from starch during ripening can be accounted for as sugars and carbon dioxide.

Hypoxia has three major effects on the metabolism of [U-^{14}C]glucose. *First*, in low oxygen tension there was a greater incorporation of label into hexose sugars (35 and 40% as opposed to 29%) and a concomitant reduction in the labelling of sucrose (10 and 14% as opposed to 29%). Thus low oxygen tension alters the partitioning of carbon between sucrose and hexose sugars. This is consistent with our net flux measurements, which show a reduced rate of sucrose accumulation, but an increased rate of hexose accumulation in low oxygen tension (Fig. 1, Table 1). *Second*, in low oxygen tension, a lower proportion of the metabolised label was found in starch (1.9 and 0% as opposed to 2.8%). This indicates that the rate of starch synthesis is reduced by hypoxia. *Third*, there is only a small reduction in the labelling of fructose during the chase, suggesting that the rate of re-entry of hexose into the hexose-phosphate pool has been reduced in low oxygen tension. The first two effects were more marked in 10% than in 15% oxygen.

We can use the specific activity of the fructose at the end of the pulse to estimate the rate of conversion of fructose to hexose monophosphate, as shown in Table 4. The rate of entry of fructose into the hexose monophosphate pool was unaltered by incubation in 15% oxygen. However, treatment with 10% oxygen led to a marked reduction in this flux ($P < 0.05$). As we have previously argued (Hill and ap Rees 1994), we can estimate the rate of conversion of internal hexose to hexose monophosphate as twice the rate of fructose conversion, 4.0 and 1.6 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$ for 15 and 10% oxygen, respectively. The rates of net hexose accumulation are 1.2 and 2.1 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$, so that the rates of hexose synthesis, and thus sucrose breakdown are 5.2 and 3.7 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$, respectively, for 15 and 10% oxygen. As the net rates of sucrose accumulation are 2.1 and 1.0 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$, then the rates of sucrose synthesis are 7.3 and 4.7 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$, respectively, for 15 and 10% oxygen. The rate of starch synthesis can be estimated by comparing the extent of labelling of starch and sugars (Hill and ap Rees 1994). The rate of starch synthesis is 0.3 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$ in 15% oxygen. In 10% oxygen, starch synthesis could not be detected. The rate of starch breakdown can be calculated from the rate of synthesis and the rate of net breakdown, and is 6.9 and 4.1 μmol hexose $\cdot gFW^{-1} \cdot h^{-1}$, respectively in 15 and 10% oxygen.

Glycolysis and the oxidative pentose-phosphate pathway. In order to estimate the flux through the two pathways of carbohydrate oxidation under hypoxia, we supplied [1-^{14}C]-, [3,4-^{14}C]- and [6-^{14}C]glucose to cores of climacteric banana fruit for 6 h in the presence of 15 or 10% oxygen. The detailed distribution of label is shown in Table 5. Again, the overall distribution of label was similar to that found in air (Hill and ap Rees 1994). As we found in

Table 2. Effect of oxygen concentration on metabolite contents of climacteric banana fruit. Banana fruit were allowed to ripen in air, then transferred to an atmosphere containing 10 or 15% oxygen as the climacteric rise began. At the climacteric peak, 8–12 h later, cores of tissue were rapidly removed from the fruit, freeze-clamped and extracted in 1.0 M perchloric acid, before measurement of the metabolites shown. Values are the mean ± SE of the number of samples shown. Fisher's *P* values were calculated using Students *t*-test. NS = not significant

Compound[a]	Metabolite content (nmol · g FW^{-1}) after incubation in:		
	Air (n = 6) I	15% oxygen (n = 3) II	10% oxygen (n = 3) III
Glc6P	161 ± 4	135 ± 4	110 ± 3
Glc1P	12 ± 2	9 ± 1	6 ± 1
Fru6P	51 ± 2	46 ± 5	29 ± 3
Total hexoseP	225 ± 6	190 ± 9	145 ± 4
ATP	53 ± 2	48 ± 3	47 ± 4
ADP	33 ± 1	33 ± 2	25 ± 3
AMP	8 ± 1	14 ± 1	19 ± 1
Total adenylates	94 ± 3	95 ± 1	91 ± 2
Pyruvate	25 ± 3	37 ± 2	21 ± 2
Glc6P/Glc1P	16 ± 3	16 ± 2	17 ± 1
Fru6P/Glc6P	0.32 ± 0.01	0.34 ± 0.03	0.26 ± 0.03
ATP/ADP	1.65 ± 0.07	1.49 ± 0.19	1.94 ± 0.31
AEC	0.74 ± 0.01	0.68 ± 0.02	0.65 ± 0.02
	Fisher's *P* values I vs II	I vs III	II vs III
Glc6P	< 0.01	< 0.001	< 0.01
Glc1P	NS	NS	NS
Fru6P	NS	< 0.001	< 0.05
Total hexoseP	< 0.05	< 0.001	< 0.05
ATP	NS	NS	NS
ADP	NS	< 0.05	NS
AMP	< 0.01	< 0.001	< 0.05
Total adenylates	NS	NS	NS
Pyruvate	< 0.05	NS	< 0.01
Glc6P/Glc1P	NS	NS	NS
Fru6P/Glc6P	NS	NS	NS
ATP/ADP	NS	NS	NS
AEC	< 0.01	< 0.001	NS

