The Phenylacetic Acid Uptake System of *Aspergillus nidulans* is under a *cre*A-Independent Model of Catabolic Repression which Seems to be Mediated by Acetyl-CoA

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The filamentous fungus *Aspergillus nidulans* is able to grow on phenylacetic acid (PhAc) as the sole carbon source and has a highly specific phenylacetic acid transport system mediating the uptake of this aromatic compound. This transport system is also able to transport some phenoxyacetic acid (PhOAc), although less efficiently. Maximal uptake rates were observed at 37°C in 50 mM phosphate buffer (pH 7.0). Under these conditions, uptake was linear for at least 1 minute, with *Km* values for PhAc and PhOAc of 74 and 425 μM, respectively. The PhAc transport system is strongly induced by PhAc and, to a lesser extent by PhOAc and other phenyl derivatives. The utilization of glucose (and other sugars), glycerol or acetate results in a substantially reduced uptake. This negative effect caused by certain carbon sources is independent of the *cre*A gene, the regulatory gene mediating carbon catabolite repression. Negative regulation by acetate is prevented by a loss-of-function mutation in the gene encoding acetyl-CoA synthetase, strongly suggesting that this regulation is mediated by the intracellular pool of acetyl-CoA.

The last step in the biosynthetic pathway of penicillin G (benzylpenicillin) and penicillin V (phenoxymethylpenicillin) in the filamentous fungi *Penicillium chrysogenum* and *Aspergillus nidulans* involves at least three different reactions: (i) uptake of aromatic compounds to be incorporated as the penicillin-side chain; (ii) activation of these molecules to CoA thioesters and (iii) exchange of the L-α-aminoadipic moiety present in the isopenicillin N (IPN) molecule by phenylacetic (penicillin G) or phenoxyacetic (penicillin V) acids (Fig. 1)[1,2]. It was believed that these aromatic compounds are only incorporated into the cell through a passive diffusion mechanism[3]. However, we have demonstrated the existence of an active and inducible transport system responsible for the uptake of PhAc in *P. chrysogenum*[4~6] and, more recently, in the bacterium *Pseudomonas putida*[7]. Some years later, HILLENGA *et al.*[8] proposed the existence in *P. chrysogenum* of a passive diffusion mechanism responsable for the cellular incorporation of this aromatic compound, whereas ERIKSEN *et al.*[9], in the same fungus, showed the existence of an uptake system which has saturating kinetics as we had been reported. Although it is generally accepted that some aromatic compounds, containing a short acyl-chain, could cross the lipid bilayer to a certain extents by passive diffusion[4,8~10], this incorporation is not enough to assure cellular growth and, therefore, permeases should be needed for optimal growth in natural environments[11].

