JOURNAL OF BACTERIOLOGY, May 1988, p. 2383–2384
0021-9193/88/052383-02$02.00/0

Vol. 170, No. 5

Induction of High-Affinity Phenol Uptake in Glycerol-Grown *Trichosporon cutaneum*

MONIKA MÖRTBERG, ÅKE SPÅNNING, AND HALINA Y. NEUJAHR*

Department of Biochemistry, The Royal Institute of Technology, S-100 44 Stockholm, Sweden

Received 6 July 1987/Accepted 10 February 1988

Two uptake systems for phenol are identified in *Trichosporon cutaneum*. One is an inducible, high-affinity system, sensitive to protonophores. It is induced coordinately with phenol hydroxylase but can operate independently of phenol metabolism. The other is a constitutive, low-affinity system with different specificity and different pH optimum. It is not sensitive to protonophores.

Phenol-grown cells of *Trichosporon cutaneum* contain a specific energy-dependent uptake system for phenol, driven by a proton gradient (5). Phenol interferes competitively with uptake of glycerol by glycerol-grown cells. Such cells take up phenol by a low-affinity system as compared with the high-affinity uptake by phenol-grown cells (5). The investigation reported here was undertaken to study the relation between the low-affinity and high-affinity uptake systems for phenol and their connection to the induction of the phenol-degrading pathways.

Kinetic parameters of uptake and its induction were determined with cells from steady-state continuous cultures (8). When reproducibility of the growth state and nutrient supply was not of crucial importance, flask cultures (5) were used. The carbon source used was phenol or glycerol. Phenol was determined with aminoantipyrin (8), and glycerol was determined with glycerol kinase (EC 2.7.1.30) according to the method described in reference 1. Cells collected from continuous cultures were washed and suspended at room temperature in the minimal growth medium without carbon source or thiamine (pH 6.8). Incubations with [^{14}C]phenol were at 28°C for 0.5 min. Uptake of [^{14}C]phenol in cells from flask cultures was studied as described before (5). Parameters of uptake were determined by kinetic analysis with an HP-85 desk computer, using the nonlinear regression algorithm of Marquardt (4) adapted for computers according to the method of Nash (6). For studies of induction of high-affinity phenol uptake and of phenol hydroxylase (EC 1.14.13.7), cells were incubated in the minimal growth medium in the presence and absence of anisomycin (10 μg ml^{-1}). Phenol concentration was maintained at 1 mM. Cell samples were removed after the indicated time intervals, analyzed for the rate of uptake of 20 μM phenol, permeabilized, and assayed for phenol hydroxylase (8).

We have previously reported a half-saturation constant for phenol uptake in *T. cutaneum* of 235 ± 30 μM (5). This value was obtained by using phenol concentrations not exceeding 2.0 mM. At these phenol concentrations, additional components of the uptake system could not be detected. Figure 1 (main diagram) shows Eadie-Hofstee plots of uptake rates in cells taken from steady-state continuous cultures on phenol and glycerol. The concentrations of [^{14}C]phenol in these studies were 1 μM to 20 mM. The plots are clearly biphasic, indicating at least two uptake systems for phenol in cells grown on either carbon substrate. The K_m values are listed in Table 1.

To saturate the high-affinity uptake system alone, 20 μM [^{14}C]phenol was used (Fig. 1, inset). The data show that the high uptake rates in cells grown on phenol coincide with the sensitivity of the uptake system to the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and that this sensitivity becomes insignificant when all phenol in the fermentor has been replaced by glycerol. There was no detectable effect of CCCP in corresponding experiments with 5 mM instead of 20 μM phenol.

The specificity of the low-affinity phenol uptake system by glycerol-grown cells differed from that of the high-affinity system of phenol-grown cells. Thus, resorcinol, which interfered with the uptake of phenol by glycerol-grown cells, had no such effect on phenol-grown cells (cf. reference 5). Cells grown on sucrose behaved similarly to cells grown on glycerol. In both types of cells, the low-affinity uptake systems for phenol exhibited a maximum around pH 7.5. This was in contrast to phenol-grown cells, in which the uptake rate increased with pH over the range 5.5 to 8.5 (5).

