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## SODIUM-DEPENDENT UPTAKE OF TAURINE IN ENCAPSULATED *STAPHYLOCOCCUS AUREUS* STRAIN M

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A novel uptake system for the unusual sulfonated amino acid taurine was discovered in the prokaryote, encapsulated *Staphylococcus aureus* strain M. This strain has been shown previously to contain taurine in its capsular polysaccharide. Taurine uptake by whole cells incubated in buffer showed a saturable dependency upon  $\text{Na}^+$  and taurine uptake was itself a saturable process, stimulated by glucose, and markedly affected by temperature. No evidence was found for the inducibility of taurine uptake. In the presence of 10 mM NaCl Lineweaver-Burk plots revealed a  $K_m$  of 42  $\mu\text{M}$  and  $V_{\max}$  of 4.6 nmol/min per mg dry weight for taurine uptake at 37°C. Increasing concentrations of  $\text{Na}^+$  decreased the  $K_m$  of the system and appeared to increase the  $V_{\max}$ . Of various other cations tested only  $\text{Li}^+$  supported marked taurine uptake. Excess unlabelled taurine did not cause efflux of radioactivity taken up. Taurine was taken up into cold trichloroacetic acid-soluble material and did not chromatograph as taurine, indicating rapid metabolism during or closely following uptake. Taurine uptake appeared to occur via a highly specific system because amino acids representing the major known groups of amino acid transport systems in *S. aureus* did not inhibit taurine uptake, and uptake was only slightly diminished by the structurally closely related compounds hypotaurine and 3-amino-1-propane sulfonic acid. Sulfhydryl group reagents, electron transport inhibitors, an uncoupler and inhibitors of  $\text{Na}^+$ -linked transport processes inhibited taurine uptake. A variety of other metabolic inhibitors had little effect on taurine uptake.

### Introduction

Taurine (2-aminoethanesulfonate), although unusual in prokaryotes, has been reported to be a component of the encapsulated *Staphylococcus aureus* M strain capsular polysaccharide [1,2]. Smiley and Wilkinson [3] have recently shown that radiolabelled taurine added to growth medium was taken up by a variety of encapsulated and unencapsulated *S. aureus* strains during overnight growth. In the encapsulated M strain most of the

radioactivity taken up was localized in a cold trichloroacetic acid-soluble, i.e. pool metabolites, fraction in a form other than taurine. Some incorporation into capsular polysaccharide material occurred, but none into other cellular macromolecules.

It was of interest to study the way in which taurine was taken up by this organism. Here we present a characterization of the taurine uptake system. Taurine appears to be taken up by an  $\text{Na}^+$ -dependent system, highly specific for taurine, distinct from previously described amino acid transport systems in *S. aureus*.

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Abbreviations: HOQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; CCCP, carbonyl cyanide *m*-chlorophenylhydrazine; DCCD, *N,N'*-dicyclohexylcarbodiimide.

crobiology, New Orleans, LA, March 1983 (Bieber, E.J. and Wilkinson, B.J. (1983) Abstr. Annu. Meet. Am. Soc. Microbiol. 1983 K 116, p. 196).

## Methods and Materials

### *Organism, growth conditions and preparation of cell suspensions*

The encapsulated *S. aureus* M strain was used in the experiments [4] and was generally grown in Phytone peptone-yeast extract- $K_2HPO_4$ -glucose medium [5] which appears not to contain taurine because it was undetectable by amino acid analysis [3,5]. In experiments investigating the inducibility of taurine uptake the organism was also grown in Brain Heart Infusion broth (Difco Laboratories, Detroit, MI, U.S.A.), which contains taurine at a level of about 0.3 mM when constituted [3,5]. Cultures (100 ml) were initiated with a 1% (v/v) inoculum from an overnight culture and were grown with shaking (200 rpm) at 37°C for about 3.5 h to an  $A_{580\text{nm}}$  of about 1.0 (exponential phase).

