ACTIVE SULFATE TRANSPORT IN *SACCHAROMYCES CEREVISIAE*

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Received 18 September 1973

1. Introduction

Active sulfate transport systems have been well characterized in some procaryotes [1–8] and some filamentous eucaryotes [9–12]. During the course of our investigation in sulfur biofractionation in *Saccharomyces cerevisiae* (unpublished results [13]) we found it necessary to measure sulfate permeation. Unfortunately, no data have been published on yeast and this is the first report on sulfate permeation in this unicellular eucaryote.

The results of this preliminary study indicate that there is an active sulfate uptake mechanism in *Saccharomyces cerevisiae.*

2. Materials and methods

2.1. *Culture conditions*

Saccharomyces cerevisiae (dried Fleishmann's Baker's Yeast) was grown in 500 ml yeast extract–glucose medium of Jollow et al. [14] under a nitrogen atmosphere at 28°C.

2.2. *Measurement of the initial velocity of sulfate uptake*

Cells were harvested after 10 hr and washed with a synthetic medium consisting of: K_2HPO_4 1.25 g, KH_2PO_4 0.875 g, NaCl 0.1 g, NH_4Cl 0.4 g, KNO_3 0.78 g, $MgCl_2$ 0.5 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, glucose 50 g, biotin 2 μg, inositol 2 mg, niacin 400 μg, *para*-aminobenzoic acid 200 μg, pyridoxine 400 μg, thiamine·HCl 400 μg, riboflavine 200 μg and calcium pantothenate 400 μg per litre of medium [13]. The cells were then taken up in the synthetic medium, diluted to an $O.D._{600nm}$ of 1.0 and incubated at 28°C in a nitrogen atmosphere for 4 hr to deplete the intracellular sulfate pool.

Initial rates of sulfate uptake were measured employing 5 ml quantities of synthetic medium containing 10^{-5} M to 2×10^{-3} M [^{35}S]sulfate (specific activity of the [^{35}S]sulfate was 20 μCi/100 μmoles). At zero time, 5 ml of sulfate-depleted cells were added to an equal volume containing the sulfate, mixed well, and 1 ml samples were withdrawn at 15 sec intervals, over a 2 min period, for rapid filtration on Millipore filters (25 mm, 0.45 μ pore size). After the sample had filtered, the filter was washed with 80 ml non-radioactive medium containing the same concentration of sulfate as that being tested. After liquid scintillation counting (32% efficiency) the initial rates of sulfate uptake were determined from a time *vs* counts plot.

2.3. *Measurement of initial velocity of sulfate, glucose and methionine uptake in the presence and absence of inhibitors*

Similar experiments were carried out to study the effect of inhibitors, temperature and of energy depletion. In all of these studies a final concentration of 10^{-4} M sulfate was used and the inhibitors were at the same concentration. For the energy depletion studies the cells were starved of sulfate for 3 hr and of sulfate and glucose for an additional hour.

To measure the specificity of selenate inhibition, methionine and glucose transport were followed in the presence and absence of 10^{-3} M selenate and compared to sulfate uptake under identical conditions.

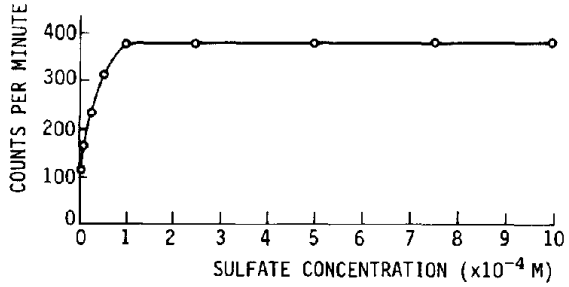

Fig. 1. Effect of sulphate concentration on the initial velocity of sulfate uptake.

3. Results

3.1. *Effect of sulfate concentration on the initial velocity of sulfate uptake*

The initial velocity of sulfate uptake exhibited a pattern of concentration dependence as shown in fig. 1. The uptake rate increased until a concentration of 1×10^{-4} M sulfate was reached; the system was then saturated and uptake levelled off.