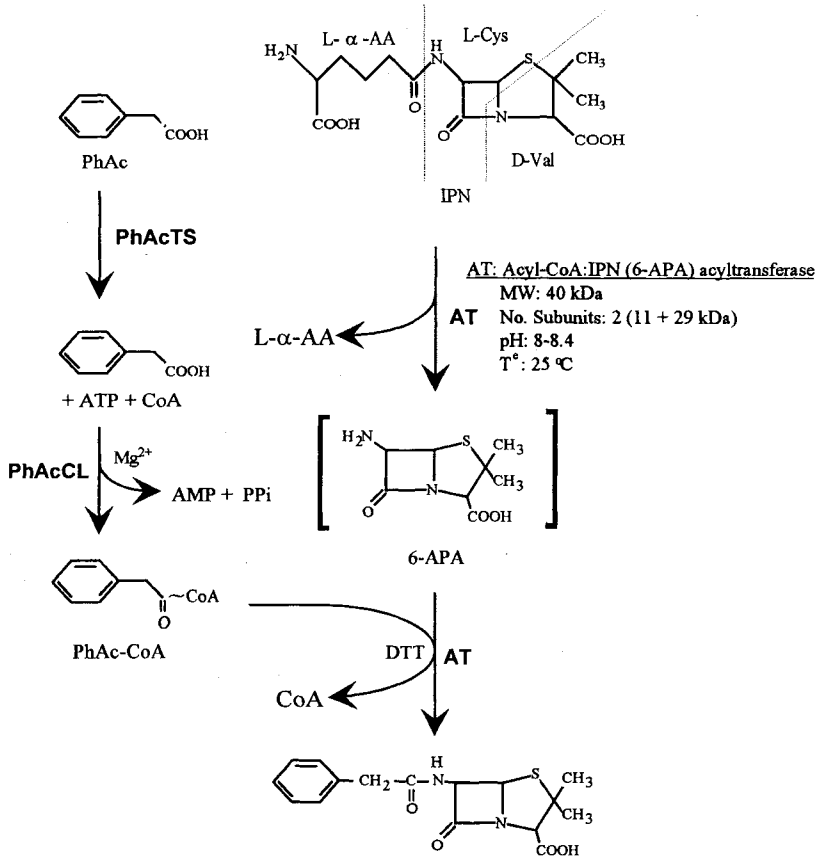
The importance of the PhAc-transport system in the biosynthesis of certain hydrophobic penicillins together with the knowledge of the mechanisms controlling the uptake of this aromatic acid seemed to be very interesting in order to obtain further information about the regulation of secondary metabolites in fungi. We were therefore prompted to broaden our study to include *A. nidulans*. This fungus was selected as a model for three different reasons: a) Unlike *P. chrysogenum*, *A. nidulans* has a sexual cycle, which would facilitate genetic analysis; b) *A. nidulans* is able to grow in minimal medium containing PhAc as the sole carbon source even when the pH of the medium was 8.5 (suggesting the existence of a phenylacetic acid transport system) and c) *A. nidulans*, like *P. chrysogenum*, is able to synthesize penicillin G and V. In summary, the study of the PhAc transport system in this fungus could be useful to clarify the implications of *cre*A gene in the carbon catabolic regulation of this uptake, a point which remains obscure in *P. chrysogenum* and is one of the objectives of this work.

Materials and Methods

Materials

Phenyl derivatives were obtained from Aldrich Química (Madrid, Spain), Lancaster Synthesis Ltd.

Fig. 1. Last three steps in the biosynthetic pathway of benzylpenicillin.



IPN, Isopenicillin N; PhAc, phenylacetic acid; L-α-AA, L-α-aminoadipic acid; PhAcCL, phenylacetyl-CoA ligase; PhAc-CoA, phenylacetyl-CoA; 6-APA, 6-aminopenicillanic acid; PhAcTS, phenylacetic acid transport system and AT, acyl-CoA-IPN(6-APA)-acyltransferase.

(Strasbourg, France); Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, Mo. U.S.A.).

[1-^{14}C]Phenylacetic acid (24 mCi/mmol) was from Sigma (U.S.A.), [1-^{14}C]phenoxyacetic acid (10 mCi/mmol) was supplied by ICN (U.S.A.) and D-[*U*-^{14}C]-xylose (76 mCi/mmol) was from the Radiochemical Centre (Amersham, U.K.).

All other products used were of analytical quality or high-performance liquid chromatography (HPLC) grade.

Strains

The strains of *A. nidulans* used in this work and their origins are summarized in Table 1.

Table 1. Strains of *A. nidulans* used in this work.

Mutant	Genotype	Source
Wild type	*bi*A1	M. A. PEÑALVA (Madrid)
*fac*A303	*bi*A1; *fac*A303	J. CLUTTERBUCK (Glasgow)
*cre*A^{d}30	*bi*A1; *cre*A^{d}30	F.G.S.C.
*cre*B15	*bi*A1; *cre*B15	F.G.S.C.
*cre*C27	*bi*A1; *cre*C27; *w*A7	F.G.S.C.
*fpa*D11	*ad*F17; *fpa*D11; *paba*A1; *y*A2	F.G.S.C.

F.G.S.C.: Fungal Genetics Stock Center. Department of Microbiology. University of Kansas Medical Center.