Cells were harvested at  $15380 \times g$  for 15 min at 4°C and were washed once by resuspension and centrifuging in cold 0.1 M Tris-HCl buffer, pH 7.0. If the  $A_{580\text{nm}}$  of the culture was 1.0 the pellet was resuspended in 5 ml 0.1 M Tris-HCl, pH 7.0, otherwise proportional adjustments were made, and was kept on ice for uptake studies. The dry weight of the suspension was determined by diluting a sample of the suspension and reading off against a previously prepared  $A_{580\text{nm}}$  vs. dry weight calibration curve.

### *Uptake studies*

The basic design of uptake assays was after that of Kaback [6]. A typical assay mixture contained 50 mM Tris-HCl, pH 7.0, 20 mM glucose, 10 mM NaCl, 11.7  $\mu\text{M}$  (0.01  $\mu\text{Ci}$ ) [ $1,2\text{-}^{14}\text{C}$ ]taurine (spec. act. 56.08 mCi  $\cdot$  mmol $^{-1}$ , New England Nuclear, Boston, MA, U.S.A.), bacteria (about 215  $\mu\text{g}$  dry weight) in a final volume of 100  $\mu\text{l}$  in  $10 \times 75$  mm test tubes. Assays were carried out in duplicate, generally at 37°C, and uptake was usually initiated by adding bacteria which had been prewarmed at 37°C for 5 min. Tubes were incubated stationary since no difference in uptake was noted when the tubes were shaken. When the effects of inhibitors were being studied the cells were preincubated

with the inhibitors in assay mixtures for 5 min at 37°C and uptake was initiated by addition of [ $^{14}\text{C}$ ]taurine. Uptake was terminated by dilution of the assay mixture with 2.5 ml of room temperature (22°C) 0.05 M Tris-HCl, pH 7.0 and rapid vacuum filtration on a prewetted Millipore type HA, 0.45  $\mu\text{m}$  filter (Millipore Corp., Bedford, MA, U.S.A.) in a multiple manifold apparatus (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.). The assay tube was rinsed twice with 2.5 ml 0.05 M Tris-HCl, pH 7.0 which was also used to wash the filter. Radioactivity was determined by placing filters in 10 ml Scinti Verse I cocktail (Fisher Scientific Co., Fairlane, NJ, U.S.A.) and counting them in a Beckman LS-8100 scintillation counter (Beckman Instruments Inc., Fullerton, CA, U.S.A.). Initial uptake rates were determined after 1 min of incubation.

### *Preparation of cold trichloroacetic acid-soluble extract and its chromatographic analysis*

Cells were allowed to take up [ $^{14}\text{C}$ ]taurine for 15 min at 37°C and then were collected by vacuum filtration. Filters were cut up and were extracted with 10% (w/v) trichloroacetic acid for 15 min at 0°C. The cold trichloroacetic acid extract was extracted five times with ether to remove trichloroacetic acid and was then lyophilized. The residue and standard [ $^{14}\text{C}$ ]taurine were subjected to chromatographic analysis as described by Smiley and Wilkinson [3].

### *Reagents*

Inhibitors and amino acids were purchased from Sigma Chemical Co., Saint Louis, MO, U.S.A. When necessary inhibitors were dissolved in 95% (v/v) ethanol and control assay mixtures were constituted with an equal volume of ethanol when these were being tested.

## Results

### *Establishment of experimental conditions for studying taurine uptake*

In initial studies our intent was to use experimental conditions for studying uptake similar to those described by Short and Kaback [7] in their studies of amino acid transport in *S. aureus*. Taurine uptake by the M strain was very poor in

cells suspended in 0.05 M potassium phosphate, pH 7.3 containing 20 mM glucose. In an experiment to investigate the effect of azide on uptake it was found that, contrary to expectations, taurine uptake was significantly stimulated by 10 mM sodium azide. Marked uptake was noted in 0.05 M sodium phosphate buffer. This suggested that taurine uptake might be an  $\text{Na}^+$ -stimulated or -dependent system. Accordingly, the buffer system was changed from potassium phosphate to Tris-HCl to avoid potential complications involving  $\text{K}^+$ .

