BBA 75364

SPECIFICITY OF TRANSPORT PROCESSES FOR SULFUR, SELENIUM, AND MOLYBDENUM ANIONS BY FILAMENTOUS FUNGI*

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(Received July 21st, 1969)

SUMMARY

1. Inorganic SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-} and MoO_4^{2-} enter mycelia of Penicillium and Aspergillus species by a common energy-, temperature-, pH-, and concentration-dependent permease. Evidence for a single permease is as follows: (a) All four anions exhibit reciprocal competitive inhibition. (b) Mycelia grown on sulfur sources that repress the sulfate permease (*e.g.*, L-methionine) show similar low $S_2O_3^{2-}$, SeO_4^{2-}, and MoO_4^{2-} transport rates. Mycelia grown on sulfur sources that derepress the sulfate permease (*e.g.*, L-djenkolic and L-cysteic acids) transport all four anions rapidly. (c) Sulfur starvation results in coincident derepression of SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-}, and MoO_4^{2-} transport to at least 40 times the base level. Throughout the derepression period the SeO_4^{2-}/SO_4^{2-}, MoO_4^{2-}/SO_4^{2-} and SeO_4^{2-}/MoO_4^{2-} transport rate ratios remain constant. (d) A mutant defective in SO_4^{2-} transport was equally defective in SeO_4^{2-} and MoO_4^{2-} transport.

2. In addition to the sulfate (thiosulfate, selenate, molybdate) permease the mycelia possess distinct permeases for SO_3^{2-} and $S_4O_6^{2-}$. The sulfite and tetrathionate permeases are under metabolic control by some intracellular sulfur-containing metabolite.

3. Inorganic $S_2O_3^{2-}$ enters the mycelium of a sulfate (thiosulfate) permease-negative mutant at 1–5 % of the wild-type rate at low extracellular $S_2O_3^{2-}$ concentrations. $S_2O_3^{2-}$ transport by the mutant is not inhibited by SO_4^{2-}. The v_{max} for $S_2O_3^{2-}$ transport by the mutant is almost the same as that of the sulfate permease-positive parent (about 2–3 μmoles/g per min). The K_m value, however, is about 30-fold higher (2 mM *vs.* 59 μM). The tetrathionate permease may be responsible for $S_2O_3^{2-}$ transport in the mutant under standard assay conditions. However, $S_2O_3^{2-}$ is unstable at the pH values of most fungal cultures. Consequently, most of the sulfur incorporated from $S_2O_3^{2-}$-containing media in long-term growth studies is probably in the form of breakdown products (SO_3^{2-}, SO_4^{2-}, S^{2-}).

4. S^{2-} uptake is 2,4-dinitrophenol- and azide-sensitive, but shows a low Q_{10} (1.15 *vs.* 2.1 for the sulfate permease), is non-saturable, and does not depend on the degree of sulfur sufficiency of the mycelium.

5. No SCN^- uptake could be detected.

Abbreviations: APS, adenosine 5'-phosphosulfate; PAPS, 3'-phosphoadenosine 5'-phosphosulfate.

* The research described in this paper is part of a thesis to be submitted to the Graduate School of the University of California, Davis, by John W. Tweedie in partial fulfillment of the requirements for the Ph.D. degree in Comparative Biochemistry.

INTRODUCTION

The characteristics of the inorganic SO_4^{2-} transport system of *Penicillium chrysogenum* have been described by YAMAMOTO AND SEGEL[1]. These authors showed that SO_4^{2-} transport was inhibited by both SeO_4^{2-} and $S_2O_3^{2-}$ and that SeO_4^{2-} entered the myselium *via* a sulfur-regulated permease (probably the sulfate permease).

The experiments described in this paper were designed to determine the number of distinct permeases that fungi possess for the naturally occurring inorganic Group VI anions. In particular, we were interested in determining whether MoO_4^{2-}, a biologically important structural analog of SO_4^{2-}, is also transported by the sulfate permease. Molybdenum is a component of several enzymes and one might expect that the transport of inorganic MoO_4^{2-} (a likely precursor of enzyme-bound molybdenum) would be regulated independently of SO_4^{2-} transport. We also hoped to establish how inorganic $S_2O_3^{2-}$ enters the mycelium of sulfate (thiosulfate) permease-negative mutants.

A preliminary report of this work has been presented[2].

MATERIALS AND METHODS

Organisms used

Several strains of Penicillium and Aspergillus were used in this work: *P. chrysogenum*, PS75 and *Penicillium notatum*, 832 are wild-type strains. *P. notatum*, 38632M is a mutant which lacks a sulfate permease and at least one of the SO_4^{2-}-activating enzymes (unpublished results). *P. notatum* 38632R is a spontaneous revertant isolated from *P. notatum* 38632M. The revertant has wild-type levels of the sulfate permease but still lacks one of the SO_4^{2-}-activating enzymes. *Aspergillus nidulans*, strain *eta*, lacks one of the enzymes involved in the reduction of 3-phosphoadenosine 5'-phosphosulfate (PAPS) to SO_3^{2-} but has wild-type levels of the sulfate permease.