Culture Media and Growth Conditions

Spores of *A. nidulans* were obtained as previously reported[12]. Fungal cultures were carried out in 2,000-ml Erlenmeyer flasks containing 400 ml of minimal medium (MM)[13] with the following composition (g/liter): KH_2PO_4, 13.6; $(NH_4)_2SO_4$, 3; $MgSO_4 \cdot 7H_2O$, 0.3; $FeSO_4 \cdot 5H_2O$, 0.001. The medium was adjusted to pH 7.0 with 10 M KOH and sterilized (120°C, 15 minutes). Glucose (0.2% w/v) or the different carbon sources used in the experiments were autoclaved separately. When required, the media were supplemented with the substances needed by each different mutant. Each Erlenmeyer flask was inoculated with 2×10^6 spores/ml and incubated on a rotary shaker (250 rpm) at 37°C for different times (between 0 and 40 hours). In some cases

(to study the appearance of the PhAc transport system), 5 mM PhAc was also supplied to the broth.

Glucose Consumption

Residual D-glucose was measured by the glucose oxidase method[14].

Determination of PhAc

The content of [1-^{14}C]PhAc inside the cells was analyzed in cell-free extracts by HPLC (see below).

HPLC Equipment and Chromatography Procedure

Samples taken at different times from the uptake mixture were disrupted by sonication, quickly centrifuged (31000 g, 5 minutes) and aliquots of 50 μl were taken and analyzed using a high-performance liquid chromatograph (Spectra Physics SP8800) equipped with a variable-wave-length ultraviolet/visible detector (SP8450), a integrator (SP4290) and a microparticulate (10-μm pore size) reverse-phase column (Nucleosil C-18 250 mm × 4.6 mm inner diameter: Phenomenex Laboratories U.S.A.). The mobile phase was 0.05 molar HK_2PO_4, pH 4-CH_3CN (99:1, by vol). The flow rate was set at 2.5 ml/minute and the eluate was monitored at 254 nm. Under these conditions the retention times for 2,5-diOH-PhAc, 3,4-diOH-PhAc, 4-OH-PhAc, 2-OH-PhAc and PhAc were 6.6, 11.0, 20.3, 25.7 and 44.8 minutes, respectively. Phenylacetyl-CoA was also evaluated by HPLC as reported previously[7]. In cell-free extracts we identified a single labelled compound with the retention time of phenylacetic acid. However, we neither found labelled hydroxy- derivatives of PhAc nor radioactive phenylacetyl-CoA.

PhAc-uptake in *A. nidulans*

Mycelia grown in the above media and conditions were harvested at the required time, filtered under vacuum and washed three times with sterile distilled water. Aliquots of 35 mg (wet weight) (about 10 mg of dry weight and equivalent to 2.5 mg of protein) were resuspended in 25-ml Erlenmeyer flasks containing 1.4 ml of 0.05 M phosphate buffer, pH 7.0, and preincubated at 37°C for 5 minutes in a thermostatically controlled bath at 160 strokes/minute before adding PhAc (51.4 μM containing 0.05 μCi of labeled PhAc) or PhOAc (56.4 μM containing 0.1 μCi of labeled PhOAc). To calculate Vmax and *Km* values these concentrations were changed. Incubations were carried out for 30 seconds (or the required time); halted by adding 10 vol of water, rapidly filtered through Millipore filters (0.45 μm pore size), and washed with 3 × 10 ml of sterile distilled water. The filters were dissolved in 10 ml of scintillation fluid and counted as described[4]. To characterize this transport system, several effectors were added to the uptake mixture at 2.5 mM or at the indicated final concentration. In these cases, the effector tested was added 2 minutes before [^{14}C]PhAc or [^{14}C]PhOAc. When arsenate was tested, the buffer employed was HCl-Tris (50 mM) pH 8.0.

Uptake is given as nmol of [^{14}C]PhAc (or [^{14}C]-PhOAc)/minute/mg of protein or as nmol of [^{14}C]PhAc (or [^{14}C]PhOAc)/mg of protein) when different uptake periods were employed.

To study the eflux of PhAc, the fungus was incubated in the presence of labelled PhAc for 5 minutes and at this time mycelia were harvested, washed and resuspended in the same buffer without PhAc. Measurements of the residual radioactivity were carried out for different times (between 0 and 30 minutes).