[a]AEC = adenylate energy charge

Table 3. Effect of oxygen concentration on the metabolism of [U-^{14}C]glucose by cores of climacteric banana fruit. Prior to the experiment, fruit were ripened in air, then transferred to 15% or 10% oxygen as the climacteric rise began. At the climacteric peak, 8–12 h later, cores of pulp were removed from the fruit and incubated in 0.3 mM [U-^{14}C]glucose (74 GBq · mol^{-1}) for 4 h at 21°C (pulse) and for a further 4 h in 0.3 mM glucose (chase). The medium and the gas phase in the reaction vessel contained either 15% or 10% oxygen. Values are the mean ± SE of three fruit. nd = not detected

Fraction	^{14}C recovered per fraction as % total metabolised:			
	15% oxygen		10% oxygen	
	4-h pulse	4-h pulse + 4-h pulse	4-h pulse	4-h pulse + 4-h chase
CO_2	4.1 ± 0.3	8.3 ± 0.6	3.9 ± 0.8	7.9 ± 0.3
Water-soluble substances	73.1 ± 8.3	55.3 ± 0.6	68.7 ± 8.2	79.7 ± 1.5
Neutral components	65.0 ± 12.0	43.0 ± 0.4	79.5 ± 3.7	70.5 ± 7.4
Sucrose	10.5 ± 1.1	9.4 ± 0.8	13.8 ± 1.7	7.1 ± 0.8
Glucose	17.5 ± 1.1	13.7 ± 0.6	20.0 ± 3.5	12.1 ± 1.2
Fructose	17.5 ± 1.1	13.7 ± 0.6	20.0 ± 3.5	12.1 ± 1.2
Oligosaccharides	3.7 ± 0.2	3.8 ± 0.3	11.1 ± 1.9	18.7 ± 2.1
Acidic components	7.6 ± 0.5	6.8 ± 0.3	12.1 ± 2.9	5.9 ± 1.0
Basic components	2.2 ± 0.1	3.1 ± 0.4	1.3 ± 0.2	4.1 ± 1.2
Water-soluble substances	22.7 ± 8.1	36.3 ± 1.1	16.2 ± 2.8	12.3 ± 1.4
Starch	1.9 ± 0.1	nd	nd	nd
[^{14}C]glucose metabolised:				
as kBq	2.39 ± 0.29	2.29 ± 0.25	1.20 ± 0.26	0.76 ± 0.04
as % ^{14}C supplied	3.4 ± 0.4	3.2 ± 0.4	1.6 ± 0.3	1.0 ± 0.1

our previous study (Hill and ap Rees 1994), a significant proportion of the ^{14}C was recovered in insoluble components that we did not identify. As we argued above with respect to the labelling of the oligosaccharide fraction, this is likely to represent equilibration of label, rather than a significant net flux. There were two major differences. *First*, as with [U-^{14}C]glucose, the hexose sugars were labelled more heavily than sucrose under hypoxia. *Second*,

Table 4. Effect of oxygen concentration on the rate of re-entry of fructose into the hexose phosphate pool in cores of climacteric banana fruit. The specific activities of fructose at the end of the pulse are calculated from measurements of the fructose content and its labelling in the samples described in Table 3. This was used to calculate the rate of fructose metabolism during the subsequent 4-h chase. Values are the mean ± SE of three fruit from the same hand. Control data from Hill and ap Rees (1994) are included for comparative purposes

Oxygen concentration (%)	Fructose content at end of pulse ($\mu mol \cdot gFW^{-1}$)	^{14}C in fructose at end of pulse ($Bq \cdot gFW^{-1}$)	Specific activity of fructose at end of pulse ($Bq \cdot \mu mol^{-1}$)	^{14}C lost from fructose during 4-h chase ($Bq \cdot gFW^{-1}$)	Rate of fructose re-entry ($\mu mol \cdot gFW^{-1} \cdot h^{-1}$)
21[a]	11.0 ± 0.5	129 ± 20	11.6 ± 1.3	90 ± 9	1.9 ± 0.1
15	36 ± 4	181 ± 19	5.1 ± 0.1	41 ± 4	2.0 ± 0.2
10	51 ± 9	350 ± 108	6.6 ± 1.0	24 ± 9	0.8 ± 0.2