Permease assay

The cell cultivation and permease assay methods used were identical to those described by BENKO *et al.*[3].

Chemicals

Carrier-free $^{35}SO_4^{2-}$ was obtained from Nuclear Science and Engineering Corp. $(NH_4)_2{}^{99}MoO_4$ in 5 M NH_4OH was obtained from New England Nuclear Corp. (specific activity 300–500 mC/g).

$Na_2{}^{35}SSO_3$ and $Na_2S^{35}SO_3$ were obtained from Schwartz Bioresearch Corp. (specific activity 15–30 mC/mmole).

$Na_2{}^{35}S_4O_6$ was prepared from $^{35}SSO_3^{2-}$ either by oxidation with a solution of I_2 in KI or by the exchange reaction between a small amount of labeled $S_2O_3^{2-}$ and a large excess of unlabeled $Na_2S_4O_6$ (K and K Laboratories).

$Na_2{}^{35}S$ and $Na_2{}^{35}SO_3$ (specific activities 20–30 mC/mole) were obtained as solids from New England Nuclear Corp.

$NaCN^{35}S$ was obtained from New England Nuclear Corp.

$H_2{}^{75}SeO_3$ (in 1 M HCl) was obtained from New England Nuclear Corp. (specific activity 15–25 C/g selenium). This was converted to $H_2{}^{75}SeO_4$ by the following pro-

cedure: 5 ml of H_2SeO_3 in 1 M HCl (0.12 mg selenium per ml) was concentrated to 0.5 ml in a stream of air at 40°. 10 ml of H_2O_2 (80 % w/v) was added and the solution was heated at 75° for 12 h. The volume was then reduced to 0.5–1.0 ml as described above. Another 10-ml aliquot of H_2O_2 was added and the mixture heated at 75° for an additional 12 h. KOH (concentrated solution) was added to bring the solution to neutrality and the K_2SeO_4 was taken to dryness under a stream of air at room temperature. The solid material was taken up in an appropriate amount of deionized water. This method gave complete conversion of SeO_3^{2-} to SeO_4^{2-} as shown by high-voltage paper electrophoresis at pH 8.1. The recovery, based on radioactivity, was better than 95 % in all preparations. Attempts to convert SeO_3^{2-} to SeO_4^{2-} by the method of VIRUPAKSHA AND SHRIFT[4] resulted in considerable loss of radioactivity (probably *via* volatile selenium compounds).

Enzyme assays

Nitrate reductase was assayed by measuring the production of NO_2^- according to the method of NASON AND EVANS[6].

RESULTS AND CONCLUSIONS

Identity of the sulfate, thiosulfate, selenate, and molybdate permeases

When *A. nidulans* strain *eta* was grown on sulfur sources that repress the active transport of SO_4^{2-} (methionine, $Na_2S_2O_3$) the transport of SeO_4^{2-}, MoO_4^{2-} and $S_2O_3^{2-}$ was also repressed. Growth on sulfur sources which have been shown to derepress the SO_4^{2-} transport system (L-djenkolic acid, L-cysteic acid)[1] also derepress the system involved in the transport of the other three anions. The results suggested that all four Group VI anions were transported by a sulfur-regulated permease(s).

We also studied the development of transport ability under conditions of sulfur starvation (which derepresses the SO_4^{2-} transport system). If all four anions were transported by the same system we would expect that the ratio of the transport rates for any two anions would remain constant throughout the development of the system. As shown in Table I, this was what was observed. The constancy of these ratios over a period in which the rate of transport increased at least 40-fold very strongly suggests that these anions are transported by the same system. In a separate experiment, we established that the transport rate ratios for SO_4^{2-} and $S_2O_3^{2-}$ also remained essentially constant throughout the derepression period.

Finally, a mutant of *P. notatum* (strain 38632M) which lacks the SO_4^{2-} transport system was also deficient in its ability to transport $S_2O_3^{2-}$, SeO_4^{2-} and MoO_4^{2-}. Under the same conditions, a wild-type strain (*P. notatum*, 832) transported all four anions. (The sulfate permease-negative mutant was in fact able to transport $S_2O_3^{2-}$ at about 1–5 % of the wild-type rate under conditions of sulfur deficiency.)