Fig. 1 shows the effect of NaCl (10 mM) on taurine uptake. In the absence of NaCl there was little uptake of taurine indicating that the uptake system was  $\text{Na}^+$ -dependent. Uptake in the presence of NaCl (10 mM) was further stimulated by the presence of glucose (20 mM) (Fig. 2). Virtually no uptake of taurine occurred under these conditions in the presence of KCl (10 mM) in the place of NaCl (data not shown; see Table I). In the presence of 0.1 mM NaCl and KCl and 1 mM NaCl and KCl the rates of uptake were 94 and 81.9%, respectively, of the rates in the presence of NaCl alone. In the presence of NaCl and glucose the rate of uptake was linear for at least a minute (Fig. 2). Virtually all of the taurine in the assay mixture had been taken up by 15 min. The results indicate that endogenous metabolism was able to support some taurine uptake, but that this was augmented by the exogenous energy source glucose.

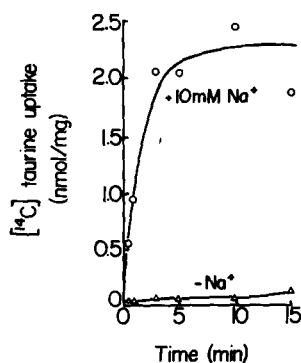


Fig. 1. Effect of NaCl on taurine uptake. Taurine uptake activity was assayed in the presence of  $11.7 \mu\text{M}$  taurine as described in Materials and Methods. Glucose was omitted from the assay mixture.

TABLE I

EFFECT OF VARIOUS CATIONS ON THE INITIAL RATE OF TAURINE UPTAKE

10 and 100 mM concentrations of various cations were included in the assay mixture and uptake was determined in the presence of  $11.7 \mu\text{M}$  taurine, 10 mM NaCl and 20 mM glucose as described in Materials and Methods.

Cation concn. (mM)	Taurine uptake (nmol/min per mg dry weight)	% of control
None	0.25	100
NaCl 10	0.29	238
100	1.02	408
KCl 10	0.10	40
100	0.03	12
LiCl 10	0.39	156
100	0.73	292
RbCl 10	0.11	44
100	0.09	32
CsCl 10	0.10	40
100	0.06	24
$\text{NH}_4\text{Cl}$ 10	0.08	32
100	0	0
$\text{CaCl}_2$ 10	0.17	68
100	0.36	144
$\text{MgCl}_2$ 10	0.07	28
100	0.03	12

Effect of various concentrations of NaCl on taurine uptake

This is shown in Fig. 3. NaCl concentrations up

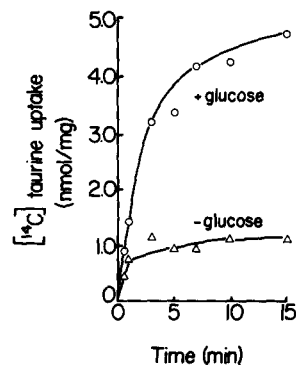


Fig. 2. Effect of glucose on taurine uptake. Taurine uptake activity in the presence of  $11.7 \mu\text{M}$  taurine and 10 mM NaCl was assayed as described in Materials and Methods. When present, glucose was at a concentration of 20 mM.

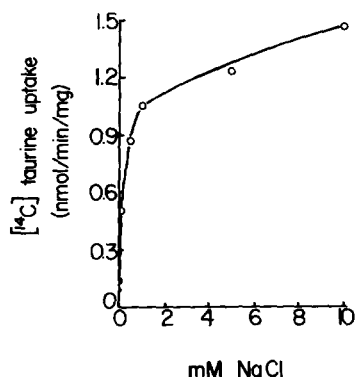


Fig. 3. Effect of NaCl concentration on the initial rate of taurine uptake. Taurine uptake activity in the presence of 11.7  $\mu$ M taurine and 20 mM glucose was assayed as described in Materials and Methods in assay mixtures constituted with varying amounts of NaCl.

to 1 mM caused large increases in taurine uptake, and an NaCl concentration of 10 mM appeared to be approaching a saturating concentration.