[a] Hill and ap Rees 1994

Table 5. Effect of oxygen concentrtion on the metabolism of specifically labelled [^{14}C]glucose by cores of climacteric banana tissue. Prior to the experiment, fruit were ripened in air, then transferred to 15% or 10% oxygen as the climacteric rise began. At the climacteric peak, 8–12 h, later, cores of pulp were incubated in 0.3 mM [1-^{14}C]-, [3,4-^{14}C]- and [6-^{14}C]glucose (74 $GBq \cdot mol^{-1}$) for 6 h at 21°C. The medium and the gas phase in the reaction vessel contained either 15% or 10% oxygen. For each oxygen concentration, values are for a single set of replicate samples each containing tissue from three fruit from the same hand. nd = none detected

Fraction	^{14}C recovered per fraction as % total metabolised:					
	15% oxygen			10% oxygen		
	C-1	C-3,4	C-6	C-1	C-3,4	C-6
CO_2	5.3	4.7	1.7	11.0	5.7	1.4
Water-soluble substances	56.9	65.4	61.9	69.1	60.9	58.3
Neutral components	54.8	48.7	46.3	49.3	79.4	86.6
Sucrose	11.5	9.0	10.5	14.3	17.3	7.8
Glucose	17.7	16.5	16.0	21.7	26.0	27.6
Fructose	17.7	16.5	16.0	21.7	26.0	27.6
Oligosaccharides	3.3	3.5	4.0	16.1	13.4	13.5
Acidic components	3.9	5.6	6.2	1.1	5.9	9.5
Basic components	1.6	2.0	2.4	3.6	2.4	3.2
Water-insoluble substances	37.8	29.9	36.3	19.9	14.9	11.8
Starch	2.4	1.9	1.8	nd	nd	nd
Protein	4.8	5.0	5.2	1.1	10.8	5.5
[^{14}C]glucose metabolised:						
as kBq	4.39	3.13	3.51	1.10	0.37	1.25
as % ^{14}C supplied	6.7	4.9	5.2	1.7	1.6	1.8

in 10% oxygen there was a marked increase in the CO_2 release from carbon atom 1. This is indicative of an increased proportion of the respiratory flux occurring via the oxidative pentose-phosphate pathway.

We estimated the flux through the oxidative pentose-phosphate pathway from the difference in the contributions of glucose carbons 1 and 6 to respired CO_2. We estimated the combined flux through glycolysis and the pentose-phosphate pathway from the labelling by glucose carbons 3 and 4 of the products of these pathways, viz. CO_2, acidic and basic components of the soluble fraction, and protein. This gives the rate of oxidative pentose-phosphate pathway CO_2 production as 0.09 and 0.08 μmol $hexose \cdot gFW^{-1} \cdot h^{-1}$ for 15 and 10% oxygen, respectively. The CO_2 production due to the citric-acid cycle is therefore 0.36 and 0.12 μmol $hexose \cdot gFW^{-1} \cdot h^{-1}$. The rates of entry of Glc6P and formation of Fru6P and triose phosphate can be estimated if the stoichiometry of the oxidative pentose phosphate pathway is taken as (ap Rees 1980a):

$$3Glc6P \rightarrow 2Fru6P + 1TrioseP + 3CO_2$$

Recycling between triose phosphates and hexose monophosphates. To estimate the extent to which triose phosphates are converted to hexose monophosphates under hypoxia we supplied [U-^{14}C]glycerol to cores of climacteric banana fruit incubated for 4 h in 15 or 10% oxygen. We analysed the detailed distribution of label and the results are shown in Table 6. Compared to incubation in air (Hill and ap Rees 1994), there was a reduction in the proportion of the label that was converted to CO_2 (22% and 1% as opposed to 49%). There was a concomitant increase in the labelling of sugars under hypoxia (10% and 12% as opposed to 9%). This suggests that the flux from triose phosphates to hexose monophosphates was increased, at least following treatment with 10% oxygen.

We further investigated the flux between triose phosphates and hexose monophosphates by determining the relative labelling of carbons 1 and 6 in the glucosyl and fructosyl moieties of sucrose and in fructose from cores that had been supplied with [1-^{14}C]- and [6-^{14}C]glucose (Table 5). Redistribution of label between the 1 and 6 carbon atoms is a measure of the flux between triose phosphates and hexose monophosphates (Hatzfeld and Stitt 1990). The results are shown in Table 7. In 15% oxygen, the degree of randomisation found in the hexose sugars was very similar to that found in air. However, the degree of randomisation increased markedly on incubation in

Table 6. Effect of oxygen concentration on the metabolism of [U-^{14}]glycerol by cores of climacteric banana fruit. Prior to the experiment, fruit were ripened in air, then transferred to 15% or 10% oxygen as the climacteric rise began. At the climacteric peak, 8–12 h later, cores of pulp were incubated in 0.3 mM [U-^{14}C]glycerol (63 GBq · mol^{-1}) for 4 h at 21°C. The medium and the gas phase in the reaction vessel contained either 15% or 10% oxygen. Values are the mean ± SE of three fruit from the same hand. nd = none detected