The identity of the $S_2O_3^{2-}$, SeO_4^{2-}, SO_4^{2-} and MoO_4^{2-} transport system in wild-type molds was also shown by the fact that they reciprocally inhibit each others transport at pH 6.0 and above. However, at pH 5.0 and below MoO_4^{2-} did not inhibit SO_4^{2-} transport.

pH-dependence of transport

The reason for MoO_4^{2-} not inhibiting SO_4^{2-} transport below pH 6 is suggested from the data described by Fig. 1, which shows the pH-dependence of transport of

TABLE I

EFFECT OF SULFUR STARVATION ON THE SO_4^{2-}, SeO_4^{2-} AND MoO_4^{2-} TRANSPORT ACTIVITY OF MYCELIUM

A. nidulans, strain *eta*, was grown for 48 h on citrate No. 3 synthetic medium containing $Na_2S_2O_3$ (1 g/l) as sole sulfur source. The mycelium was then filtered and washed 3 times and then resuspended at 20 g/l wet wt. (about $^1/_2$ the original mycelial density) in citrate No. 3 medium *minus* a sulfur source. At the indicated intervals during sulfur starvation aliquots of the mycelial suspension were taken and washed and the uptake of the various anions was measured. The assay medium contained 1 g (wet wt.) of mycelium per 50 ml of 0.05 M potassium ammonium phosphate buffer (pH 6.0). The initial concentration of labeled substrate was 0.1 mM. Mycelial samples (10 ml of assay suspension) were taken at 30-sec intervals for the first 2 min and immediately filtered and extracted in approx. 50 ml of boiling water. The extract suspension was then filtered and the mycelium dried at 100° for 8 h. The filtrate was made up to 100 ml and a 1-ml aliquot taken for the determination of radioactivity. Radioactivity was determined in a liquid scintillation counter (Tricarb, Packard Instrument Co.). For counting ^{99}Mo, which decays to the radioactive products ^{99m}Tc and ^{99}Tc, the discriminators were set to exclude radiations from the daughter nuclides. The scintillation solution used was that of BRAY[5].

Period of sulfur starvation (h)	*Anion transport rate (μmoles/g per min)*			*Ratio of rates*		
	$^{35}SO_4^{2-}$	$^{75}SeO_4^{2-}$	$^{99}MoO_4^{2-}$	$\frac{SeO_4^{2-}}{SO_4^{2-}}$	$\frac{MoO_4^{2-}}{SO_4^{2-}}$	$\frac{SeO_4^{2-}}{MoO_4^{2-}}$
0	0	0	0	—	—	—
2	0.037	0.055	0.015	1.5	0.41	3.7
3	0.120	0.195	0.044	1.6	0.37	4.4
5	0.459	0.733	0.177	1.6	0.39	4.1
7	1.024	1.770	0.362	1.7	0.35	4.9
9	0.977	1.500	0.350	1.5	0.36	4.3

the four anions. Above pH 6.0 the transport rates of all four anions show a close correspondence as would be expected if they were transported by the same system. However, below pH 6 the transport rate of MoO_4^{2-} drops off much more rapidly than the other three. The marked decrease very likely results in part from the protonation of MoO_4^{2-} to form $HMoO_4^-$. The titration curve for molybdate shows a pK_a at approx. 5.3 for $MoO_4^{2-} \rightleftharpoons HMoO_4^-$. However, protonation to a nontransportable ionic form cannot be the sole explanation because at this pH the transport rate is only about 10% of maximum. Another possible explanation for the decrease is the acid-stimulated formation of polymolybdates which are not transported by the permease.

Concentration-dependence of transport

The effect of substrate concentration on transport rate was determined for all four anions over a 10000-fold range of external substrate concentration (1 μM–10 mM). The transport system was shown to be saturable for each anion. The results obtained for SO_4^{2-}, SeO_4^{2-} and $S_2O_3^{2-}$ transport in *A. nidulans*, strain *eta*, over a narrow concentration range are shown in Fig. 2 plotted according to the Lineweaver–Burk method.

The v_{max} values for SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-} and MoO_4^{2-} for *P. notatum* 38632R grown on both low djenkolic acid and high methionine media are shown in Table II.

Energy-dependence of transport

The transport of SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-} and MoO_4^{2-} was inhibited 90–95 % by the addition of 1 mM 2,4-dinitrophenol or NaN_3 10 min prior to the determination of the transport rate (substrate concentration of 0.1 mM).