PhAc-transport System Induction Experiments

A. nidulans was cultured in the above medium and conditions and when glucose was exhausted (14 hours), mycelia were starved for a further 2 hours and then 5 mM PhAc (or 5 mM PhOAc) was added. The appearance of the PhAc transport system was measured at 90 minutes intervals. The effect of different molecules on the induction of this transport system was analyzed by adding the required compound to the minimal medium. In this kind of experiment, *A. nidulans* was grown in 2 liter-Erlenmeyer flasks containing 400 ml of the same medium (see above). When glucose was exhausted, cells were harvested, washed, and 0.5 g of wet mycelia were transferred to 250 ml-Erlenmeyer flasks containing 50 ml of MM and incubated at 37°C for 2 hours. At this time, PhAc (or other inducers) as well as different carbon sources were added. In all cases, the quantity of effector was equivalent, in carbon concentration, to 5 mM glucose.

Results and Discussion

Phenylacetic Acid Transport System of *Aspergillus nidulans*

Addition of 5 mM PhAc to *A. nidulans* cultures starved of a carbon source elicited a strong uptake of PhAc (Fig. 2). In cultures containing a mixture of glucose and PhAc,

Fig. 2. Time-course of appearance of PhAc (△) and PhOAc (■) transport system; growth (▼), glucose (▽) and PhAc consumption (●) in *A. nidulans* cultured in MM.

Arrow indicates the time at which [1-^{14}C]PhAc was added.

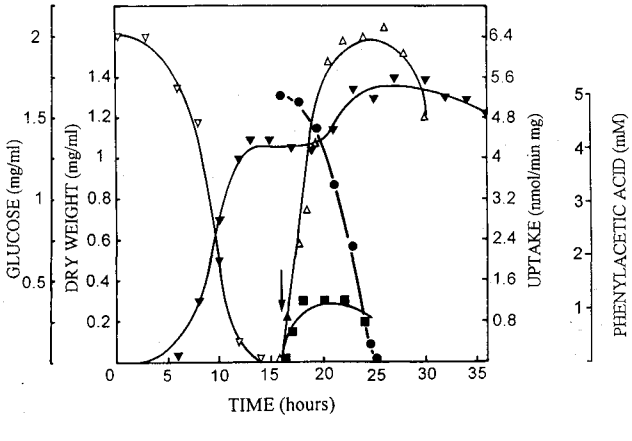
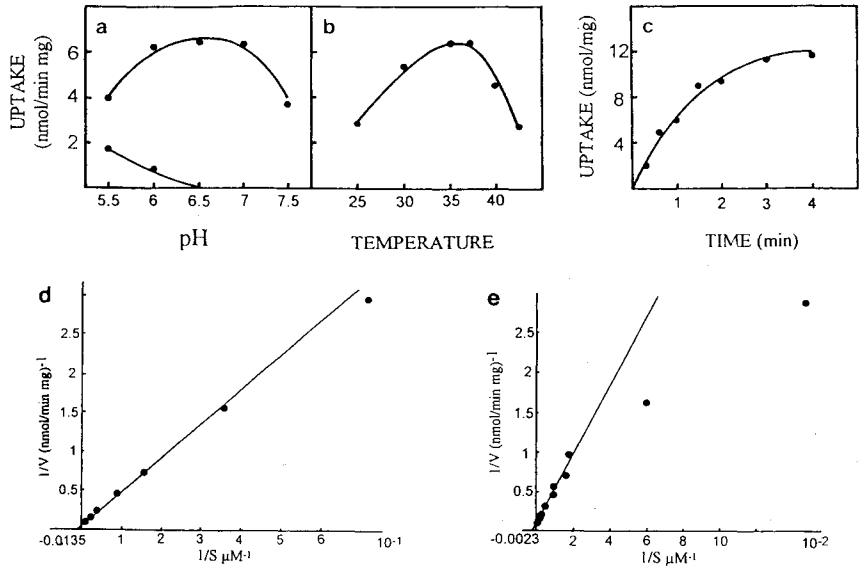


Fig. 3. Effect of different pH values on active transport (top) and passive diffusion (bottom) of PhAc (a). Assay of PhAc transport system at different temperatures (b); at different times (c); kinetics of PhAc-transport (1/v *vs.* 1/s) (d); and kinetics of the PhOAc-transport system (1/v *vs.* 1/s) (e).