#### Effect of various cations on taurine uptake

Various cations, at 10 and 100 mM concentrations, were tested for their ability to stimulate the initial rate of taurine uptake (Table I). Besides NaCl only LiCl markedly stimulated taurine uptake. 100 mM  $\text{CaCl}_2$  showed slight stimulation

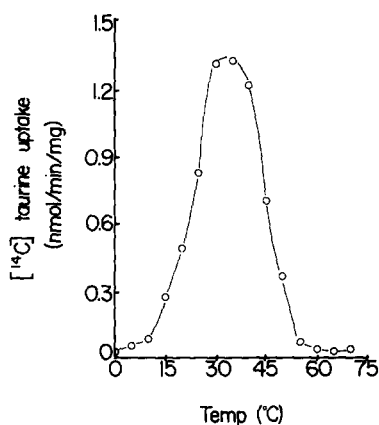


Fig. 4. Effect of temperature on the initial rate of taurine uptake. Cell suspensions in assay mixtures constituted without taurine were preincubated at the various temperatures for 5 min. Uptake in the presence of 11.7  $\mu$ M taurine, 10 mM NaCl and 20 mM glucose was initiated by adding taurine and was measured as described in Materials and Methods.

whereas other cations had no effect or appeared to be inhibitory

#### Effect of temperature on taurine uptake

This is shown in Fig. 4. Taurine uptake was markedly affected by temperature with an optimum about 37°C after which uptake decreased rapidly, possibly due to inactivation of the uptake system. There was negligible taurine uptake at 0°C indicating little non-specific binding of taurine to cells was occurring.

#### Effect of pH on taurine uptake

pH values between 6.0 and 8.0 had little effect upon the initial rate of taurine uptake. The initial rates of uptake at pH values of 6, 7 and 8 were 0.47, 0.50 and 0.50 nmol/min per mg, respectively.

#### Kinetic parameters of taurine uptake

Increasing concentrations of NaCl increased the affinity of the uptake system for taurine (Fig. 5).  $K_m$  values obtained in the presence of 0.1, 1 and 10 mM NaCl were 122, 53 and 42  $\mu$ M, respectively. Corresponding  $V_{max}$  values were 2.7, 2.3 and 4.6 nmol/min per mg dry weight, indicating that  $\text{Na}^+$  also affected the capacity of the system.

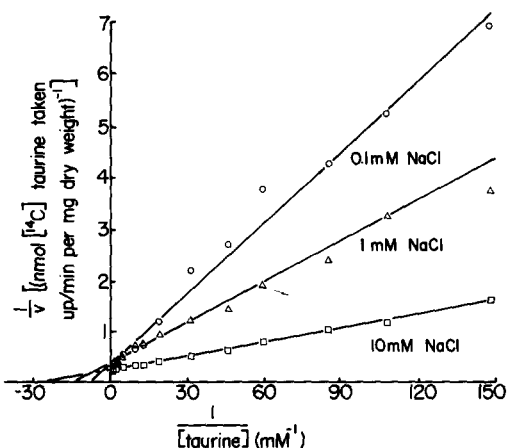


Fig. 5. Lineweaver-Burk plots of taurine uptake in the presence of various concentrations of NaCl. Taurine uptake activity was assayed as described in Materials and Methods in the presence of 11.7  $\mu$ M taurine, 20 mM glucose and various concentrations of NaCl.  $K_m$  and  $V_{max}$  values were calculated using a computer program for a least-squares regression analysis.

### Investigations of the inducibility of taurine uptake

Peptone-yeast extract- $K_2HPO_4$ -glucose medium does not contain taurine yet cells grown under these conditions take up taurine efficiently. The effects of taurine on the possible inducibility of higher levels of taurine uptake were tested by growing the cells in the presence and absence of various concentrations of taurine in the presence and absence of added NaCl, and in Brain Heart Infusion broth which contains taurine. None of these conditions led to an enhanced rate of taurine uptake, indicating that taurine uptake is a constitutive property of this strain.

### Identity of radioactivity taken up

(a) *Failure to cause efflux of radioactivity taken up.* Cells were allowed to accumulate  $[1,2-^{14}C]$ taurine, then 10 mM (1000-fold excess) unlabelled taurine was added to the cell suspension at the point indicated by the arrow (Fig. 6). Small amounts of radioactivity were lost from the cells indicating that the taurine taken up was not freely exchangeable with external taurine.