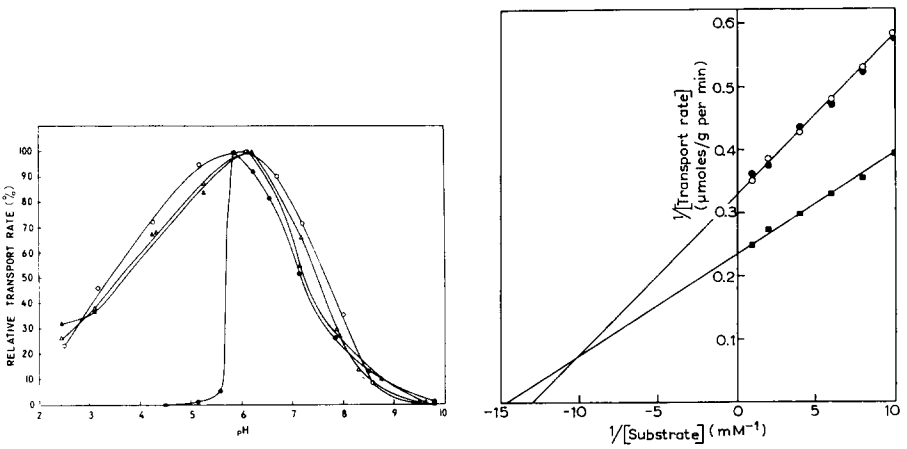

Fig. 1. pH-dependence of SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-}, and MoO_4^{2-} transport by *A. nidulans*, strain *eta*, grown on low djenkolic acid medium. The buffers used were: pH 2–4, 0.05 M phosphate; pH 4–5.5, 0.05 M citrate; pH 5.5–7.5, 0.05 M phosphate; pH 7.5–9, 0.05 M Tris–HCl; pH 9–10, 0.05 M glycine–NaOH. △—△, SO_4^{2-}; ○—○, SeO_4^{2-}; ▲—▲, $S_2O_3^{2-}$; ●—●, MoO_4^{2-}.

Fig. 2. Reciprocal plot of SO_4^{2-}, $S_2O_3^{2-}$, and SeO_4^{2-} transport by low L-djenkolic acid-grown mycelium of *A. nidulans*, strain *eta*. ○—○, SO_4^{2-}, $K_m = 75$ μM; ●—●, $S_2O_3^{2-}$, $K_m = 75$ μM; ■—■, SeO_4^{2-}, $K_m = 77$ μM.

TABLE II

MAXIMUM TRANSPORT RATES OF SO_4^{2-}, $S_2O_3^{2-}$, $S_4O_6^{2-}$, SeO_4^{2-} AND MoO_4^{2-} BY *P. notatum* 38632R.

P. notatum 38632R was grown for 36 h in citrate No. 3 medium containing 1 g/l L-methionine as sulfur source. The mycelium was filtered and washed and assayed immediately for transport of the indicated anion *via* Method II (ref. 3). One half of the wet mycelial pad was also suspended in 1 l of citrate No. 3 medium containing no sulfur source. After 10 h the sulfur-starved mycelium was filtered and washed and the appropriate anion transport rates determined by Method II (ref. 3).

Labeled substrate (1 mM)	*Transport rate (μmoles/g per min)*	
	Sulfur sufficient	*Sulfur deficient*
$^{35}SO_4^{2-}$	0.032	1.81
$S^{35}SO_3^{2-}$	0.023	1.34
$^{75}SeO_4^{2-}$	0.037	1.98
$^{99}MoO_4^{2-}$	0.022	0.96
$^{35}S_4O_6^{2-}$	0.026	1.50
$^{35}S^{2-}$	7.15	7.20

Temperature-dependence of transport

The transport of SO_4^{2-}, $S_2O_3^{2-}$ and MoO_4^{2-} shows a marked temperature-dependence (Q_{10} of 2.1) with optimum at 30°. The drop in transport rate at low temperatures was reversible. However, after preincubation of the mycelium at 50° there was

no recovery of the ability to transport any of the three anions. The high Q_{10} of Group VI anion uptake and its temperature lability is characteristic of an enzyme-like, carrier-mediated process.

Other possible routes of MoO_4^{2-} transport

Above pH 5.0 MoO_4^{2-} can be transported by the SO_4^{2-} transport system and it is possible that this is a route for the entry of the minute amounts of molybdenum required for the growth of the organism. However, MoO_4^{2-} transport *via* the sulfate permease is undetectable below pH 5.0. Furthermore, MoO_4^{2-} transport *via* the sulfate permease would be regulated by the sulfur nutritional status of the organism. Consequently, one might expect the fungi to possess a specific molybdate permease.

Nitrate reductase is a molybdenum-containing enzyme which is inducible by NO_3^-. Consequently, we tried to induce a molybdate permease in *P. notatum* 38632M by growing the organism on medium with NO_3^- as sole nitrogen source. Following a shift from ammonium phosphate to NO_3^--containing medium the level of nitrate reductase rose from zero to a maximum after 3 h. During this period we could detect no transport of MoO_4^{2-} at pH 5.0 or 6.0.

Transport of other Group VI sulfur anions

As shown earlier, $S_2O_3^{2-}$ is transported predominantly *via* the sulfate permease. However, *P. notatum*, strain 38632M, which lacks the sulfate permease will grow well on $S_2O_3^{2-}$ as sole sulfur source and can transport $S_2O_3^{2-}$ at about 1–5 % of the wild-type (or revertant) rate at the usual external assay concentrations (10–100 μM). $S_2O_3^{2-}$ transport by this mutant is not inhibited by SO_4^{2-}, which indicates that $S_2O_3^{2-}$ is not transported by a sulfate permease present at a very low level in the mutant. This conclusion is supported by the data in Fig. 3, which shows that the K_m for $S_2O_3^{2-}$ transport by the mutant is about 30 times higher than the K_m for $S_2O_3^{2-}$ transport by the revertant.

Fig. 4 shows the derepression of $S_2O_3^{2-}$ and $S_4O_6^{2-}$ transport in *P. notatum* 38632M during a period of sulfur starvation. Sulfur starvation does not result in an increase in the rate of SO_4^{2-} transport by this organism.

