The Bursting of the Hyphal Tips of *Dendryphiella salina* and the Relevance of the Phenomenon to Repression of Glucose Transport

By J. D. THORNTON, MARY F. J. GALPIN AND D. H. JENNINGS

Botany Department, The University, P.O. Box 147, Liverpool L69 3BX

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SUMMARY

Observations on *Dendryphiella salina* have confirmed that bursting of hyphal apices is brought about by an excessive water potential gradient across the hyphal membrane. Low concentrations of the non-metabolizable sugars 3-*O*-methylglucose and L-sorbose are much more effective than metabolizable sugars in producing bursting. However, at high concentrations they have a protective effect by reducing the water potential gradient. These observations allow a reinterpretation of sugar transport kinetic data which suggested that the glucose transport system in filamentous fungi can exist in two states dependent on the repressive effect of glucose.

INTRODUCTION

The study of glucose transport into fungal hyphae is hindered by the further metabolism of the absorbed sugar. Investigators have attempted to overcome this problem by using non-metabolizable analogues, in particular 3-*O*-methylglucose. However, it cannot be assumed that this sugar is without any effect on metabolism (Jennings & Austin, 1973; MacDermott & Jennings, 1976), and, like L-sorbose, another slowly or non-metabolizable sugar, it induces a number of morphogenetic effects presumably brought about by altered metabolism (Galpin & Jennings, unpublished results). L-Sorbose also causes rapid bursting of hyphal tips (Rizvi & Robertson, 1965.) Our work shows that 3-*O*-methylglucose has a similar effect and the amount of bursting is a function of sugar concentration. One must therefore interpret with caution kinetic data for the uptake of these two sugars, since the amount of active protoplasm in otherwise equivalent samples will depend on the external concentration of sugar to which they are exposed.

METHODS

Organism and media. *Dendryphiella salina* Nicot & Pugh was maintained in culture and grown for experimental purposes as described by Allaway & Jennings (1970*a*).

Hyphal tip bursting. Mycelium was grown for three days on medium containing 0.5 % (w/v) glucose, 0.3 % (w/v) tryptone and 2.0 % (w/v) agar, made up in various concentrations of sea water. The medium was overlaid with cellophane (600 P, British Cellophane). Strips of cellophane with leading hyphae were cut out and observed microscopically. The test solution was applied to the edge of the cellophane and allowed to spread towards the hyphal tips. The time from contact with the solution to the first burst was noted and the number of burst tips was counted after 2 min.

Sugar uptake. The procedure of Jennings & Austin (1973) was used except that solutions in 250 cm³ flasks were agitated by rotary shaking at 160 shakes min⁻¹. Each result is the mean of two samples.

Mycelial carbohydrate extraction and analysis. This was done as described by Holligan & Jennings (1972*a*).

Labelled compounds and assay of radioactivity. D-[U-¹⁴C]glucose, D-[U-¹⁴C]fructose, L-[U-¹⁴C]sorbitose and 3-*O*-[U-¹⁴C]methyl-D-glucose were obtained from The Radiochemical Centre, Amersham, Buckinghamshire. The amount of radioactivity used was 1.67 μ Ci/25 cm³ and the level was determined using a Nuclear-Chicago gas flow counter with the window in position.

RESULTS

Effect of solutes on bursting of hyphal apices

Table 1 shows the effect of growth in media containing various concentrations of sea water on both the percentage of hyphae which burst in distilled water and the rate of bursting. Both increased with increasing concentration of sea water, indicating that bursting was due to an excessive water potential gradient between the inside and the outside of the hyphae. Table 2 shows the effect of various concentrations of either L-sorbitose or 3-*O*-methylglucose on the bursting of hyphal apices. At sugar concentrations of less than 10⁻² mol l⁻¹, and for mycelium which had been growing in 25 % (or stronger) sea water medium, more bursting occurred than in distilled water. At sugar concentrations of greater than 10⁻¹ mol l⁻¹, the converse was true. At the lower sugar concentrations, either the sugar was accumulated within the hyphae or the wall was weakened (Rizvi & Robertson, 1965); both would lead to an even more unfavourable water potential gradient. At the higher sugar concentrations, though the sugars may have entered the hyphae or the wall may have been weakened, there was sufficient sugar in the medium to act as a protective osmoticum. Other sugars were also able to act in this way (Table 3).