The effect of anisomycin on the induction of the high-affinity uptake system and of phenol hydroxylase in glycerol-grown cells is shown in Fig. 2. Cells removed after 6.5 h of incubation were analyzed for initial rates of phenol uptake. The kinetic parameters are listed in Table 1 together with those obtained with cells from steady-state continuous cultures (cf. Fig. 1). The results support the occurrence of two transport systems for phenol in *T. cutaneum*. The high-affinity transport system, K_m 3 to 12 μM, has a V_{max}/K_m ratio of 9 to 14 in cells grown on or incubated with phenol but a ratio of only 1.5 in cells grown on glycerol or in such cells incubated with phenol during arrested protein synthesis. Thus a major part of the high-affinity system seems to be induced in the presence of phenol and dependent on de novo protein synthesis. The low-affinity system, K_m 6 to 8 mM, shows the same V_{max}/K_m ratio (0.14 to 0.19) in phenol-grown cells as in glycerol-grown cells. This indicates a constitutive character of the low-affinity system. The concomitant induction of phenol hydroxylase and high-affinity transport raises the question of whether the appearance of the high-affinity phenol uptake represents the induction of a high-affinity transport system per se or, rather, reflects the induction of phenol hydroxylase. It would be possible to answer this question conclusively if mutants devoid of phenol hydroxylase but possessing the transport system could be obtained. However, we have not been able to find a selection method for such presumptive mutants. Under normal physiological conditions, it is suicidal for the cell to concentrate phenol intracellularly. There are, nevertheless, a number of indica-

* Corresponding author.

TABLE 1. Kinetic parameters of phenol uptake by washed cells of *T. cutaneum* grown on phenol or glycerol

Growth substrate and other conditions	Uptake system for phenol[a]			
	High-affinity		Low-affinity	
	K_m	V_{max}/K_m	K_m	V_{max}/K_m
Phenol[b]	3	9–14	7,800	0.19
Phenol[c]	12	9	8,200	0.14
Phenol with anisomycin (10 μg/ml)[c]	10	1.5	5,700	0.16
Glycerol[b]	11	1.4	6,300	0.16

[a] Conditions of uptake as in Fig. 1; K_m in micromolar phenol; V_{max} in nanomoles of phenol milligram of cells^{-1} minute^{-1}.
[b] Cells from steady-state continuous culture without C limitation, maintaining 150 to 200 μM phenol or 360 to 500 mM glycerol in the effluent.
[c] Cells after 6.5 h of incubation in flasks as in Fig. 2.

tions pointing to an inducible, phenol (phenolate)-proton symport which is not dependent on phenol hydroxylase. Thus, 2,6-dimethylphenol, which cannot be hydroxylated by phenol hydroxylase, gives competitive inhibition of [^{14}C] phenol uptake, whereas resorcinol, a good substrate of phenol hydroxylase, does not interfere with phenol uptake in phenol-grown cells (5). The inductive character of the sensitivity to CCCP, concomitant with the appearance of high-affinity phenol uptake (Fig. 1, inset), indicates that these two properties are induced simultaneously. Low concentrations of monovalent anions, e.g., chloride, severely inhibit phenol hydroxylase (7), whereas high concentrations of KCl, NaCl,

