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ENERGIZATION OF SULFATE TRANSPORT IN YEAST

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Sulfate uptake by *Saccharomyces cerevisiae* is stimulated about 12-fold by preincubation of cells with 1% D-glucose or 1% ethanol. The K_T remains unchanged (0.34–0.38 mM), the J_{max} increases from 18–20 to 195–230 and 170–185 nmol/min per g dry wt., respectively, after glucose and ethanol preincubation. The stimulation involves protein synthesis (it is suppressed by cycloheximide), has a half-time of 18 min and requires mitochondrial respiration (no or low effect in respiration-deficient mutants and those lacking ADP–ATP transport in mitochondria, as well as after anaerobic preincubation of the wild-type strain, and in low-phosphate cells). The presence of NH_4^+ and some amino acids (e.g., leucine, aspartate, cysteine and methionine) depressed the stimulation while that of cationic amino acids (typically arginine and lysine) and of K^+ increased it by 50–80%. The stimulated (i.e., newly synthesized) transport system was degraded with a half-life of about 10 min.

Introduction

Sulfate anions are transported into baker's yeast by a saturable and specific [1] active transport. The molecular basis of this appears to comprise a carrier-mediated, proton-driven mechanism [2]. While two distinct systems have been reported [3], a single carrier is probably sufficient to account for the data obtained [2]. One of the earliest observations [4] was that glucose was essential for proper uptake of sulfate anions, as is the case with amino acids (e.g., Ref. 5), phosphate anions [6] and many other solutes [7]. The character of synthesis and degradation of the sulfate-transport system brought about by various substrates and factors is discussed here.

Materials and Methods

Microorganisms. Saccharomyces cerevisiae K used in previous studies [7], the collection strain DXII, its respiration-deficient mutant DXIIA and the op_1 mutant deficient in the ADP-ATP transport system of mitochondria (a kind gift of Dr. J. Šubík of Bratislava) were cultivated in a mineral medium with yeast extract and 2% glucose (containing 1.5 g SO_4^{2-} per l) up to early stationary phase, harvested and washed three times in tap water. Yeast cells lacking in phosphate were grown without phosphate anions and in the presence of one-tenth of the yeast extract ordinarily used. This gave rise to cells containing less than 3 mg extractable P per g dry wt. [8].

Incubation and sulfate uptake. The suspension (about 10 mg dry wt./ml) was aerated for 2 h in water and then incubated with glucose and/or other additives as indicated. Then it was washed and cells were resuspended in distilled water in Erlenmeyer flasks in a Dubnoff incubator. $Na_2^{35}SO_4$ was then added and 0.2 ml samples were withdrawn at intervals, filtered through Synpor 5 filters (0.6 μm pore diameter) and washed, and the pellet with filter was transferred to a scintillation vial containing a toluene/ethanol scintillation cocktail. For anaerobic conditions suspensions were flushed for 5 min with argon containing less then 1 ppm O_2 and maintained in an atmosphere of Ar in the presence of white phosphorus.

Membrane potential measurement. The magnitude of the membrane potential was computed using the Nernst equation from the distribution of ^{3}H-labelled tetraphenylphosphonium ion (TPP^+) and, in parallel, by monitoring the distribution with a TPP^+-specific electrode (cf. Ref. 9).

Radioactivity was counted in an Isocap 2 spectrometer.

Chemicals. All compounds were of the highest available purity. They included D-glucose, ethanol and 2,4-dinitrophenol (Lachema, Czechoslovakia), amino acids (Koch-Light, U.K.), cycloheximide (Fluka, Switzerland) daunomycin and tetraphenylphosphonium (TPP^+; Serva, F.R.G.) and phenylmethylsulfonyl fluoride and antimycin (Sigma, U.S.A.). Carrier-free $Na_2^{35}SO_4$ was obtained from the Radiochemical Centre Amersham, U.K. and [^{3}H]-TPP^+ was a gift of Dr. P. Geck of Frankfurt am Main, F.R.G.

Results and Discussion

Incubation with energy-providing substrates

Uptake of inorganic sulfate is highly active during exponential growth of yeast cells. After 14 h of growth (mid-exponential) the rate of uptake was about 130 nmol/min per g dry wt., at the beginning of the stationary phase it dropped to 60 and after a 2-h aeration in water to roughly 12 nmol/min per g dry wt. When this impoverished yeast was incubated with either glucose or ethanol and washed, sulfate uptake, measured in suspension in distilled water, increased even to exceed the values of exponential-phase uptake (Table I). The low-phosphate cells apparently lack the aerobic pathway through which the sulfate uptake system is stimulated. This is confirmed by the virtual lack of activation in the respiration-deficient mutant and the decreased effect in the op_1 mutant. In normal cells, anaerobic preincubation with glucose results in a slight stimulation of sulfate uptake, to 28.1–29.3 nmol/min per g dry wt. contrasting with aerobic preincubation, leading to 138.5–144.5 nmol/min per g dry wt. Anaerobiosis during sulfate uptake itself has a negligible effect.