High-voltage paper electrophoresis of the $S_2O_3^{2-}$ used showed only a single peak of radioactivity. Thus, it was unlikely that we were observing the transport of a radioactive contaminant present at low concentrations in the labeled $S_2O_3^{2-}$. However, contaminating $S_4O_6^{2-}$ would not be detected by our electrophoretic method because of the rapid exchange of ^{35}S between $^{35}S_4O_6^{2-}$ and $^{35}S_2O_3^{2-}$.

It is possible that the fungi possess a specific thiosulfate permease in addition to the sulfate–thiosulfate–selenate–molybdate permease. However, considering the high K_m for $S_2O_3^{2-}$ transport in the sulfate permease-negative mutant, this seems unlikely. A more reasonable explanation is that the fungi possess at least one other permease for Group VI anions with a high affinity for its preferred substrate and a low affinity for $S_2O_3^{2-}$. An attempt was made to determine the number of distinct permeases that fungi possess for the most common forms of inorganic sulfur anions found in nature.

As shown in Table III, *P. notatum* will accumulate SO_4^{2-}, $S_2O_3^{2-}$, SO_3^{2-}, $S_4O_6^{2-}$ and S^{2-}, but not CNS^-. From this it is apparent that *P. notatum* possess at least one other sulfur anion permease in addition to the sulfate permease. This is

shown by the ability of the sulfate permease-negative mutant to transport SO_3^{2-} and $S_4O_6^{2-}$. The results in Table II show that SO_4^{2-}, $S_2O_3^{2-}$ and $S_4O_6^{2-}$ transport are regulated by the degree of sulfur sufficiency of the mycelium. The dependence of SO_3^{2-} transport on the sulfur nutritional status of *P. notatum* has also been demon-

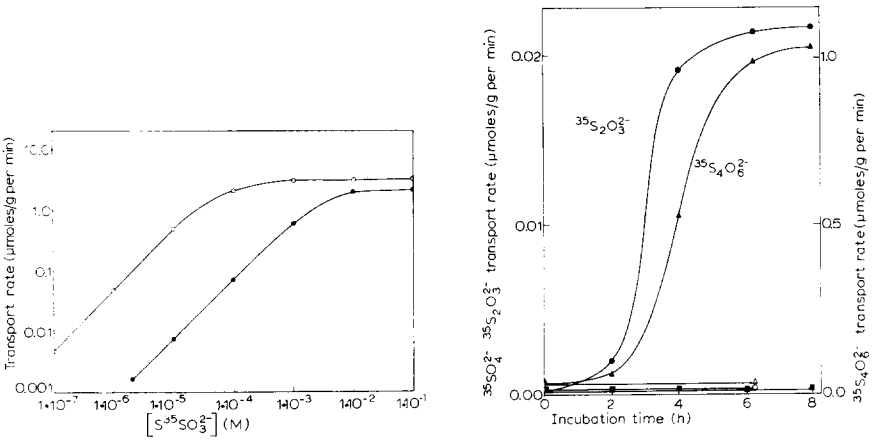

Fig. 3. Concentration dependence of $S_2O_3^{2-}$ transport in *P. notatum*, strains 38632R (○—○) and 38632M (●—●). The organisms were grown for 36 h on citrate No. 3 medium containing 100 mg/l L-djenkolic acid as sole sulfur source.

Fig. 4. Derepression of $S_2O_3^{2-}$ transport during sulfur starvation of *P. notatum* 38632M. The organism was grown for 36 h on citrate No. 3 medium with 1 g/l L-methionine as sole sulfur source. The mycelium was filtered and washed and the SO_4^{2-}, $S_2O_3^{2-}$, and $S_4O_6^{2-}$ transport rates determined. The rest of the mycelium was then divided into two parts. One part (4 g wet wt.) was resuspended in 100 ml of citrate No. 3 containing no sulfur source. Another 4-g portion was suspended in fresh citrate No. 3 with 1 g/l L-methionine. Aliquots were taken from each flask at the times shown and the SO_4^{2-}, $S_4O_6^{2-}$, and $S_2O_3^{2-}$ transport rates determined at an external substrate concentration of 0.1 mM. ●—●, $S_2O_3^{2-}$ transport, *minus* sulfur medium; ○—○, $S_2O_3^{2-}$ transport, methionine medium; ▲—▲, $S_4O_6^{2-}$ transport, *minus* sulfur medium; △—△, $S_4O_6^{2-}$ transport, methionine medium; ■—■, SO_4^{2-} transport, *minus* sulfur and methionine medium.

TABLE III

TRANSPORT OF SULFUR ANIONS BY SULFATE PERMEASE-POSITIVE AND SULFATE PERMEASE-NEGATIVE STRAINS OF *P. notatum*

Sulfur-deficient mycelia of *P. notatum*, 38632M and 38632R were grown for 2 days in synthetic citrate No. 3 medium containing L-djenkolic acid (100 mg/l) as sole sulfur source. The transport rates were determined by Method II (ref. 3).