Fraction	^{14}C recovered per fraction as % total ^{14}C metabolised:	
	15% oxygen	10% oxygen
CO_2	21.7 ± 1.8	0.6 ± 0.1
Water-soluble substances	44.9 ± 2.1	78.5 ± 4.4
Neutral components	11.1 ± 1.9	19.3 ± 4.7
Sucrose	2.3 ± 0.3	3.6 ± 1.7
Glucose	3.8 ± 0.3	4.3 ± 0.8
Fructose	3.8 ± 0.3	4.3 ± 0.8
Acidic components	21.9 ± 2.2	48.4 ± 12.1
Basic components	12.2 ± 0.9	2.2 ± 0.3
Water-insoluble substances	33.4 ± 0.3	20.8 ± 4.3
Starch	nd	nd
Protein	5.2 ± 0.4	0.9 ± 0.1
[^{14}C]glycerol metabolised:		
as kBq	3.69 ± 0.36	3.67 ± 0.66
as % ^{14}C supplied	5.5 ± 0.5	5.5 ± 1.0

10% oxygen, supporting the conclusion that the flux from triose phosphates to hexose monophosphates was greatly increased.

We estimated the flux from triose phosphates to hexose monophosphates by comparing the distribution of label following the supply of [U-^{14}C]glucose (Table 3) and [U-^{14}C]glycerol (Table 6). Since the former experiment gives the proportion of the hexose-monophosphate pool that is converted to sugars, and the latter gives the proportion of the triose-phosphate pool that is converted to sugars, we can calculate the total proportion of the triose-phosphate pool that had access to the hexose-monophosphate pool. This can then be compared with the fraction converted to CO_2 following the supply of [U-^{14}C]glycerol, to give an estimate of the flux. The fluxes calculated in this way are 0.36 and 4.5 μmol hexose · gFW^{-1} · h^{-1} for 15 and 10% oxygen, respectively. A similar calculation allows us to estimate the fluxes from triose phosphates to amino acids and protein as 0.3 and 0.6 μmol hexose · gFW^{-1} · h^{-1} for 15 and 10% oxygen.

We can also calculate the flux from triose phosphates to hexose monophosphates from the data of Table 7, using equation 2 of Hatzfeld and Stitt (1990). This gives values of 0.13 and 2.3 μmol hexose · gFW^{-1} · h^{-1}, which are in reasonable agreement with those obtained above. In the subsequent discussion we have used the mean of the values obtained by the two methods, i.e. 0.24 and 3.4 μmol hexose · gFW^{-1} · h^{-1} for 15 and 10% oxygen, respectively.

Discussion

Our measurements of the net fluxes of starch breakdown, sugar accumulation and CO_2 production in fruit ripened in air (Table 1) are similar to those we have previously reported (Hill and ap Rees 1994). Both our metabolite measurements and feeding experiments are adequately authenticated (see *Materials and methods*). For our metabolite measurements under hypoxia, we argue that the time interval between sampling and freeze-clamping was so low as to have negligible effect on metabolite contents. Consequently, we suggest that the data presented give

Table 7. Effect of oxygen concentration on the redistribution of ^{14}C in hexose units during synthesis of sucrose and fructose from [1-^{14}C]- and [6-^{14}C]glucose by cores of climateric banana fruit. Prior to the experiment fruit were ripened in air, then transferred to 15% or 10% oxygen as the climacteric rise began. At the climacteric peak, 8–12 h later, replicate samples containing tissue from three fruit were incubated in 0.3 mM [1-^{14}C]- and [6-^{14}C]glucose (74.0 GBq · mol^{-1}, respectively) for 6 h at 21°C in the presence of 15% or 10% oxygen, as described in Table 5. Fructose and sucrose were isolated from the tissue and the latter was digested with invertase. The glucosyl and fructosyl moieties were then isolated. The distribution of ^{14}C within the isolaed hexoses was determined by degradation with 6-phosphogluconate dehydrogenase. The percent redistribuion was calculated after correction for the distribution of ^{14}C in samples of authentic [1-^{14}C]- and [6-^{14}C]glucose. Control data (21% oxygen) are taken from Hill and ap Rees (1994), and are included for comparative purposes