(b) *Characteristics of radioactivity taken up.* Cells were allowed to take up  $[1,2-^{14}C]$ taurine as previously described. A parallel set of incubations was carried out where 1 ml cold 10% (w/v) trichloroacetic acid was added to the samples instead of their being filtered. The samples were maintained on ice for at least 15 min and were then filtered,

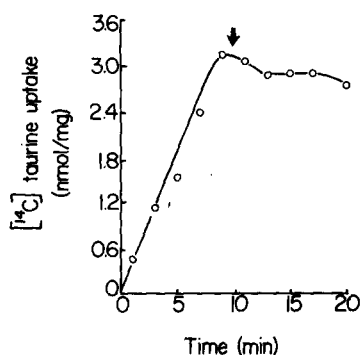


Fig. 6. Failure of excess unlabelled taurine to cause efflux of radioactivity taken up. Cells were allowed to take up taurine in the presence of 11.7  $\mu$ M taurine, 10 mM NaCl and 20 mM glucose as described in Materials and Methods then unlabelled taurine was added to a final concentration of 10 mM at the point indicated by the arrow.

the filters washed with cold trichloroacetic acid and then counted. The results of this experiment are shown in Table II. More than 95% of the radioactivity was taken up into a trichloroacetic acid-soluble form. This is compatible with our previous studies [3] where 90% of radioactivity taken up after overnight growth was present in a trichloroacetic acid-soluble form with only 10% in a cold trichloroacetic acid-insoluble, hot trichloroacetic acid-soluble (capsular polysaccharide) form.

The cells were allowed to take up taurine for 15 min and trichloroacetic acid-soluble radioactivity was subjected to chromatographic analysis (Fig. 7). In three systems the majority of the radioactivity did not co-chromatograph with authentic taurine indicating that most of the  $[1,2-^{14}C]$ taurine taken up had been metabolized to another compound either during or after uptake. In two of the systems a small peak chromatographing in the position of  $[1,2-^{14}C]$ taurine was detected.

### Effect of amino acids and compounds structurally related to taurine on taurine uptake

Glycine, L-alanine, L-valine, L-serine, L-glutamic acid, L-glutamine, L-lysine, L-arginine, L-phenyl-

TABLE II

UPTAKE OF TAURINE INTO WHOLE CELLS AND COLD TRICHLOROACETIC ACID-PRECIPITABLE MATERIAL

(a) Taurine uptake into whole cells was measured in the presence of 11.7  $\mu$ M taurine, 10 mM NaCl and 20 mM glucose as described in Materials and Methods. (b) At the same time cold trichloroacetic acid was added to a parallel set of samples which were allowed to sit on ice for at least 15 min. Trichloroacetic acid-precipitable material was collected by vacuum filtration and was washed with cold 10% (w/v) trichloroacetic acid.

Time (min)	Taurine uptake (nmol/mg dry weight)		% uptake into (b)
	(a) whole cells	(b) cold trichloroacetic acid-precipitable material	
1	0.31	0.04	12.9
3	0.82	0.03	3.7
5	1.41	0.03	2.1
10	2.43	0.04	1.6
15	3.03	0.06	2.0