A similar situation holds for hydrochloric acid (Table 4). At low concentrations of the acid, more bursting occurred than in water, while at higher concentrations the converse was true. Though hydrochloric acid may not act at the molecular level in the same way as L-sorbitose or 3-*O*-methylglucose, bursting must be caused by an excessive water potential gradient. Bartnicki-Garcia & Lippman (1972) have obtained similar results with sulphuric acid and *Neurospora crassa*, and came to similar conclusions.

Uptake of sugars in the presence of other sugars

Figure 1 shows the uptake of glucose from a 10⁻⁴ mol l⁻¹ unbuffered solution as a function of time. The curve is hyperbolic with no obvious discontinuities. When 10⁻² mol l⁻¹ L-sorbitose was present, the initial rate of uptake was significantly (*P* between 0.02 and 0.05) greater than when the sugar was absent but after between 10 and 30 min the rate was

Table 1. *Apical bursting on treatment in distilled water of hyphae of Dendryphiella salina grown in media containing sea water at various concentrations*

Each figure is the mean of at least three replicates.

Sea water in growth medium (%, v/v)	Apices burst in 2 min (%)	Time to first burst (s)
5	2	30
10	10	25
25	72	10
50	87	< 5
100	100	< 5

Table 2. *Apical bursting on treatment with water or sugar solutions of hyphae of Dendryphiella salina grown in media containing sea water at various concentrations*

Each figure is the mean of at least three replicates.

Treatment	Apices burst in 2 min (%), after growth in medium containing sea water (%), v/v):				
	5%	10%	25%	50%	100%
H ₂ O	0	16	64	84	100
L-Sorbose (mol l ⁻¹)					
10 ⁻⁴	0	0	100	100	100
10 ⁻³	0	0	95	100	100
10 ⁻²	0	0	95	100	100
10 ⁻¹	0	0	66	95	100
0.3	0	0	76	76	90
1.0	0	0	0	0	40
3-O-Methylglucose (mol l ⁻¹)					
10 ⁻⁴	0	0	100	100	100
10 ⁻³	0	0	95	100	100
10 ⁻²	0	0	86	93	100
10 ⁻¹	0	0	69	65	100
0.3	0	0	0	55	89
1.0	0	0	0	0	0

Table 3. *Apical bursting on treatment with water or sugar solutions of hyphae of Dendryphiella salina grown in media containing 25 % or 100 % sea-water*

Each figure is the mean of at least three replicates.

Treatment	Apices burst in 2 min (%) and, in parentheses, time to first burst, after growth in medium containing sea water (% v/v):	
	25%	100%
H ₂ O	74 (5-10)	87 (< 5)
Fructose (mol l ⁻¹)		
0.01	67 (10)	90 (< 5)
0.1	53 (10)	90 (< 5)
0.3	0	79 (10)
0.5	0	67 (10)
1.0	0	0
Glucose (mol l ⁻¹)		
0.01	70 (10)	95 (< 5)
0.1	52 (10)	91 (< 5)
0.3	0	91 (< 5)
0.5	0	57 (10)
1.0	0	0
L-Sorbose (mol l ⁻¹)		
0.01	—	95 (< 5)
0.1	62 (15)	95 (< 5)
0.3	69 (55)	95 (< 5)
0.5	0	95 (< 5)
1.0	0	55 (50)

Fig. 1

Fig. 2

Fig. 1. The uptake of glucose by mycelium of *Dendryphiella salina* from 10^{-4} mol l^{-1} solution in the presence (○) or absence (●) of 10^{-2} mol l^{-1} L-sorbose.