Anion (*0.1 mM*)	*Transport rate* (*μmoles/g per min*)	
	Strain 38632R	*Strain 38632M*
$^{35}SO_4^{2-}$	3.20	0.00
$^{35}S_2O_3^{2-}$	1.96	0.04
$^{35}SO_3^{2-}$	*	0.25
$^{35}S_4O_6^{2-}$	1.15	1.09
$^{35}S^{2-}$	4.40	3.20
$CN^{35}S^-$	0.00	0.00

* We were unable to measure SO_3^{2-} transport in strain 38532R because of the presence of contaminating $^{35}SO_4^{2-}$ in the $^{35}SO_3^{2-}$.

strated. S^{2-} uptake, however, is independent of the sulfur nutritional status of the organism. S^{2-} uptake is 2,4-dinitrophenol- and azide-sensitive but shows a lower temperature-dependence (Q_{10} of 1.15) than that shown for SO_4^{2-} transport. The concentration-dependence of S^{2-} uptake was determined over the range of external substrate concentration from 1 μM to 10 mM. This system does not appear to be saturable (at least up to 10 mM external substrate). S^{2-} transport was a linear function of external concentration over the range studied but the slope of the velocity *versus* concentration curve was less than that expected for a first-order-dependence on concentration. The lack of regulation, together with the low Q_{10} and the anomalous kinetic behavior of S^{2-} uptake suggests that a carrier-mediated process may not be involved in S^{2-} uptake. These factors distinguish S^{2-} uptake from the sulfate permease and the one (or more) permeases involved in SO_3^{2-}, $S_2O_3^{2-}$ and $S_4O_6^{2-}$ transport. $S_4O_6^{2-}$ transport obeys saturation kinetics ($K_m = 25$ μM, $v_{max} = 3$ μmoles/g per min).

In order to further document the number and specificity of sulfur anion permeases, reciprocal inhibition studies were carried out. The results are shown in Tables IV and V. Unfortunately many combinations of labeled substrate *plus* unlabeled potential inhibitor could not be tested because of nonenzymatic exchange and oxidation–reduction reactions that occur between several of the sulfur anions. As described earlier, SO_4^{2-} and $S_2O_3^{2-}$ show reciprocal inhibition of transport in the sulfate (thiosulfate) permease-positive revertant. However, $S_2O_3^{2-}$ transport in the sulfate permease-negative mutant is SO_4^{2-} insensitive. SO_4^{2-} transport in the revertant is not inhibited by $S_4O_6^{2-}$. Nor is $S_4O_6^{2-}$ transport in both the revertant and mutant inhibited by SO_4^{2-}. SO_3^{2-} and S^{2-} show reciprocal inhibition. Thus S^{2-} could conceivably be a substrate of a sulfite permease, but not *vice versa* (otherwise, SO_3^{2-} transport would not depend on the degree of sulfur sufficiency of the mycelium). It would appear then that the mechanisms by which SO_3^{2-} and S^{2-} are taken up by the mycelium are different. Neither SO_3^{2-} nor S^{2-} inhibit $S_2O_3^{2-}$ transport in either strain. Nor does SO_3^{2-} inhibit $S_4O_6^{2-}$ transport in either strain. These results suggest the existence of distinct permeases for SO_4^{2-} ($S_2O_3^{2-}$, *etc.*), SO_3^{2-}, $S_4O_6^{2-}$, and (possibly) S^{2-}. The results also suggest again that we were not observing the transport of contaminating SO_3^{2-} or S^{2-} from solutions of labeled $S_2O_3^{2-}$.

TABLE IV

RECIPROCAL INHIBITION OF SULFUR ANION TRANSPORT IN *P. notatum* 38632R

Sulfur-deficient mycelium was prepared and the transport rate determined by Method II (ref. 3). SO_3^{2-} transport cannot be accurately determined in the revertant because of contaminating SO_4^{2-} in the substrate.

Substrate (*0.1 mM*)	*Inhibition* (%) *caused by following anions* (*1 mM*)				
	SO_4^{2-}	SO_3^{2-}	S^{2-}	$S_2O_3^{2-}$	$S_4O_6^{2-}$
$^{35}SO_4^{2-}$	—	37*	18	95	0
$^{35}S^{2-}$	0	62	—	0	***
$^{35}S_2O_3^{2-}$	79	4	4	—	**
$^{35}S_4O_6^{2-}$	0	0	***	**	—

* Uncorrected for possible contaminating SO_4^{2-} in SO_3^{2-} inhibitor solution.