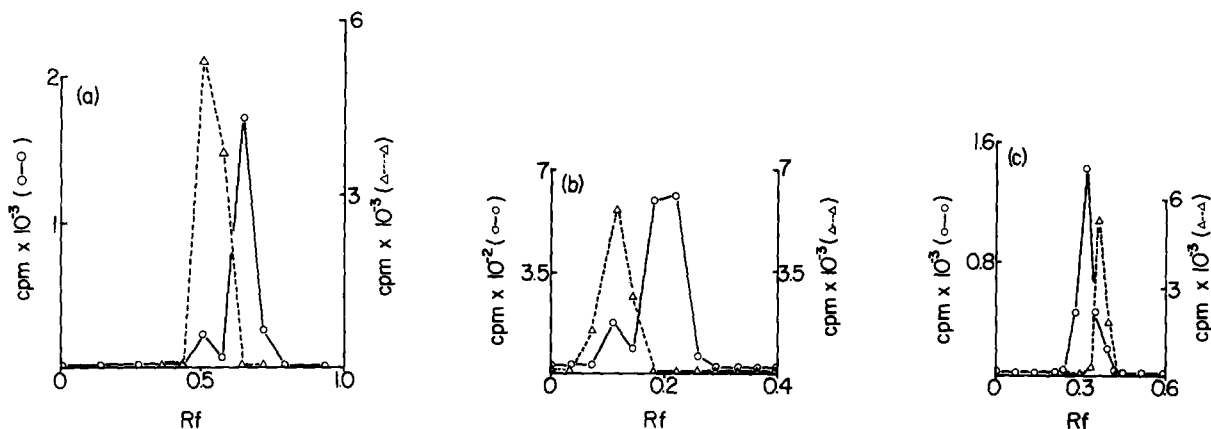


Fig. 7. Chromatographic analysis of cold trichloroacetic acid-soluble radioactivity. The cold trichloroacetic acid-soluble fraction was prepared and chromatographed as described in Materials and Methods. In all cases  $\circ$ — $\circ$  represents the cold trichloroacetic acid-soluble radioactivity and  $\triangle$ --- $\triangle$  standard  $[1,2-^{14}\text{C}]$ taurine. (a) TLC, solvent 95% ethanol/water (67:33, v/v); (b) paper chromatography, solvent butan-1-ol/acetic acid/water (3:1:1, v/v/v); (c) paper chromatography, solvent propan-2-ol/95% ethanol/water (50:50:15, v/v/v).

alanine, L-cysteine, L-methionine, L-proline, L-histidine, and  $\beta$ -alanine at 1 mM concentrations (100-fold excess) were tested for any inhibitory effects on the initial rate of taurine uptake. None of these amino acids significantly diminished taurine uptake (uptake rates were 93.2–113.6% of control). Under the same conditions uptake in the presence of 1 mM unlabelled taurine was 4.5% of control. These amino acids represent twelve previously documented groups of *S. aureus* amino acid transport systems [7]. This indicates that taurine uptake occurs by a system distinct from these amino acid transport systems. Various other compounds were tested for their effects on taurine uptake (Table III). Only hypotaurine and 3-amino-1-propane sulfonic acid showed significant inhibition.  $\beta$ -Alanine, which is known to inhibit taurine uptake in eukaryotic cells [8], only inhibited taurine uptake by 7%. Taken together these results indicate that taurine uptake occurs by a highly specific system.

#### Effect of various inhibitors on taurine uptake

A variety of inhibitors were tested for their effects on taurine uptake (Table IV). Uptake was not much inhibited by inhibitors of glycolysis (iodoacetate and fluoride) or of ATP formation (arsenate) whereas sulphydryl group reagents inhibited uptake markedly (*N*-ethylmaleimide and

*p*-chloromercuribenzoate). Some electron transport inhibitors showed moderate inhibition (HOQNO, antimycin A) and the uncoupler CCCP showed substantial inhibition. Electron transport inhibitors cyanide and azide and ATPase inhibitor DCCD were not markedly inhibitory. The effects of gramicidin D and monensin on taurine uptake were also studied (Table IV). These agents are known to inhibit  $\text{Na}^+$ -linked transport systems. Consistent with the  $\text{Na}^+$ -dependency of taurine uptake, these agents were found to be inhibitory.

TABLE III

#### EFFECT OF AMINO ACIDS AND OTHER COMPOUNDS ON THE INITIAL RATE OF TAURINE UPTAKE

1 mM concentrations of the various compounds were included in the assay mixture and uptake was determined in the presence of 11.7  $\mu\text{M}$  taurine, 10 mM NaCl and 20 mM glucose as described in Materials and Methods.

Compound	Taurine uptake (nmol/min per mg dry weight)	% of control
None	0.95	100
Taurine	0.11	11.6
Hypotaurine	0.65	68.4
L-Cysteic acid	1.00	105.3
L-Cysteine sulfinic acid	0.98	103.2
$\beta$ -Mercaptoethylamine	1.20	126.3
3-Amino-1-propane sulfonic acid	0.74	77.9

TABLE IV

## EFFECT OF VARIOUS INHIBITORS ON THE INITIAL RATE OF TAURINE UPTAKE

Inhibitors were preincubated with cells at 37°C for 5 min before initiating uptake with [ $^{14}$ C]taurine. Uptake was measured in the presence of 11.7  $\mu$ M taurine, 10 mM NaCl and 20 mM glucose as described in Materials and Methods. Typically uptake in the absence of inhibitors was about 1 nmol/min per mg dry weight.