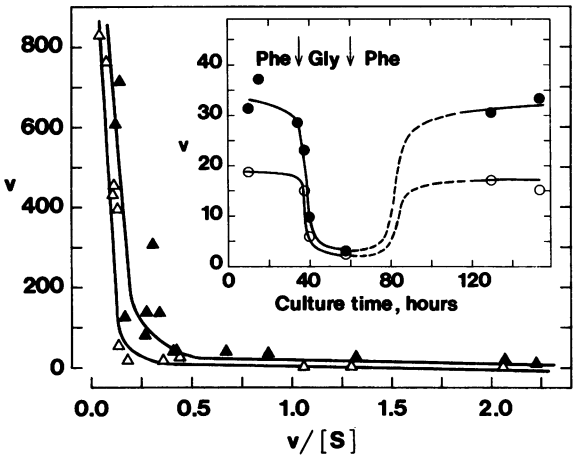

FIG. 1. Biphasic kinetics of phenol uptake in cells grown on phenol or glycerol in steady-state continuous cultures without C limitation. Main diagram: Eadie-Hofstee plots of data obtained from initial rates of [^{14}C]phenol uptake (1.0 μM to 20 mM) in washed cells grown on phenol (▲) or glycerol (△); uptake rate (v) in nanomoles of phenol milligram of cells^{-1} minute^{-1}, S and K_m in micromolar phenol. Inset: Sensitivity of phenol uptake rates to 2 min of preincubation with the protonophore CCCP (0.1 mM) in washed cells taken during substrate shifts (indicated by arrows) of phenol-glycerol-phenol (Phe ↓ Gly ↓ Phe), grown at a septic growth rate of 0.1 h^{-1}, i.e., a replacement time of 10 h. ●, Control cells; ○, cells preincubated with CCCP. The values are corrected for the inhibiting effect of ethanol, which was used to dissolve CCCP.

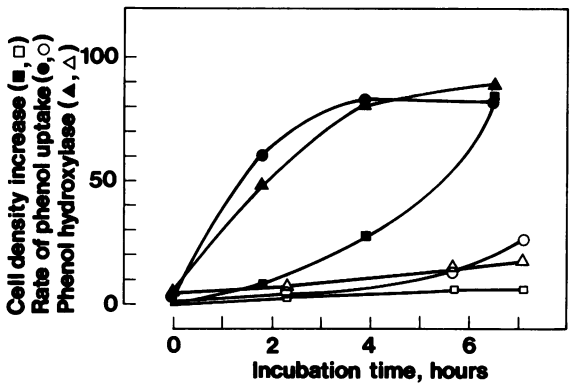

FIG. 2. Induction of the high-affinity phenol uptake system and phenol hydroxylase in cultures of *T. cutaneum* seeded with a dense inoculum of glycerol-grown cells. Incubation was in the minimal growth medium (filled symbols) or in the same medium containing anisomycin (10 μg ml^{-1}) (empty symbols). The initial cell density was 0.16 mg of cell dry weight ml^{-1}. Cell density increase is plotted in micrograms of cell dry weight milliliter^{-1} minute^{-1}; phenol hydroxylase is plotted in milliunits milligram of protein^{-1}, and rate of phenol uptake is plotted in nanomoles of phenol milligram of cells^{-1} minute^{-1}. Protein content of the permeabilized cells was about 40% of their dry weight.

or NH_4Cl do not affect phenol uptake (5). Considering these properties of the high-affinity uptake system, the simultaneous induction of phenol hydroxylase and high-affinity uptake could alternatively be explained by the assumption that the genes for uptake and hydroxylase are coinduced, regulated, or both by the same control mechanisms. Coordinate induction of nutrient-degrading enzymes and transport systems has been demonstrated in both bacteria and yeasts. The classical example is the *lac* operon in *Escherichia coli*, identified by Jacob and Monod (3). More recently, a coordinate induction of a transport system for maltose and α-glucosidase activity has been demonstrated in *Saccharomyces cerevisiae* (2).

LITERATURE CITED

1. **Bergmeyer, H. U. (ed.).** 1970. Methods of enzymatic analysis, 2nd ed., vol. 2, p. 1367–1372. Academic Press, Inc., New York.
2. **Cohen, J. D., M. I. Goldenthal, B. Buchferer, and J. Marmur.** 1984. Mutational analysis of the MAL1 locus of *Saccharomyces cerevisiae*: identification and functional characterization of three genes. Mol. Gen. Genet. **196**:208–216.
3. **Jacob, F., and J. Monod.** 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3**:318–356.
4. **Marquardt, D. W.** 1963. An algorithm for least squares estimation of non-linear parameters. J. Soc. Ind. Appl. Math. **11**:431–441.
5. **Mörtberg, M., and H. Y. Neujahr.** 1985. Uptake of phenol by *Trichosporon cutaneum*. J. Bacteriol. **161**:615–619.
6. **Nash, J. C.** 1979. Compact numerical methods for computers: linear algebra and function minimisation, p. 37–44. Adam Hilger Ltd., Bristol, United Kingdom.
7. **Neujahr, H. Y.** 1983. Effects of anions, chaotrops, and phenol on the attachment of flavin adenine dinucleotide to phenol hydroxylase. Biochemistry **22**:580–584.
8. **Spånning, Å., and H. Y. Neujahr.** 1987. Growth and enzyme synthesis during continuous culture of *Trichosporon cutaneum* on phenol. Biotechnol. Bioeng. **29**:464–468.