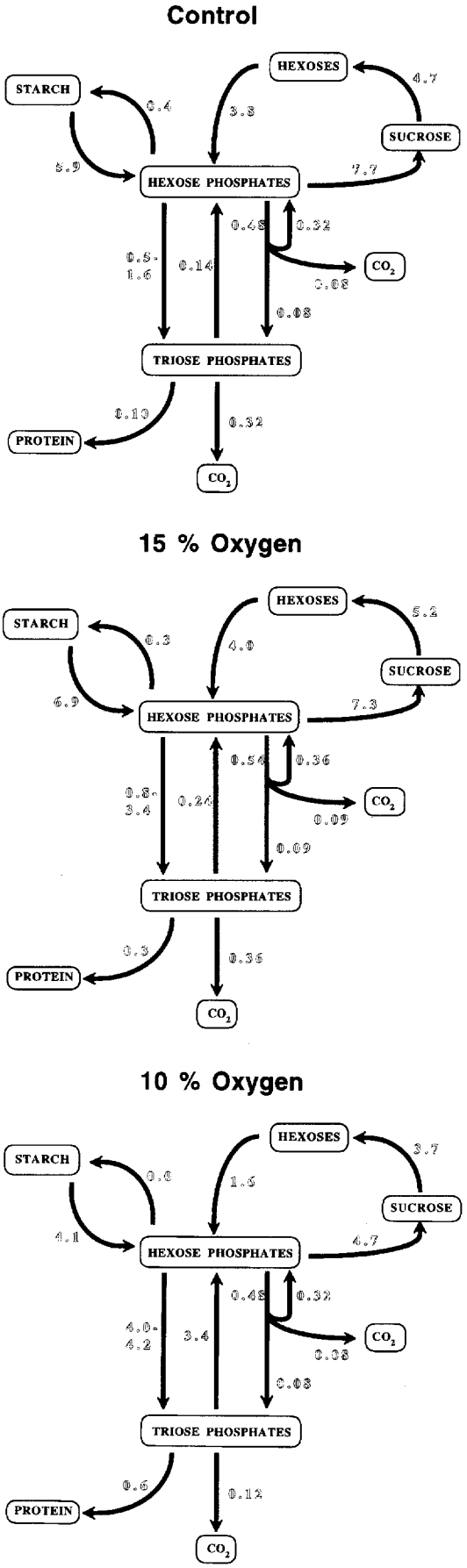
Source of hexose for analysis	% redistribution of ^{14}C following supply of [^{14}C]glucose labelled in:		% recovery of ^{14}C during analysis following supply of [^{14}C]glucose labelled in:		total ^{14}C analysed per sample (Bq) following supply of [^{14}C]glucose labelled in:	
	C-1	C-6	C-1	C-6	C-1	C-6
Control						
Sucrose						
Glucosyl	5.4	4.6	98.0	97.5	9.0	8.8
Fructosyl	2.0	3.0	97.1	97.2	9.1	9.8
Fructose	2.8	2.4	98.1	97.9	10.3	13.6
15% oxygen						
Sucrose						
Glucosyl	4.4	2.7	98.7	96.2	12.3	10.8
Fructosyl	1.7	6.8	97.1	91.6	12.0	11.4
Fructose	2.9	3.8	94.5	95.2	10.3	9.6
10% oxygen						
Sucrose						
Glucosyl	38.8	39.3	95.4	97.7	7.8	12.6
Fructosyl	39.1	40.3	98.2	97.3	8.4	6.9
Fructose	34.0	40.6	98.7	95.3	8.0	9.4

a reliable picture of the effects of hypoxia on climacteric banana fruit.

Effect of hypoxia on the fluxes of carbohydrate metabolism. Our estimates of the major fluxes of carbohydrate metabolism in cores of climacteric banana fruit during incubation in 15 or 10% oxygen are summarised in Fig. 2. The glycolytic fluxes from hexose monophosphate to triose phosphate were calculated in two ways: in the first we assumed that this flux is the only flux out of the hexose-monophosphate pool that is unaccounted for; in the second a similar assumption is made for the triose-phosphate pool. The two estimates obtained are given as a range. During incubation in 15% oxygen the flux from hexose monophosphates to triose-phosphates lies between 0.8 and 3.4 μmol hexose·gFW^{-1}·h^{-1}, while in the presence of 10% oxygen this flux is 4.0–4.2 μmol hexose·gFW^{-1}·h^{-1}.

Fluxes altered by hypoxia. Incubation in 15% oxygen has only minor effects on the fluxes of carbohydrate metabolism. There is a small reduction in the rate of sucrose synthesis. This is likely to have been brought about by the reduction in size of the hexose-phosphate pool (Table 2), acting both directly and via inactivation of sucrose-phosphate synthase (Reimholz et al. 1994). The change in size of the hexose-monophosphate pool is the result of a small increase in the net glycolytic flux from 0.4–1.5 μmol hexose·gFW^{-1}·h^{-1} in air to 0.6–3.2 μmol hexose·gFW^{-1}·h^{-1} in 15% oxygen. The mechanism by which this increase is brought about is unclear: the flux through the citric-acid cycle is unaltered or slightly increased by this treatment, and the ATP/ADP ratio is unchanged (Table 2). However, there is a significant increase in the AMP content (Table 2). This suggests that the adenylate status of the tissue has been perturbed, and that the ATP/ADP ratio is maintained via the action of adenylate kinase (see later). Consequently, the increase in the net flux from hexose monophosphates to triose phosphates could be brought about through an allosteric effect of AMP on the reactions that catalyse this flux. In animals AMP activates phosphofructokinase [PFK(ATP); Stryer 1981], but in plants the available evidence suggests that PFK(ATP) is *inhibited* by AMP (Copeland and Turner 1987). However, the recent demonstration by Thomas and Kruger (1994) that contamination of coupling enzymes by adenylate kinase may interfere with the assay of PFK(ATP), leading to an apparent ADP-dependent activity, leads us to question the validity of AMP-inhibition of PFK(ATP). Since the equilibrium position of adenylate kinase favours ADP production (Barman 1969), addition of AMP to PFK(ATP) assay mixtures will reduce the ATP concentration and increase the ADP concentration; both these effects will tend to reduce the apparent PFK(ATP) activity (Copeland and Turner 1987). Our data provide evidence in support of AMP-activiation of PFK(ATP), and we suggest that this issue needs to be re-examined in the light of the data of Thomas and Kruger (1994).