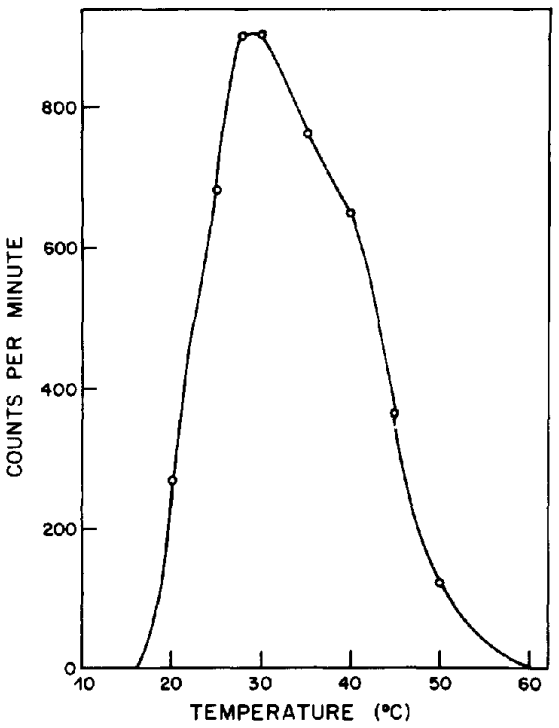

Fig. 2. Effect of temperature on the initial velocity of sulfate uptake.

Table 1
The effect of inhibitors on sulfate uptake.

Inhibitor (10^{-4} M)	% Inhibition
$CrO_4^{=}$	66%
$MoO_4^{=}$	5%
$SeO_4^{=}$	55%
$SO_3^{=}$	23%
$S_2O_3^{=}$	31%
$TeO_3^{=}$	11%

3.2. *Sulfate uptake in the presence and absence of an energy source*

Glucose starvation was responsible for a large reduction in the ability of the yeast cells to take up sulfate. Sulfate uptake was almost totally dependent on the presence of an energy source (12 cpm in the absence *vs* 390 cpm in the presence of glucose).

3.3. *Effect of temperature on sulfate uptake*

The effect of temperature on the initial velocity of sulfate uptake is shown in fig. 2. No uptake was observed at 15°C; the uptake increased to a maximum at 28–30°C then fell again to zero at 60°C.

3.4. *The effect of inhibitors on sulfate uptake*

The effect of various inhibitors on sulfate uptake is illustrated in table 1. Inhibition patterns varied depending on the growth conditions (unpublished data) and it was apparent that the degree of inhibition was concentration dependent (10^{-4} M selenate –55% inhibition *vs* 10^{-3} M selenate –85% inhibition). The specificity of selenate inhibition is restricted to sulfate for neither glucose nor methionine transport was affected, as shown in table 2.

4. Discussion

In *Saccharomyces cerevisiae* sulfate uptake is a saturable, temperature dependent, energy requiring process. Since the process is specifically inhibited by the structural analogue selenate, it meets all of the requirements of an active transport process or permease [15]. The permease in *Saccharomyces cerevisiae* closely resembles the permeases of some procaryotes [1–8] and filamentous eucaryotes [9, 10] but is unlike

Table 2
The effect of 10^{-3} M selenate on the uptake of sulfate, glucose and methionine.

cpm minus Selenate		cpm plus Selenate
450	10^{-4} M $^{35}SO_4$ (20 μCi/100 μmoles)	70
360	1.6×10^{-7} M [^{14}C]methionine (50 μCi/μmole)	370
1734	3.8×10^{-4} M [^{14}C]glucose (1 μCi/132 μmoles)	1724

the dual permease system found in *N. crassa* [11, 12]. This is the first report of a sulfate permease in a unicellular eucaryote and, as might be expected, its characteristics on an evolutionary scale seem to lie between those exhibited by procaryotes [1–8] and multicellular eucaryotes [9–12].

Acknowledgement

This work was supported by a grant from the National Research Council of Canada.

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