Inhibitor	Concn. (mM)	% of control
None		100
<i>N</i> -Ethylmaleimide	0.5	85.3
	5.0	22.5
Sodium <i>p</i> -chloro- mercuribenzoate	0.5	5.7
	5.0	1.4
Sodium iodoacetate	0.5	70.5
	5.0	70.9
Sodium arsenate	1.0	78.9
	10.0	80.3
Sodium fluoride	1.0	89.9
	10.0	77.9
Potassium cyanide	1.0	80.5
	10.0	71.3
HOQNO	0.01	75.7
	0.05	53.1
Antimycin A	0.01	82.2
	0.05	59.8
Dinitrophenol	1	87.1
	10	68.0
CCCP	0.01	67.7
	0.10	22.3
DCCD	0.01	85.0
	0.10	78.4
Gramicidin D	1 $\mu$ g/ml	56
	10 $\mu$ g/ml	16.5
Monensin	0.015	11.6
	0.150	10.7

## Discussion

Our previous studies led us to suspect that taurine might enter *S. aureus* M by an uptake system, rather than by simple diffusion [3]. Here we report the discovery of an uptake system for

taurine and describe some of its general characteristics.

Taurine uptake was dependent upon Na<sup>+</sup> and the dependency was a saturable process. Na<sup>+</sup>-dependent or -stimulated transport systems have been described in a wide variety of bacterial species including halophilic, marine and terrestrial bacteria (see Ref. 9 for a review), but this appears to be the first description of an Na<sup>+</sup>-requiring uptake system in *S. aureus*. It is worth noting that staphylococci tolerate relatively high NaCl concentrations, and inclusion of 10% (w/v) NaCl in media is used to make them selective for *S. aureus* [10]. Li<sup>+</sup> was able to replace Na<sup>+</sup> and support taurine uptake, although less effectively. Similar effects of Li<sup>+</sup> have been noted in transport of thiomethyl galactoside in *Salmonella typhimurium* [11]. Other cations were not stimulatory with the possible exception of Ca<sup>2+</sup>.

Glucose stimulated taurine uptake by freshly harvested cells maintained on ice. This suggested that uptake was an energy-requiring process. Taurine uptake was markedly temperature dependent indicating that uptake was not occurring by simple diffusion. Taurine uptake showed saturation kinetics which indicated that uptake was a carrier-mediated process. Increasing concentrations of Na<sup>+</sup> clearly decreased the  $K_m$  of the uptake system and appeared to increase the  $V_{max}$ . In other cases of bacterial Na<sup>+</sup>-dependent uptake systems Na<sup>+</sup> typically affects either the  $K_m$  or the  $V_{max}$  [9]. Hence we believe further kinetic analyses in other strains should be performed in order to make firm conclusions on Na<sup>+</sup> effects on  $V_{max}$ .

The taurine uptake system did not appear to be inducible because cells grown in the presence of taurine showed no increased capacity for taurine uptake. This indicated that the taurine uptake system was constitutively expressed.

Taurine appears to be rapidly metabolized upon its entry into the cell. Excess external unlabelled taurine was unable to cause efflux of radioactivity. Most of the radioactivity taken up was in a trichloroacetic acid-soluble form. Thus little incorporation of radioactivity into trichloroacetic acid-insoluble macromolecules such as capsular polysaccharide had occurred. However, chromatography revealed that most of the radioactivity taken up was not present as taurine. Presumably metabo-

lism of taurine occurs either during uptake as it crosses the membrane, or upon entry into the cytoplasm. Because of this metabolism it is not possible to estimate whether taurine uptake occurs against a concentration gradient. At this time we cannot be more specific than to describe the entry of taurine into *S. aureus* M as occurring via an uptake system. The uptake system we describe may well involve both transport and metabolism.