** Undeterminable because of the exchange reaction:
$^{35}S_4O_6^{2-} + {}^{32}S_2O_3^{2-} \rightleftharpoons {}^{35}S_2O_3^{2-} + {}^{32}S_4O_6^{2-}$

*** Undeterminable because S^{2-} is oxidized by $S_4O_6^{2-}$ to yield elemental sulfur.

Artifacts of $S_2O_3^{2-}$ transport

In the preceding experiments, $S_2O_3^{2-}$ transport was measured at pH 6.0 over a 2-min interval. The short incubation time and pH was chosen to minimize complications that could arise from (a) departure of transport from linearity over longer periods, (b) metabolism of the transported ion to a feedback inhibitor of the permease[1,2], (c) feedback inhibition of the permease by high intracellular concentrations of the transported ion itself (I. H. SEGEL, unpublished results), and (d) decomposition of the $S_2O_3^{2-}$ to other sulfur anions[7]. Fig. 5 shows the time-course of $S_2O_3^{2-}$ transport by strain 38632M over a 9-h period. It can be seen that the rate of ^{35}S transport increased markedly as the pH of the incubation medium dropped. The increased rate did not result from an induction of a thiosulfate permease because mycelia preincubated under identical conditions in the presence of unlabeled $S_2O_3^{2-}$ showed the usual rate of $S_2O_3^{2-}$ transport when assayed for 2 min in fresh pH 6.0 buffer containing labeled $S_2O_3^{2-}$. The increased rate shown in Fig. 5 very likely results from the slow decomposition of $S_2O_3^{2-}$ to more rapidly transported compounds. This result points

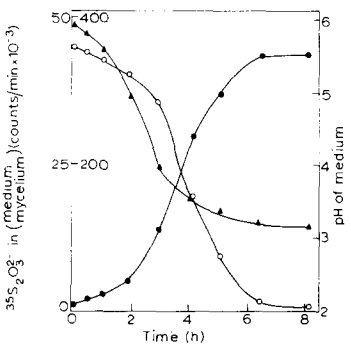

Fig. 5. $S_2O_3^{2-}$ transport by *P. notatum*, strain 38632M over a long incubation period. Standard assay conditions were used except that the buffer was supplemented with 4% glucose. The initial substrate concentration was 0.2 mM $Na_2{}^{35}SSO_3$. Almost identical results were obtained with 0.1 mM $Na_2S^{35}SO_3$. The results are expressed as counts/min per 0.5 ml of medium after filtration (○—○), or as counts/min in the mycelium from 5 ml of the suspension (●—●). ▲—▲, pH of the medium.

TABLE V

RECIPROCAL INHIBITION OF SULFUR ANION TRANSPORT IN *P. notatum* 38632M

The experimental details were the same as those described in Table IV.

Substrate (0.1 *mM*)	*Inhibition* (%) *caused by following anions* (1 *mM*)				
	SO_4^{2-}	SO_3^{2-}	S^{2-}	$S_2O_3^{2-}$	$S_4O_6^{2-}$
$^{35}SO_3^{2-}$	0	—	75	0	15
$^{35}S^{2-}$	0	63	—	0	**
$^{35}S_2O_3^{2-}$	0	0	17	—	*
$^{35}S_4O_6^{2-}$	0	0	**	*	—

* Undeterminable because of the exchange reaction:
$^{35}S_2O_3^{2-} + S_4O_6^{2-} \rightleftharpoons {}^{35}S_4O_6^{2-} + S_2O_3^{2-}$

** Undeterminable because S^{2-} reacts with $S_4O_6^{2-}$ to yield sulfur.

out the dangers inherent in long-term biological experiments involving potentially unstable sulfur compounds[7].

DISCUSSION

The SO_4^{2-} transport system in some species of Aspergillus and Penicillium is capable of transporting SO_4^{2-}, $S_2O_3^{2-}$, SeO_4^{2-}, and MoO_4^{2-} at about the same rates. Selenium is not considered to be an effective replacement for sulfur in the sulfur amino acids. HUBER *et al.*[8] have shown that SeO_4^{2-} will not completely replace SO_4^{2-} in growth media for a SeO_4^{2-}-tolerant strain of *Escherichia coli*. These authors have also shown[9] that when selenium replaces sulfur in the sulfur-containing amino acids the properties of these acids are changed. Proteins synthesized from the selenium-substituted amino acids are unlikely to be biologically active. WILSON AND BANDURSKI[10] have also shown that the product of yeast ATP sulfurylase is unstable when MoO_4^{2-} or SeO_4^{2-} are used as a substrate so it is not possible for these compounds to enter the general sulfur metabolite pool. The competitive inhibition of SO_4^{2-} transport by SeO_4^{2-} and the instability of adenosine 5′-phosphoselenate could provide an explanation for the known toxicity of high concentrations of SeO_4^{2-} (ref. 11).

We are not sure whether the transport of MoO_4^{2-} by the SO_4^{2-} transport system is physiologically significant. Molybdenum is a component of nitrate reductase, which is induced in fungi grown in the presence of NO_3^-. Since no MoO_4^{2-} transport *via* the sulfate permease could be detected below pH 5.0, it may be necessary for the fungi to have some other means of accumulating molybdenum at low pH levels. The fact that MoO_4^{2-} transport by the SO_4^{2-} system is controlled by the level of sulfur nutrition of the organism could also be disadvantageous to the fungi. Under conditions of adequate sulfur nutrition the sulfate permease is repressed and SO_4^{2-} transport is very slow. However, even under these conditions of low pH and adequate sulfur nutrition MoO_4^{2-} transport could still conceivably be rapid enough to supply the organism with the very small amounts of molybdenum required.