The progress curve of the stimulation reaction with time is S-shaped, with a reproducibly estimated half-time of 18 min at 30°C. (Fig. 1) both with D-glucose and with ethanol as stimulant.

The preincubation with glucose or ethanol apparently does not lead to the synthesis of a new type of

TABLE I

ENTRY OF 1 mM Na_2SO_4 AFTER 1-h PREINCUBATION OF DIFFERENT STRAINS OF *SACCHAROMYCES CEREVISIAE* WITH WATER, D-GLUCOSE OR ETHANOL (1%)

Values of initial uptake rate are means of 2–12 separate experiments and are expressed in nmol/min per g dry wt.

Strain	Characteristic	Preincubated for 1 h with	Initial uptake rate
K	Distillery yeast, aneuploid, CCY 21-4-60	water	12.4
		glucose	144.5
		ethanol	138.6
K	Low-phosphate	water	<2
		glucose	40.3
		ethanol	<2
DXII	Collection strain, CCY 21-4-13	water	9.2
		glucose	108.3
		ethanol	99.5
DXII op_1	Lacks mitochondrial transport system for ATP/ADP	water	5.2
		glucose	44.4
		ethanol	39.6
DXIIA	Respiration-deficient, ρ^-, CCY 21-4-19	water, glucose ethanol	<2

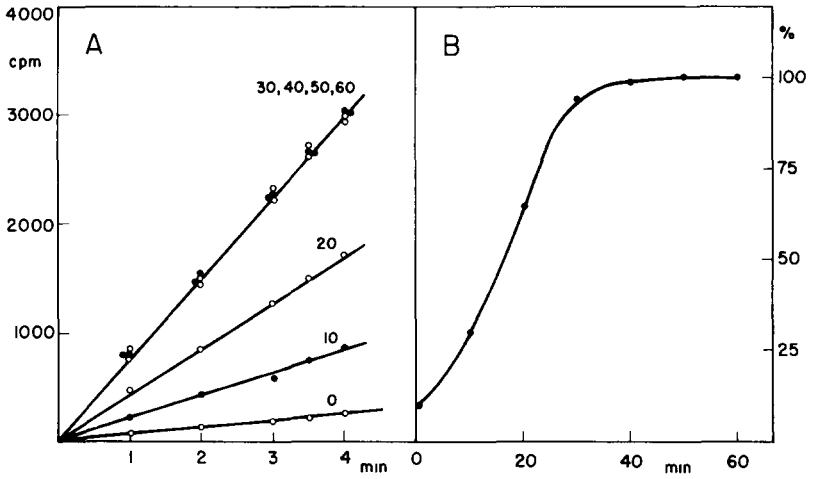

Fig. 1 Stimulation of sulfate uptake by preincubation with glucose. Uptake curves of 1 mM $Na_2^{35}SO_4$ are shown in panel A, in samples preincubated for 0–60 min with glucose as indicated on the curves. In panel B, the rates are plotted (in %) against time of preincubation.

transport system, since the K_T (half-saturation constant) values do not change after such treatment (0.38 mM without preincubation, 0.34 mM after both glucose and ethanol preincubation, with an S.D. of ±0.06 mM). At the same time, the J_{max} (maximum rate of transport) values rise from 18–20 in the control to 195–230 and 170–185 nmol/min per g dry wt. after glucose and ethanol preincubation, respectively.

It should be noted that there is absolutely no growth of cells during the preincubation period.

Preincubation with nitrogen-containing compounds

Ammonium ions are powerful inhibitors of the glucose-stimulation effect on amino acids (unpublished results from this laboratory) as well as on xanthine uptake in *Schizosaccharomyces pombe* 972 h^- [10] while they do not alter the glucose stimulation with disaccharides or phosphate [8]. Their effect and those of several amino acids and inhibitors on sulfate transport are shown in Table II. Somewhat surprisingly, NH_4^+ as well as representatives of neutral and anionic amino acids suppressed the stimulation by glucose, while cationic amino acids were highly active in enhancing the stimulation further. 2-Aminoisobutyric acid, an analog that is neither metabolized nor incorporated into proteins, was ineffective. Preincubation with 10 mM KCl, which leads to an accumulation of K^+ in cells, had an effect practically identical with that of L-lysine.

Now it was to be established whether the cationic agents present intracellularly somehow activate the

TABLE II

ENTRY OF 1 mM Na_2SO_4 AFTER 1-h PREINCUBATION OF *SACCHAROMYCES CEREVISIAE* K WITH SUBSTRATES AND INHIBITORS

The control value after preincubation with glucose alone was 144.5 nmol/min per g dry wt. (=1.0).