Fig. 2. The uptake of fructose by mycelium of *Dendryphiella salina* from 10^{-4} mol l^{-1} solution in the presence (○) or absence (●) of 10^{-2} mol l^{-1} L-sorbose.

Table 4. *Apical bursting on treatment with hydrochloric acid of hyphae of Dendryphiella salina grown in media containing sea water at various concentrations*

Each figure is the mean of at least three replicates.

Treatment	Apices burst in 2 min (%), after growth in medium containing sea water (% v/v):				
	5%	10%	25%	50%	100%
H ₂ O	0	16	64	84	100
HCl (mol l^{-1})					
10^{-3}	31	95	100	100	100
5×10^{-3}	40	72	61	57	80
10^{-2}	24	51	49	38	54
5×10^{-2}	0	0	0	0	0

reduced. The form of the curve is a less exaggerated version of that exhibited when fructose was absorbed (Fig. 2), where the rate of uptake was initially rapid, declined and then increased again after 60 min. The form of the curve was the same whether the mycelium had been grown in a medium containing fructose or glucose. L-Sorbose stimulated the initial rate of uptake but after 10 min, the rate of fructose uptake was slower than when L-sorbose was absent. The same pattern of uptake was shown by L-sorbose itself (Fig. 3) with a rapid initial rate of uptake which fell to zero after 10 min followed by a resumption of uptake after about 60 min. When glucose was present, the initial rate of L-sorbose uptake was higher than when glucose was absent, but subsequent to the first 10 min there was little further uptake.

We have always observed these patterns of uptake for both L-sorbose and fructose and for glucose uptake, when no other sugars were present. However, the pattern of 3-*O*-methylglucose uptake could resemble both that for glucose and that for L-sorbose and fructose (Fig. 4), though we are not clear why there should be differences between the two experiments. The presence of calcium in the medium, which is known to reduce efflux of the

Fig. 3

Fig. 3. The uptake of sorbose by mycelium of *Dendryphiella salina* from 10^{-4} mol l^{-1} solution in the presence (○) or absence (●) of 10^{-2} mol l^{-1} glucose.

Fig. 4

Fig. 4. The uptake of 3-O-methylglucose by mycelium of *Dendryphiella salina* from 10^{-4} mol l^{-1} solution. Experiment 1: (●). Experiment 2: in the presence (○) or absence (□) of 10^{-2} mol l^{-1} calcium chloride.

Table 5. Uptake of ^{14}C -labelled sugar by mycelium of *Dendryphiella salina* in the presence of other (unlabelled) sugars

The uptake of ^{14}C -labelled sugar (at 10^{-4} mol l^{-1}) in the presence of a different unlabelled sugar (at 10^{-2} mol l^{-1}) was measured at various times. Results are expressed as a percentage of the control.

^{14}C -labelled sugar	Unlabelled sugar	Time (min)		
		10	60	150
Glucose	Fructose	52	55	81
	L-Sorbose	132	90	100
Fructose	L-Sorbose	141	101	89
	Glucose	154	121	68
L-Sorbose	Fructose	118	91	71
	Fructose*	162	118	61
3-O-Methylglucose	Glucose	41	34	25
	L-Sorbose	52	59	51

* Mycelium grown in medium in which fructose was the sugar supplied.

sugar (Jennings & Austin, 1973), altered the pattern quantitatively but not qualitatively – the rate of uptake was higher with calcium present but the ion did not stop the reduction in rate after between 10 and 30 min.

Using mycelium incubated for 10 min in media containing L-sorbose or 3-O-methylglucose alone, the concentration of these sugars within the mycelium was calculated, assuming an intracellular water/dry weight ratio of 2.54 (Slayman & Tatum, 1964). It was found that the concentration inside the hyphae was always higher than that outside by a factor of approximately 25.