Incubation in 10% oxygen results in major changes in metabolic fluxes (Fig. 2). Starch synthesis is reduced to undetectable levels, even though the Glc1P and ATP contents are unaltered. There are three possible explana-



tions for the reduction in starch synthesis. *First*, the plastidic pools of Glc1P and/or ATP have been reduced. This would imply that the plastidic pools of these metabolite are not in equilibrium with those in the cytosol. Our previous work has demonstrated that this may be the case, at least for Glc1P (Hill and ap Rees 1995). *Second*, changes in the contents of 3-phosphoglyceric acid and/or inorganic phosphate could have allosterically inactivated ADP glucose (ADPGlc) pyrophosphorylase (Preiss 1988). *Third*, there is an uncharacterised allosteric effect on either ADPGlc pyrophosphorylase, starch synthase or branching enzyme. The rate of starch breakdown is also reduced under 10% oxygen (Fig. 2). The mechanism for this change is unclear, but, if the reduction in starch synthesis leads to an increase in the ADPGlc content of the plastids, then ADPGlc-inhibition of starch phosphorylase is a possible explanation (Kruger and ap Rees 1983).

The rate of sucrose synthesis is reduced in 10% oxygen. As with 15% oxygen, this is likely to be due to the reduction in the contents of Glc6P and Fru6P (Table 2). There is also a reduction in the rate of conversion of glucose and fructose into hexose phosphates. This occurs despite the fact that the ATP content is unchanged (Table 2), and that the glucose and fructose contents have increased (Fig. 1). Since the AMP content is substantially increased in 10% oxygen, this could be due to AMP-inhibition of hexokinase and fructokinase. The hexokinases and fructokinases from potato tubers are weakly inhibited by AMP (Renz and Stitt 1993). However, this suggestion seems unlikely, since treatment with 15% oxygen, which also causes an increase in the AMP content, does not alter the flux between hexoses and hexose monophosphates. An alternative explanation is that the distribution of hexoses between the cytosol and the vacuole is altered in 10% oxygen, so that the cytosolic glucose and fructose concentrations are reduced. Recently Heineke et al. (1994) have provided compelling evidence that hexose sugars are preferentially accumulated in the vacuole of tobacco leaves.

The rate of CO_2 production by the citric-acid cycle is considerably reduced in 10% oxygen (Fig. 2). This is presumably due to a reduced rate of electron transport because of a reduction in the activity of cytochrome oxidase. This will lead to a build up of mitochondrial NADH, that will inhibit the citric-acid-cycle dehydrogenases (Pascal et al. 1990). The most striking effect of incubation in 10% oxygen is the massive increase in the flux between triose phosphates and hexose monophosphates. We will discuss this in detail later.

Fluxes unaltered by hypoxia. The only flux that we found to be unaffected by hypoxia is that through the oxidative pentose-phosphate pathway (Fig. 2). This is despite a reduction in the content of the substrate for this pathway Glc6P. The simplest explanation for this is that flux through this pathway is regulated by the demand for NADPH (ap Rees 1980b), and this demand does not change, even in 10% oxygen. The relative insensitivity of the oxidative pentose-phosphate pathway to hypoxia has also been demonstrated in *Echinochloa* (Rumpho and Kennedy 1983).

Sucrose recycling is reduced with the availability of ATP. Our data provide evidence that, under conditions of low ATP synthesis rates, there are regulatory mechanisms which operate to reduce the rate of sucrose synthesis. These mechanisms do not operate via changes in the ATP/ADP ratio in this tissue, but, in fact, act to maintain a fixed ATP/ADP ratio. In contrast, when the rate of sucrose synthesis is artificially altered by the supply of exogenous glucose to banana tissue, respiration is stimulated by a fall in the ATP/ADP ratio (Hill and ap Rees 1995).

Despite the reduction in the unidirectional rate of sucrose synthesis, there is only a minor fall in net sugar accumulation under hypoxia: in 10% oxygen the unidirectional rate is reduced by 39% (Fig. 2), whereas the net sugar accumulation rate (sucrose + glucose + fructose) is only reduced by 17% (Table 1). This is because of the significant reduction in the rate at which sugars are recycled (Table 4). Thus a potential function of sucrose recycling in sugar-accumulating tissues may be to allow net sugar accumulation to be relatively insensitive to changes in the rate of ATP synthesis. If sugar accumulation is a simple linear pathway, then ATP consumption and sugar accumulation can only change in parallel to one another. In contrast, the existence of a cycle allows ATP consumption to fall dramatically, without a major change in the rate of sugar accumulation.