PhAc was not taken up until glucose had been exhausted, indicating that the sugar prevents induction of this uptake system. We therefore made cultures under controlled conditions in which glucose exhaustion would occur at 14 hours after inoculation and routinely induced the PhAc transport system by adding 5 mM PhAc after 16 hours of growth (see Fig. 2). Under these conditions and when PhAc was used as the carbon source, uptake was very efficient as shown by the resulting increase in mycelial dry weight (Fig. 2). The phenylacetic acid transport system thus induced was also able to transport PhOAc, although with lower efficiency (20%) than for PhAc (see Fig. 2).

Maximal uptake rates were observed in 50 mM phosphate buffer, pH 7.0 at 37°C. Under these conditions, the *Km* values were 74 μM for PhAc and 425 μM for PhOAc (see Fig. 3). At pH values lower than 5.5, a passive influx was observed in non-induced mycelia (see Fig. 3a) but this could not be seen when pH values were higher. Similar results have been reported for the penicillin producer *P. chrysogenum* Wis 54-1255[4]. PhOAc also induced a PhAc uptake system (see below), although in this case the uptake rates were lower (see Table 2). The similar properties of PhAc uptake elicited by both of these aromatic compounds (see below) strongly suggests that both induce the same transport system, even though PhOAc cannot be utilised by the fungus as a carbon source and therefore represents a gratuitous inducer.

Table 2. Induction of the PhAc transport system in *A. nidulans* by different phenylderivatives.

Compound tested	Growth	Relative uptake capacity (%)
Phenylacetic acid	+	100
2-Hydroxyphenylacetic acid	+	26
3-Hydroxyphenylacetic acid	+	40
4-Hydroxyphenylacetic acid	+	60
2-Cl-phenylacetic acid	−	59
3-Cl-phenylacetic acid	−	15
4-Cl-phenylacetic acid	−	23
2-Methylphenylacetic acid	−	36
3-Methylphenylacetic acid	−	65
4-Methylphenylacetic acid	−	75
Phenoxyacetic acid	−	23
3-Phenylpropionic acid	+	ND
3-(2-Hydroxy)phenylpropionic acid	+	ND
3-(3-Hydroxy)phenylpropionic acid	+	ND
3-(4-Hydroxy)phenylpropionic acid	+	ND
3-Phenoxypropionic acid	−	ND
4-Phenylbutyric acid	+	ND
4-Phenoxybutyric acid	+	ND
Phenylalanine	+	ND
Tyrosine	+	ND

In this table and in the following ones, 100% corresponds to 5.48 nmol/minute/mg dry weight and all the data are the average of three different determinations. (+) or (−) growth indicates that *A. nidulans* is able (+) or unable (−) to use this compound as the sole carbon source. ND: Not detected.

Table 3. Effect of different molecules on the uptake of PhAc by *A. nidulans*.

Compound	PhAc uptake (%)	Compound	PhAc uptake (%)
Control (without addition)	100	3-Phenylpropionic acid	7
2-Hydroxyphenylacetic acid	17	3-(2-Hydroxy)phenylpropionic acid	39
3-Hydroxyphenylacetic acid	17	3-(3-Hydroxy)phenylpropionic acid	35
4-Hydroxyphenylacetic acid	21	3-(4-Hydroxy)phenylpropionic acid	10
2-Methylphenylacetic acid	38	2-Phenylpropionic acid	70
3-Methylphenylacetic acid	9	3-Phenoxypropionic acid	66
4-Methylphenylacetic acid	13	4-Phenylbutyric acid	26
2-Methoxyphenylacetic acid	85	2-Phenylbutyric acid	30
3-Methoxyphenylacetic acid	38	4-Phenoxybutyric acid	64
4-Methoxyphenylacetic acid	40	2-Phenoxybutyric acid	40
Phenoxyacetic acid	19	Phenol	56
Benzoic acid	40	2-Hydroxyphenol	81
2-Hydroxybenzoic acid	83	3-Hydroxyphenol	78
2-Aminobenzoic acid	75	4-Hydroxyphenol	81
4-Aminobenzoic acid	50		

All compounds were tested at 2.5 mM.