Data for a number of experiments concerned with the effect of one sugar (at 10^{-2} mol l^{-1})

Table 6. The concentration of mannitol and arabitol in the medium after exposure of mycelium of *Dendryphiella salina* for 180 min to 3-*O*-methylglucose in the presence or absence of L-sorbose

Sugar concn (mol l ⁻¹)		Sugar alcohol concn [nmol (mg dry wt) ⁻¹]		
3- <i>O</i> -Methyl-glucose	L-Sorbose	Mannitol	Arabitol	Total
10 ⁻⁴	—	0.60	0.63	1.23
10 ⁻⁴	10 ⁻⁴	0.47	0.52	0.99
10 ⁻⁴	10 ⁻³	0.42	0.43	0.85

Fig. 5. The loss of radioactivity from mycelium of *Dendryphiella salina* pretreated for 180 min with 10⁻⁴ mol l⁻¹ 3-*O*-[¹⁴C]methylglucose. Mycelium in water, (□); in 10⁻⁴ mol l⁻¹ unlabelled 3-*O*-methylglucose, (○); in 10⁻² mol l⁻¹ glucose, (●).

on the uptake of another (at 10⁻⁴ mol l⁻¹) are summarized in Table 5. When the uptake of glucose or fructose was measured in the presence of L-sorbose or vice versa, the amount absorbed after 10 min was always greater than when using the sugar alone. However, this was not found for 3-*O*-methylglucose or glucose in the presence of fructose. After 60 min, there might still be stimulation of uptake, though it was always less than at 10 min. At 150 min, except in the case of glucose in the presence of L-sorbose, the uptake of all sugars was reduced by the presence of another. The exceptional case of glucose is due to the fact that this sugar was exhausted from the medium both in the presence and absence of L-sorbose.

The medium was analysed at the end of the experiment concerned with the effect of L-sorbose on the uptake of 3-*O*-methylglucose. The mannitol and arabitol content is given in Table 6. Both sugar alcohols were lost into the medium but the total amount of polyol lost varied inversely with the total concentration of sugars in the experimental solution.

Figure 5 shows that when 3-*O*-[¹⁴C]methylglucose was absorbed, it did not diffuse out of the mycelium but freely exchanged with unlabelled 3-*O*-methylglucose and with glucose in the medium. Glucose brought about almost total exchange.

DISCUSSION

Hyphal apices of *Dendryphiella salina* will burst if they absorb sufficient sugar to establish an excessive water potential gradient between the hyphal interior and the external medium. This is particularly so when the internal water potential is already low due to growth in

a medium of low water potential. Bartnicki-Garcia (1973) has discussed how an excessive water potential gradient might be brought about.

Both 3-*O*-methylglucose, which is known not to be metabolized (Jennings & Austin, 1973), and L-sorbose, which we presume is not metabolized by *D. salina*, were more effective in causing bursting than the metabolizable sugars, glucose and fructose. In no case was there bursting in the presence of the highest concentrations of sugar used (1.0 mol l^{-1}); the maximum amount of bursting occurred at the lower concentrations. At high external sugar concentrations, the rate of sugar uptake was not sufficient to generate an excessive water potential gradient, given that there was already a low water potential in the external medium. At low external sugar concentrations, the water potential of the medium was not low enough to counteract the fall in the water potential of the hyphae. Consequently an excessive gradient was quickly established and the hyphae burst. This interpretation is also supported by the data for hydrochloric acid, where there is a similar relationship between the water potential of the growth medium and the concentration of solute causing bursting.

Sugars which are metabolized, i.e. glucose and fructose, caused less bursting than did 3-*O*-methylglucose and L-sorbose. This may be because glucose and fructose are in part converted into insoluble compounds once they are absorbed (Allaway & Jennings, 1970*b*; Holligan & Jennings, 1972*b*; MacDermott & Jennings, 1976).