A side effect of this mechanism is a change in the water relations of the tissue. Although, in 10% oxygen, the rate of total net sugar accumulation falls by 17% when expressed in hexose equivalents, the rate of synthesis of osmotically active sugar molecules actually increases by 14%. It is interesting to note that, ultimately, fruit ripened under 10% oxygen accumulate less sugar than those in air (Fig. 1), and tempting to speculate that this may be due to an osmotic restriction on sugar accumulation. Therefore, hexose accumulation in the vacuole may be a short-term mechanism for preventing excessive respiration of carbohydrate during periods of low ATP synthesis. When ATP synthesis rates recover, the hexose sugars could then be recycled back into the hexose-monophosphate pool and used once again for sucrose synthesis. The detailed mechanisms underlying the accumulation of hexose sugars in banana fruit under hypoxia remain to be elucidated.

The extent to which hypoxia-induced hexose accumulation is general to plant tissues as a whole is unclear. However, there is evidence to suggest that sucrose recycling plays an important role in the regulation of carbohydrate metabolism in plants. In the cotyledons of *Ricinus*, a tissue involved in transferring nutrients between the endosperm and the developing seedling, sucrose recycling appears to function to allow buffering between import and export rates (Geigenberger and Stitt 1991). Similarly, in sugar-cane suspension cells, sucrose recycling allows the rate of sucrose accumulation to be varied sensitively in

Fig. 2. The effect of oxygen concentration on the fluxes of carbohydrate metabolism in climacteric banana fruit. Fluxes were calculated as described in the text, and are expressed as mmol hexose $\cdot (gFW)^{-1} \cdot h^{-1}$. The control values are from Hill and ap Rees (1994), and are included for comparative purposes

response to changes in the nutrient status of the cells (Wendler et al. 1990)

Adenylate kinase acts to maintain the ATP/ADP ratio. During hypoxia the ATP/ADP ratio in banana tissue is remarkably stable (Table 2). However, there were substantial increases in the AMP content (Table 2), suggesting that a net flux through the adenylate-kinase reaction was compensating for in part the reduction in the rate of mitochondrial ATP synthesis. The mass action ratio of the adenylate-kinase reaction changed from 0.39 in air to 0.62 and 1.43 in 15 and 10% oxygen respectively. In rice and soybean cultures, incubation in anoxic environments leads to increases in the content of AMP, although in soybean there is also a decrease in the ATP/ADP ratio (Mohanty et al. 1993). In rice, which is relatively tolerant of anoxia, the ATP/ADP ratio remains unchanged and the mass-action ratio of adenylate kinase increases from 0.33 in air to 0.52 in anoxia (Mohanty et al. 1993). This is similar to the behaviour of banana fruit in hypoxia. This tissue is likely to be tolerant of hypoxic stress, since its demand for oxygen is high, and the peel represents a significant diffusive barrier to gases (Banks 1983); some degree of hypoxia is likely to be the usual state for this tissue. Therefore, we suggest that one of the metabolic features which allow some plant tissues to tolerate anoxia is a high adenylate kinase activity, which allows ATP to be synthesised at the expense of ADP.

A consequence of the above hypothesis is that the AMP content may act as an important intracellular signal of hypoxia in tolerant plant tissues. As we have already discussed, AMP acts allosterically on hexokinase and fructokinase (Renz and Stitt 1994), and potentially PFK(ATP) (Copeland and Turner 1987; but see our earlier discussion). In addition AMP could act via changes in the content of fructose-2,6-bisphosphate (Fru2,6bisP), since AMP inhibits fructose-2,6-bisphosphatase (Macdonald et al. 1989).

Pyrophosphate: Fructose-6-phosphate phosphotransferase [PFK (PP_i)] and metabolism in hypoxia. The most striking feature of the data presented in this paper is the massive increase in the rate of recycling of triose phosphates to hexose monophosphates observed in 10% oxygen (Table 7; Fig. 2). Since there is no cytosolic fructose-1,6-bisphosphatase in banana fruit (Ball et al. 1991), the flux in the gluconeogenic direction is almost certainly catalysed by PFK (PP_i). Two lines of evidence suggest that the glycolytic flux is also catalysed by this enzyme under hypoxia. *First*, since the maximum catalytic activity of PFK(ATP) is only 6 μmol hexose · $gFW^{-1} \cdot h^{-1}$ (Ball et al. 1991), the flux of 4.0–4.2 μmol hexose · $gFW^{-1} \cdot h^{-1}$ is sufficiently close to the maximum to make it unlikely that the whole flux is catalysed by this enzyme. *Second*, the rate of ATP synthesis is insufficient to supply the ATP required for PFK(ATP) to catalyse the flux. The maximum rate of ATP synthesis in 10% oxygen, assuming 38 molecules of ATP for each molecule of hexose oxidised, is 4.6 μmol · $gFW^{-1} \cdot h^{-1}$. The rate of ATP consumption by sucrose synthesis and hexose phosphorylation in 3.9 μmol · $gFW^{-1} \cdot h^{-1}$. Thus PFK(ATP) can at most catalyse 17% of the flux from hexose monophosphates to triose phosphates. Therefore, we argue that under hypoxia the major part of the glycolytic flux is catalysed by PFK (PP_i), rather than PFK(ATP) which is likely to catalyse the major part of the glycolytic flux in air (Ball et al. 1991; Hill and ap Rees 1995).