Induction Specificity

A. nidulans was grown in glucose minimal medium until the sugar had been exhausted, and then mycelia were washed and transferred to several media containing different aromatic compounds to test their ability to induce the PhAc uptake system. The highest levels of induction were obtained with PhAc, 3- and 4-methyl-PhAc and 4-hydroxy-PhAc (Table 2). Several Cl- and methyl-derivatives were also able to induce uptake to different extents although they were not metabolised (Table 2). Interestingly, neither phenylpropionate (or hydroxy-derivatives) nor phenylbutyrate, which are used by the fungus as carbon sources, were able to induce the system, indicating a strong specificity for the acetyl moiety of PhAc. Conversely, however, 4-phenylbutyrate strongly induced the *P. chrysogenum* PhAc uptake system[5].

It is worth noting that Phe or Tyr did not act as inducers of the PheAc transport system (Table 2), suggesting that the physiological role of this uptake is not to transport the structurally-related aromatic amino acids. Indeed, a mutation (*fpa* D11, see Table 1) preventing Phe uptake[15] has no effect on the induction of PhAc transport system, and Phe or Tyr are not recognised by this uptake system (see below).

To test the ability of this PhAc uptake system to recognize different compounds, we used PhAc-induced-mycelia in competition experiments with ^{14}C-PhAc and an excess of the relevant compound. Table 3 shows that although different aromatic compounds inhibit ^{14}C-PhAc uptake to different extents, indicating that the uptake system is mediated by a protein and does not correspond to a passive diffusion mechanism[8], the uptake system is highly specific for PhAc. The highest inhibitions were observed with 3-phenylpropionic acid, 3-methyl PhAc, and 3(4-hydroxy)phenylpropionic acid. Interestingly, neither Phe nor Tyr were able to compete with PhAc uptake (Table 4). This was also the case for a number of amino acids, sugars (glucose, fructose, sucrose, lactose), glycerol and acids (acetic, lactic and TCA intermediates), with the exception of xylose, which competed with PhAc to a significant extent (61%) (Table 4). However, mycelia induced with PhAc were unable to transport D-[*U*-^{14}C]xylose and mycelia grown in D-xylose (0.2% w/v) as the sole carbon source were un-

Table 4. Effect of different sugars, amino acids and metabolic intermediates on phenylacetic acid transport system (PhAcTS) of *A. nidulans*.

Compound	PhAc uptake (%)	Compound	PhAc uptake (%)
Control (without addition)	100	Oxalacetic acid	101
		Arginine	106
Glucose	91	Aspartic acid	97
Fructose	91	Asparagine	102
Xylose	39	Glutamic acid	100
Sucrose	98	Glutamine	113
Lactose	81	Lysine	104
Glycerol	86	Tyrosine	103
Lactic acid	89	Phenylalanine	93
Acetic acid	80	Valine	105
Citric acid	100	Cysteine	120

All effectors were tested at 2.5 mM.

able to transport PhAc (data not shown).

Effect of Different Reagents and Metabolic Inhibitors on the PhAc Transport System