Substance added during preincubation with 1% D-glucose	Concentration (mM)	Relative uptake rate
None	–	1.00
NH_4^+	110	0.35
L-Arginine	10	1.45
L-Lysine	10	1 80
L-Leucine	10	0.25
L-Aspartate	10	0 40
L-Methionine	10	0 36
L-Cysteine	10	0.43
2-Aminoisobutyrate	10	1.00
2,4-Dinitrophenol	0 5	0.50
Antimycin	0.004	0.23
Iodoacetamide	0.5	0.12
Daunomycin	0.4	0.11
Cycloheximide	0 4	0.10

actual transport process (say, by exchanging with the protons reportedly accompanying sulfate during entry). The presence of these cations (arginine, lysine, K^+) in the external medium during sulfate uptake should then inhibit the uptake by competing with the protons. In fact, none of these agents (including leucine as a potential activator), added at a 10-times higher concentration than sulfate, had any detectable effect on the rate of sulfate uptake. The effects of the cationic additives bear apparently no relation to the membrane potential ($\Delta\varphi$) of preincubated yeast. The values of $\Delta\varphi$ found after 1 h preincubation with water, glucose alone, glucose plus ammonium chloride, and glucose plus arginine were 70, 81, 71 and 68 mV, respectively. The nature of the effects thus remains obscure and must be sought in some metabolic interference with the glucose stimulation of sulfate uptake.

The stimulation described here is obviously a process involving protein synthesis outside mitochondria (therefore, the term 'activation' has been avoided) since 0.4 mM cycloheximide suppresses all the effects of glucose stimulation as well as the superimposed positive or negative influences of amino acids, the values of sulfate uptake being in all cases about 12 nmol/min per g dry wt. Daunomycin, an inhibitor of RNA transcription, had a similar effect. The requirement for energy in the stimulation by glucose is documented by the powerful depression caused by 2,4-dinitrophenol and antimycin, as well as iodoacetamide, attesting to the essential role of glucose degradation in the stimulatory process.

Sulfate as such does not appear to act as inducer. Starvation for sulfate apparently increases the avidity for its uptake [4], but this is not the case observed here where, in the absence of sulfate (washed and aerated stationary cells), its subsequent uptake was less than in its presence.

Degradation of the sulfate uptake system

To establish the 'inducible' nature of the synthesis of the sulfate system we 'de-induced' the system by incubating cells possessing the stimulated sulfate transport in the presence of various factors and measured the residual sulfate uptake (Table III). To begin with, both the glucose-stimulated and the ethanol-stimulated uptakes decay in water at 30°C with a half-life of 10–11 min, further pointing to the

TABLE III

INITIAL RATE OF UPTAKE OF 1 mM SULFATE STIMULATED BY 1-h PREINCUBATION WITH 1% D-GLUCOSE (A) OR 1% ETHANOL (B)

After preincubation, cells were washed and postincubated for another hour with the substance shown. After another washing the uptake of sulfate was measured. Values of sulfate uptake after preincubation with D-glucose or ethanol, which were both 140 nmol/min per g dry wt. with no postincubation, were set equal to 1.0.

Added during postincubation	A	B
D-Glucose (1%)	1.10	–
Ethanol (1%)	0.80	0.96
Water	0.22	0.30
Cycloheximide (0.4 mM)	0.23	0.31
Cycloheximide (0.4 mM) and D-glucose (1%)	0.23	0.47

identity of the systems thus synthesized (Fig. 2). Cycloheximide has no further effect which suggests that: (1) no inducer is present any more; (2) no protein synthesis, such as that of a protease, is involved here (contrast with the maltose uptake system [1]).

The presence of 2 mM phenylmethylsulfonyl fluoride, an inhibitor of proteases with a serine active centre, led to insignificant retardation of the decay of the sulfate transport system, so that no conclusion

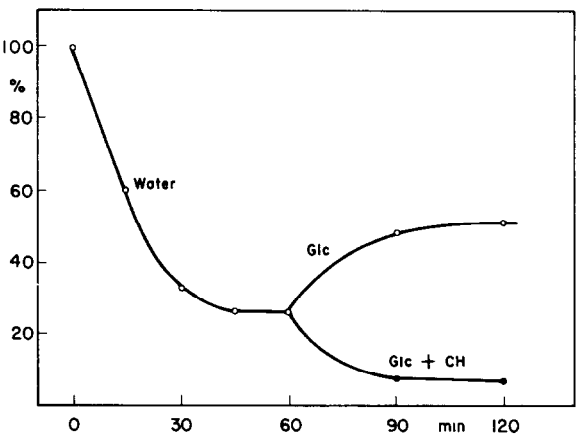

Fig. 2. Initial rates of uptake J_{S_0} (in percent) of 1 mM Na_2SO_4. At time zero, J_{S_0} after preincubation for 1 h with 1% D-glucose. Between time zero and 60 min, incubation of cells in water. At 60 min, either 1% D-glucose (○) or 1% D-glucose plus 0.4 mM cycloheximide (●) were added.

about the involvement of the serine protease can be made.

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