There are two possible mechanisms that result in a change from PFK(ATP) to PFK(PP_i); either an inhibition of PFK(ATP) or an activation of PFK. The former could be brought about directly through AMP inhibition of PFK(ATP) (Copeland and Turner 1987), although as we discussed earlier, the effect of AMP may not yet be established. A more likely mechanism for the switch to PFK(PP_i) is through an increase in Fru2,6bisP, a potent activator of this enzyme (Stitt 1990). An increase in the content of this activator may result from the increase in the content of AMP (Table 2), because the latter inhibits fructose-2,6-bisphosphatase, the enzyme responsible for the breakdown of Fru2,6bisP (Macdonald et al. 1989). A problem with this hypothesis is that, as Stitt (1990) has argued, the AMP concentrations required to inhibit fructose-2,6-bisphosphatase are unphysiologically high. However, if we assume that the majority of the AMP is cytosolic in ripening banana fruit, and that the cytosol accounts for 5% of the volume of the fruit (Winter et al. 1994), we can estimate the maximum AMP concentration in the cytosol to be 0.2 mM in air and 0.4 mM in 10% oxygen. These values are of the same order as the K_i for spinach leaf fructose-2,6-bisphosphatase, which is 0.95 mM (Macdonald et al. 1989). The interpretation may be affected by compartmentation of AMP, as, in leaf protoplasts, up to 65% of the total AMP is plastidic (Stitt et al. 1982). A second problem with the hypothesis that AMP-induced changes in Fru2,6bisP cause the increased flux through PFK(PP_i) is the high Fru2,6bisP content of ripening banana fruit in air (ca. 100 pmol · gFW^{-1}; Ball and ap Rees 1988). This means that the concentration in the cytosol is about 2 μM and, since the in-vitro K_a of potato PFK(PP_i) is 0.005 μM (Stitt 1989), the enzyme should be fully activated in air. A similar problem exists in potato, where transgenic tubers with elevated activities of fructose-6-phosphate 2-kinase and, consequently increased contents of Fru2,6bisP, show altered fluxes, despite the fact that the Fru2,6bisP content of the control tubers should be sufficient to fully activate PFK(PP_i) (P. Scott, M. Bettey and N.J. Kruger, Department of Plant Sciences, University of Oxford, UK; personal communication). This suggests either that the in-vitro kinetic properties of PFK(PP_i) do not adequately reflect the properties in vivo, or that the measured total Fru2,6 bisP content represents an over-estimate of the free concentration in the cytosol.

There are number of lines of evidence which suggest that PFK(PP_i) may be involved in metabolism under anoxia. Germination of rice seeds in anoxia leads to both an increase in the Fru2,6bisP content and in the maximum catalytic activity of PFK(PP_i) (Mertens et al. 1990). A similar increase in the maximum catalytic activity of PFK(PP_i) under anoxia has been reported for rice suspension cultures (Mohanty et al. 1993). An anoxia-induced increase was not observed with soybean suspension cells, which are much less capable of coping with anoxia than those of rice (Mohanty et al. 1993). Finally, the maximum

catalytic activity of PFK(PP_i) has been shown to increase under anoxia in leaves of *Typha angustifolia*, a marsh plant able to grow in anoxia (E. Barker and T. ap Rees, unpublished data). However, in the same study the activity did not change in the leaves of *Glyceria maxima*, a marsh plant susceptible to anoxia. Thus, the evidence is accumulating in support of the hypothesis that PFK(PP_i) is involved in adaptation to hypoxic and anoxic stress. The advantage gained by using this enzyme rather than PFK(ATP) is that the ATP-yield of glycolysis is increased particularly during fermentation (Mertens 1991). Theodorou and Plaxton (1994) have suggested a similar role for PFK(PP_i) during phosphate starvation, a condition in many ways analogous to anoxia.

In conclusion, we have demonstrated that low oxygen tension can be used as a means to perturb fluxes of carbohydrate metabolism in ripening banana fruit. This has identified an important role for PFK(PP_i) in the adaptation to hypoxic stress. We are now in the position to apply top-down metabolic control analysis to this system. This will form the subject of a subsequent paper.

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