The effects caused by different monothiols (MSH, GSH) and dithiols (DTT, GSSG) on the PhAc transport system were studied (see Table 5). None of them had any significant effect on the rate of transport, suggesting that an extracellular environment containing reduced thiols might not be necessary for the uptake of PhAc[16]. However, the thiol-modifying reagents *N*-ethylmaleimide (NEM), 5,5′-dithiobis nitrobenzoic acid (DTNB) and iodoacetate inhibited PhAc uptake (51, 70 and 65%, respectively). These data indicate that reduced thiol groups are essential for the catalytic function of this transport system[4,7,17,18]. When metabolic inhibitors were tested, we observed that cyanide and azide decreased the uptake of PhAc (65 and 84%, respectively) and that uptake was also inhibited when uncoupling agents (protonophores), such as carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) (83%), 2,4-dinitrophenol (68%) and 4-nitrophenol (73%) were tested. These results suggest that the PhAc transport system is an energy-dependent system probably mediated by a H^+ gradient. Similar results have been reported for the transport of different molecules in several other biological systems[7,16~19]. It is interesting to note the lack of effect caused by arsenate, even when this compound was tested at 10 mM (final concentration) in the absence of phosphate (see Materials and Methods) indicating that it is not energized by a phosphorylated compound.

Table 5. Effect of uncouplers, metabolic inhibitors, thiol-containing and thiol-modifying reagents on the PhAcTS in *A. nidulans*.

Compound	PhAc uptake (%)
Control without addition	100
β-Mercaptoethanol (MSH)	91
Dithiothreitol (DTT)	96
Oxidized glutathione (GSSG)	103
Reduced glutathione (GSH)	104
N-Ethylmaleimide	49
5,5′-Dithiobisnitrobenzoic acid (DTNB)	30
Iodoacetate	36
Potassium cyanide	35
Sodium azide	16
FCCP (100 μM)	17
2,4-Dinitrophenol (1 mM)	32
4-Nitrophenol (1 mM)	27
2-Nitrophenol (1 mM)	91
Arsenate (10 mM)	92

All compounds were tested at 2.5 mM or at the concentration indicated between bracketts.

Additional proof that PhAc-transport is an active process arose from the study of the [1-^{14}C]PhAc eflux (see Materials). No efflux of labelled PhAc was observed even after 30 minutes of incubation. It could be expected that [1-^{14}C]PhAc,which was detected as free acid inside the cells (see Materials), would be released to the medium if uptake were mediated by passive diffusion.

Regulation by Carbon Sources of Induction of PhAc Uptake

Addition of D-glucose, D-fructose, D-xylose, L-fructose or glycerol at the time of PhAc induction prevented the appearance of the PhAc transport system, while addition of pyruvate, oxaloacetate or citrate had no effect on induction (Fig. 4). As indicated above, the fact that the addition of phenylacetate before glucose had been exhausted from the cultures did not result in induction of the PhAc transport system, suggested that induction might be regulated by carbon catabolite repression[20]. However, the fact that glycerol (a derepressing carbon source in *cre*A-mediated carbon catabolite repression) prevented induction, suggested the existence of a *cre*A-independent mode of carbon regulation. A similar effect of glycerol has been described in *P. chrysogenum* for both PhAc transport and penicillin biosynthesis[21]. In *A. nidulans*, Espeso and coworkers have shown the existence of a *cre*A-independent mechanism that negatively regulates the transcription of *Ipn*A, a penicillin biosynthetic gene[22]. This negative regulatory circuit, is also independent of *cre*B and *cre*C, two genes possibly encoding membrane proteins, whose mutation results in carbon catabolite derepression of several enzymes which are under catabolic repression in the wild type[23,24].

Fig. 4. Effect of different carbon sources on the induction of PhAc in *A. nidulans*.

(a) Control without addition (●), +D-fructose (▲), +D-glucose (△), +D-mannose (▽) and +D-xylose (■); (b) control (●), +pyruvate (▲), +sodium acetate (■), +potassium acetate (▼), +ammonium acetate (△), +citrate (▽) and oxalacetate (□); (c) control (●), +lactose (□), +glycerol (■), +sucrose (△), and +maltose (▽).

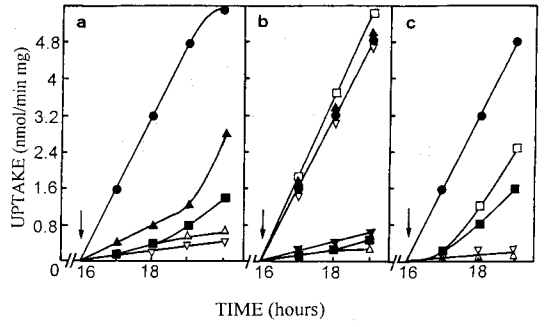


Table 6. Uptake of PhAc by different mutants of *A. nidulans*.