These data for hyphal bursting allow an interpretation of the stimulation of uptake of one sugar by the presence of another. When one sugar alone is present at $10^{-4} \text{ mol l}^{-1}$, absorption will lead to an unfavourable water potential gradient. On the other hand, when another sugar is present at $10^{-2} \text{ mol l}^{-1}$, the gradient is prevented from becoming unfavourable. Fewer tips burst and therefore more of the labelled sugar can be absorbed. Equally, one could expect less carbohydrate in the medium, if this is released as a result of bursting.

In interpreting the results for the uptake of the labelled sugar, it is assumed that the unlabelled sugar does not compete for the same transport sites. If there is competition, though fewer tips burst there could still be a reduction in the amount of labelled sugar which is absorbed. This could explain the reduced uptake after 10 min of 3-*O*-methylglucose in the presence of glucose or L-sorbose. The extent to which competition is detectable at 10 min will depend upon the balance between the increased amount absorbed per unit dry weight and the decrease due to competition. However, after 10 min, when one might anticipate little further disruption of the tips, any reduction in uptake of the labelled sugar by the presence of another is most likely to be due to competition. From the results obtained over the period of 150 min, it would seem that there is competition between glucose and fructose, fructose and L-sorbose, glucose and L-sorbose, glucose and 3-*O*-methylglucose and 3-*O*-methylglucose and L-sorbose.

If this interpretation of our data is correct, it has implications for the use of non-metabolizable sugars for transport studies, in particular for kinetic aspects. The data have shown that the amount of bursting is dependent upon the concentration of the analogue and the presence of other osmotically active material (e.g. buffer) in the medium. Thus if buffer is present, there may be little bursting at very low concentrations of the non-metabolizable sugar, but at higher concentrations there is likely to be an increase. The consequence will be a reduction in the amount of active protoplasm per unit dry weight and therefore also a reduction in the rate of uptake per unit dry weight.

This explanation could account for the observation of Neville, Suskind & Roseman (1971) that the relationship between the uptake of 3-*O*-methylglucose and concentration is biphasic, which they suggested was due to the presence of two uptake systems. We would interpret the high affinity system ($K_m 0.07 \text{ mM}$) which is operative at low concentrations as representing

the capacity of hyphae to absorb 3-*O*-methylglucose when the bulk of the hyphal tips are intact due to the protecting effect of 10^{-2} mol l⁻¹ buffer which was used in these particular experiments. On the other hand, the low affinity system (K_m 10 mM) operative at much higher sugar concentrations represents the capacity of hyphae to absorb 3-*O*-methylglucose when a significant proportion of tips have burst. The difference in V_{max} (approx. 5 nmol mg⁻¹ min⁻¹ for the high affinity system and approx. 12 nmol mg⁻¹ min⁻¹ for the low affinity system) observed by Neville *et al.* (1971) is of the right order to be accounted for by the above hypothesis if it is remembered that the percentage changes in uptake after 10 min reported in this paper are confounded by competition between sugars for the transport site.

It is interesting that the relationship between the uptake of sugar and time can differ for different sugars, cf. glucose and fructose, and even for the same sugar in different experiments, e.g. 3-*O*-methylglucose. The form of the relationship for fructose and L-sorbose could be interpreted, on the basis of previous hypotheses (Scarborough, 1970*a, b*; Schneider & Wiley, 1971*a, b*; Schulte & Scarborough, 1975), as arising from rapid equilibration of the sugar across the membrane by facilitated diffusion followed by, at around 45 min, removal of glucose repression of the transport system such that it becomes active, allowing more sugar to be absorbed. In our experiments, we have found that, while it is true that the transport system does allow free exchange of sugar across the membrane, uptake after 10 min brings about an accumulation of sugar in the mycelium. Furthermore, this was observed irrespective of whether the mycelium was grown in glucose or fructose.

Therefore it seems that the time course for the uptake of fructose cannot be explained by derepression of the transport system. At this stage an alternative explanation cannot be put forward, but we have some indication (Galpin & Jennings, unpublished results) that cyclic AMP may be implicated.

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