$S_4O_6^{2-}$ is a product of $S_2O_3^{2-}$ oxidation by the thiobacilli[12] and is likely to comprise a considerable proportion of the sulfur sources in an environment populated by these aerobes. Consequently, the transport of this metabolite by filamentous fungi could provide an important sulfur source for these organisms. The fungi we have tested can utilize $S_4O_6^{2-}$ as the sole sulfur source.

The transport of $S_2O_3^{2-}$ by the SO_4^{2-} transport system could have physiological significance since $S_2O_3^{2-}$ is produced by some anaerobic S^{2-}-utilizing bacteria[12]. This $S_2O_3^{2-}$ could then diffuse to a more aerobic environment where it could be utilized by filamentous fungi. The transport of $S_2O_3^{2-}$ by the sulfate permease-negative mutant is difficult to explain. SPENCER *et al.*[13] also noted that a sulfate permease-negative mutant of *A. nidulans* would grow well on $S_2O_3^{2-}$. These authors attribute $S_2O_3^{2-}$ uptake in the mutant to either a defective sulfate permease that is "leaky" for $S_2O_3^{2-}$, or to rapid diffusion of $S_2O_3^{2-}$ through the mycelial membrane. Free diffusion seems unlikely because (with our mutant, at least) $S_2O_3^{2-}$ transport shows saturation kinetics (Fig. 3) has a Q_{10} of 2, and is clearly under metabolic control (Fig. 4). The question then is what permease is responsible for $S_2O_3^{2-}$ transport in the sulfate permease-negative mutant? The simplest answer is that fungi possess a specific thiosulfate permease in addition to the sulfate–thiosulfate permease. The v_{max}

values of the two permeases are quite similar (Fig. 3). Consequently, the specific permease, because of its higher K_m value, would be undetectable in sulfate–thiosulfate permease-positive organisms. Another possibility is that $S_2O_3^{2-}$ transport in sulfate–thiosulfate permease-negative mutants is mediated by an organic thiosulfate permease. *P. chrysogenum*, for example, has been shown to transport cysteine-*S*-sulfate *via* a sulfur-regulated, saturable permease[14]. However, this permease has not been thoroughly characterized and it may, in fact, be the cystine permease (G. E. SKYE and I. H. SEGEL, unpublished results). A third possibility is that the defective sulfate–thiosulfate permease is indeed leaky for $S_2O_3^{2-}$, but not for SO_4^{2-}. Such leakiness might occur if the mutant lacks a binding protein component of the permease. If $S_2O_3^{2-}$, but not SO_4^{2-} can react directly with a (hypothetical) "transporter" portion of the permease, then $S_2O_3^{2-}$ could conceivably be transported with the same ultimate v_{max}. If the binding protein functions as a concentrating agent, then it would be reasonable to expect a significantly higher K_m value for $S_2O_3^{2-}$ transport in the absence of the binding protein. $S_2O_3^{2-}$ transport would also be relatively SO_4^{2-} insensitive. A fourth possibility is that $S_2O_3^{2-}$ is oxidized (to $S_4O_6^{2-}$ or SO_3^{2-} *plus* SO_4^{2-}) or reduced (to SO_3^{2-} and S^{2-}) by a surface enzyme. The reaction products might then react directly with their respective transporter molecules, bypassing the binding proteins. Because the binding proteins seem to be the sites of competitive inhibition[15], $S_2O_3^{2-}$ transport by this process would be unaffected by external SO_4^{2-}, SO_3^{2-}, *etc.* A final and most likely explanation is that $S_2O_3^{2-}$ is transported by one of the other Group VI anion permeases, but with a greatly reduced affinity. The tetrathionate permease would be a likely candidate for this role. $S_4O_6^{2-}$ transport by the sulfate permease-negative mutant is derepressible and insensitive to SO_4^{2-}.

Although the tetrathionate permease may transport $S_2O_3^{2-}$ into sulfate permease-negative mutants under our assay conditions, growth on $S_2O_3^{2-}$ may not involve the tetrathionate permease to any great extent. In most fungal cultures the pH frequently drops below 5.5. $S_2O_3^{2-}$ is unstable at pH values much below 6.0. Consequently, it is likely that a large portion of the sulfur that mycelia incorporate from $S_2O_3^{2-}$-containing media (over long incubation periods) is not $S_2O_3^{2-}$, but rather breakdown products such as SO_3^{2-} and S^{2-}.

ACKNOWLEDGMENTS

This work was supported by United States Public Health Service Research Grant GM-12292 and National Science Foundation Research Grants GB-5376 and GB-7736.

We greatly appreciate the technical assistance of Miss Trudy Wood, Miss Marilyn Holby, Miss Gretchen Bradfield, and Miss Tina Meyn is various phases of this work.

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