Strain	Carbon source			
	—	PhAc	PhAc+ glucose	PhAc+ glycerol
Wild type	0.4	13.7	0.6	2.3
*cre*A^{d} 30	0.5	13.9	1.3	2.9
*cre*B 15	1.1	11.9	7.2	8.3
*cre*C 27	0.6	10.2	4.6	5.6

PhAc uptake is given as nmol/minute/10 mg dry weight. Mutants *cre*A^{d} 30, *cre*B 15 and *cre*C 27 are strains of *A. nidulans* altered in catabolic repression (derepressed).

Table 7. Uptake of PhAc by the *fac*A 303 mutant of *A. nidulans*.

Carbon source	Strain	
	Wild type	*fac*A 303
—	0.4	0.3
PhAc	13.7	12.3
PhAc+glucose	0.5	0.4
PhAc+glycerol	2.1	1.1
PhAc+acetate	0.6	12.5
PhAc+hexanoate	1.2	0.2

*fac*A 303 mutant is defective in acetyl-CoA synthetase. PhAc uptake is given as nmol/minute/10 mg dry weight.

It has been suggested that the *cre*B15 and *cre*C27 gene products are not true transcriptional repressors and that mutants carrying this kind of mutation could be altered in membrane permeability and hence in different transport systems. If this were the case, the uptake of the molecule responsible for catabolic repression could be diminished and, therefore, not effect could be caused since the repressor cannot be taken-up[25]. However, no alterations in glucose uptake have been found in these mutants[23~26]. Moreover, we did not observe any significant differences in mycelia growth when the fungus was cultured in the presence of glucose and neither were alterations observed in glucose uptake (in a concentration range between 17 μM and 1 mM) or in PhAc transport (data not shown). Table 6 indicates that the repression of the PhAc uptake is not mediated by the *cre*A gene product, since the derepressed mutant (*cre*A^{d} 30) can be regulated, like the wild type, by glucose and glycerol, suggesting the involvement of a different gene (*cre*B or *cre*C) in the control of this system. Thus, mutants *cre*B and *cre*C are able to induce PATS even in the presence of a concentration of glucose which repressed the induction of the transport system in the wild type and in *cre*A^{d} 30 mutant (see Table 6). A similar hypothesis was proposed, in pioneering work, by HYNES and KELLY studying pleiotropic mutants of *A. nidulans* altered in carbon metabolism[24].

Acetate strongly repressed PhAc uptake (see Fig. 4). A negative role of acetyl-CoA on sugar transport has been previously described by ROMANO and KORNBERG[27], and indeed we have previously presented evidence pointing to the repression of PhAc uptake by acetyl-CoA in *P. chrysogenum*[28]. If the negative effect of acetate were indeed mediated through acetyl-CoA, the absence of acetyl-CoA synthetase would prevent this effect. Table 7 shows that this is in fact the case. The addition of acetate abolished PhAc induction with the wild type strain, but had no effect with a *fac*A 303-mutant strain (see Table 1) lacking in acetyl-CoA-synthetase[29,30]. This strain is not a defective transport mutant since acetate is able to induce certain enzymes involved in it own catabolism (isocitrate lyase, acetamidase and other related enzymes) suggesting that it is taken up by the cells[31]. In this mutant, acetate is able to induce enzymes which are specifically produced in presence of acetate (like isocitrate lyase)[29] but it is unable to synthesize those enzymes induced by acetyl-CoA[31]. The mutation had no effect when glucose, glycerol or hexanoate were added instead since their conversion to acetyl-CoA does not require acetyl-CoA synthetase. These results support our hypothesis that transition from primary (growth) to secondary metabolism (penicillin production and other events) could be mediated by either the intracellular pool of acetyl-CoA or by the acetyl-CoA/CoA ratio in these two fungi[1,28].

Acknowledgments

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