Taurine uptake does not appear to occur via any of the previously described *S. aureus* amino acid transport systems [7]. Excess unlabelled amino acids did not diminish the rate of taurine uptake. It is noteworthy that none of these transport systems have been reported to be  $\text{Na}^+$ -dependent or -stimulated in *S. aureus*, which further reinforces the idea that taurine uptake occurs through a distinct system. However, the possible dependency on  $\text{Na}^+$  or stimulatory effects of  $\text{Na}^+$  on these systems may well not have been specifically tested. The taurine uptake system appears to be a highly specific one since only slight inhibition of uptake was seen with two structurally closely related molecules, hypotaurine and 3-amino-1-propane sulfonic acid.

Taurine uptake was inhibited substantially by *N*-ethylmaleimide and sodium *p*-chloromercuribenzoate which suggests the involvement of protein SH groups in the process. The inhibitors of glycolysis, iodoacetate and fluoride, had little inhibitory effect on uptake of taurine suggesting glycolysis plays little role in energizing uptake. Arsenate had little inhibitory effect suggesting that ATP itself is not directly involved in energizing uptake. The electron transport chain inhibitors, HOQNO and antimycin A, inhibited taurine uptake significantly suggesting respiration is involved in energizing uptake. However, cyanide and azide which also inhibit electron transport were not potent inhibitors of taurine uptake. The uncoupling agent CCCP markedly inhibited taurine uptake, although another uncoupler dinitrophenol caused little inhibition of uptake. The inhibitory effect of CCCP suggests that the energy coupling of uptake may involve, perhaps indirectly, generation of a proton motive force. DCCD, which inhibits proton translocating ATPase, was not particularly inhibitory suggesting that ATPase activity was not important in energizing taurine uptake.

Uptake of taurine appears to be coupled to an electrochemical gradient of  $\text{Na}^+$ . Gramicidin and monensin which both dissipate the  $\text{Na}^+$  electrochemical gradient inhibited taurine uptake. Perhaps the simplest mechanism to suggest for the energy coupling involved in taurine uptake would be that  $\text{Na}^+$  ions are extruded by antiport for protons. Taurine uptake then occurs via an  $\text{Na}^+$ -dependent cotransport or symport system in response to the  $\text{Na}^+$  gradient and electrical potential. However, we are aware of the variety of  $\text{Na}^+$  extrusion systems that exist in different bacteria [9,12] and more work will be necessary to further define the  $\text{Na}^+$  extrusion system in *S. aureus*.

Studies of the effects of inhibitors on uptake in whole cells always lead to concern about access of the agents to targets in the membrane or cytoplasm. This problem may be further compounded in that the M strain possesses a large negatively-charged capsule surrounding its cell wall. This might influence the uptake of small molecular weight compounds, although pore sizes in the capsule appear to be large [13]. In general taurine uptake seems to be not particularly susceptible to a variety of inhibitors and as such is reminiscent of *N*-acetylglucosamine transport in *Bacillus subtilis* where only partial inhibition of transport was noted with a range of inhibitors [14].

Further elucidation of the characteristics of the uptake system, including the mechanism of energy coupling, should ensue if vesicles capable of taurine uptake were available. Thus far we have been unable to prepare vesicles from the M strain capable of taurine or glycine uptake using a variety of procedures [7,15,16], and taking the precaution of adding protease inhibitors which have been reported to protect an  $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$  in *Streptococcus faecalis* vesicles [17] (Werkmeister, Bieber and Wilkinson, unpublished observations).

An  $\text{Na}^+$ -dependent taurine uptake system seems to be a common property of staphylococci. Such a system was detected in most, but not all, *S. aureus* strains examined, and in a variety of coagulase-negative staphylococcal species (Werkmeister, Bieber and Wilkinson, unpublished observations). Although taurine may be incorporated into surface molecules in a few strains [3], taurine may have a more general role as a staphylococcal nutrient. The natural environment



of staphylococci is the body of warm-blooded animals in which taurine is present in high concentrations [18]. Taurine contains carbon, nitrogen and sulfur and we have speculated previously that taurine might play an important role in the sulfur metabolism of staphylococci [3].

### Acknowledgements

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