Survey of Taurine Uptake and Metabolism in *Staphylococcus aureus*

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Taurine has been reported to be a component of the capsular polysaccharide of the encapsulated M strain of *Staphylococcus aureus*. This led to a study of the uptake and metabolism of [1,2-¹⁴C]taurine in a variety of encapsulated and unencapsulated *S. aureus* strains. Taurine was taken up by all strains studied. A discrepancy between uptake measured as depletion of radioactivity from growth medium and as cell-associated radioactivity suggested that taurine may be catabolized to CO₂ in some strains. In most strains, cell-associated radioactivity was located mainly in cold TCA-soluble (pool metabolites) fractions. About 90% of the cell-associated radioactivity was present in the pool metabolites fraction in the M strain, and about 10% in hot TCA-soluble (nucleic acid–teichoic acid–capsular polysaccharide) fraction. Radioactivity in spent medium and the capsular polysaccharide-containing fraction appeared to be present as taurine in this strain. Radioactivity in the pool metabolites fraction of three of the strains examined did not chromatograph as taurine, indicating that taurine was converted into other cell metabolites. One strain incorporated radioactivity from taurine into cellular macromolecules, thus revealing a heterogeneity of staphylococcal taurine metabolism.

INTRODUCTION

Taurine (2-aminoethane sulphonate) is a β -amino acid that has a wide distribution and abundance in animals (Jacobsen & Smith, 1968). However, there is limited information on the occurrence, function and metabolism of taurine in bacteria. Taurine catabolism has been studied in a few different bacteria (see Shimamoto & Berk, 1979, 1980). Taurine has also been identified as a constituent of bacterial cell surface polymers. Kelly & Weed (1965) reported that taurine was present in the cell walls of a variant, or possibly symbiont, of *Bacillus subtilis*. In the most thoroughly documented example, Liao *et al.* (1974) and Liao & Hash (1977) reported taurine to be a constituent of the capsular polysaccharide of *Staphylococcus aureus* strain M (Melly *et al.*, 1974; Scott, 1969). Also, Ohtomo *et al.* (1980) have reported the presence of taurine in what are referred to as 'cell surface teichoic acid preparations' from encapsulated *S. aureus* strains.

The M strain capsule confers resistance to phagocytosis upon this organism (Melly *et al.*, 1974; Peterson *et al.*, 1978), and the mechanism of this phenomenon has been studied extensively in this strain (Wilkinson *et al.*, 1979*a, b*). In one of these studies (Wilkinson *et al.*, 1979*a*) taurine could not be detected upon analysis of cell walls retaining capsular polysaccharide isolated from the M strain grown in medium free from added taurine. A preliminary study of taurine metabolism (Wilkinson *et al.*, 1981) showed that exogenously

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supplied radiolabelled taurine could be taken up by the M strain, and that most of the radioactivity was extractable by 0.01 M-HCl at 100 °C and was dialysable.

We felt it worthwhile to undertake a study of staphylococcal taurine metabolism in view of the potential importance of taurine as a surface component, and lack of knowledge of the metabolism of a molecule to which staphylococci are commonly exposed in their natural environment, the bodies of warm-blooded animals. In this paper we survey aspects of taurine metabolism in various *S. aureus* strains, with more detailed work on the M strain in particular.

METHODS

Organisms and cultural conditions. The following *S. aureus* strains were used: encapsulated strain M and its unencapsulated derivative M variant (Melly *et al.*, 1974; Wilkinson & Holmes, 1979), encapsulated strain Smith diffuse (Finkelstein & Sulkin, 1958; Hunt & Moses, 1958; Wilkinson & Holmes, 1979), well-known unencapsulated laboratory strain H and its microencapsulated derivative T (Park *et al.*, 1974; Wu & Park, 1971), encapsulated strain NS58D (Yoshida, 1971), and encapsulated coagulase-negative strains 11587 and SA222 (King & Wilkinson, 1981). Although strains 11587 and SA222 are coagulase-negative, DNA-DNA hybridization studies have shown them to be *S. aureus* strains (W. E. Kloos, personal communication). Strain NS58D was kindly provided by Dr C. L. San Clemente, Michigan State University, U.S.A., and strains 11587 and SA222 by Dr W. E. Kloos, North Carolina State University, U.S.A. The strains were grown in either Brain Heart Infusion (BHI) broth (Difco) or PYK medium consisting of (l⁻¹): 5 g Phytone-Peptone (BBL), 5 g Bacto Yeast Extract (Difco) and 3 g K₂HPO₄, pH 7.2. Inocula were 1% (v/v) from overnight cultures. Cultures were grown with shaking (200 r.p.m.) at 37 °C for 18 h.

Uptake and distribution of [1,2-¹⁴C]taurine. Organisms were grown in 25 ml medium in a 50 ml Erlenmeyer flask supplemented with 1.0 µCi [1,2-¹⁴C]taurine (specific activity 56.08 mCi mmol⁻¹; 2.08 GBq mmol⁻¹; New England Nuclear). BHI medium contains about 0.3 mM-*taurine*, whereas taurine was not detected in PYK medium upon amino acid analysis of acid-hydrolysed powders (Wilkinson *et al.*, 1981; R. Greenburg, U.S.D.A. Laboratories, Philadelphia, Pa., U.S.A., personal communication). Unlabelled taurine was added to PYK medium to a concentration of 0.1 mM. Cells were harvested by centrifugation at 13800 g for 10 min at 4 °C and were resuspended in 5 ml distilled water. The cells were fractionated by the procedure of Park & Hancock (1960) as modified by Wilkinson & White (1973). The fractions have been designated as follows (Park & Hancock, 1960): cold TCA-soluble, pool metabolites fraction; ethanol-soluble, lipid fraction; hot TCA-soluble, nucleic acid-teichoic acid-capsular polysaccharide fraction; trypsin-soluble, protein fraction; trypsin-insoluble, peptidoglycan fraction. To determine uptake of radioactivity, duplicate 0.1 ml samples of uninoculated growth medium, spent growth medium and resuspended cells were counted (Saleh & White, 1979). After each extraction in the cell fractionation scheme, the pellets were resuspended in 5 ml H₂O, and duplicate 0.1 ml samples were counted, except for the final peptidoglycan pellet which was resuspended directly in scintillation cocktail. Samples were counted in 10 ml Beckman GP Scintillation Cocktail (Beckman Instruments) in a Beckman LS-8100 scintillation counter.

Identification of radioactivity. Portions (3–5 ml) of fractions containing the greatest amounts of radioactivity, i.e., spent growth medium, cold TCA, and hot TCA-soluble fractions, were dialysed against six changes of 500 ml distilled water in 2 d at 4 °C. The dialysis tubing had a molecular weight cut-off of about 12000. Samples were taken for determination of radioactivity before and after dialysis, and the volume in the dialysis bag was measured after dialysis to obtain total radioactivity.

Fractions were also subjected to paper chromatography and TLC. Samples were chromatographed on Whatman no. 1 paper or Eastman Chromagram Sheets (13255 cellulose without fluorescent indicator; Eastman Kodak). Before chromatography, TCA was removed from the cold and hot TCA-soluble fractions by five extractions with ether. When necessary, fractions were concentrated by lyophilization. The hot TCA-soluble fraction was hydrolysed in 4 M-HCl for 4 h at 105 °C and HCl was removed as described by Wilkinson *et al.* (1978). Unlabelled taurine was used as a standard and was detected by dipping chromatograms in ninhydrin (0.25%, w/v in acetone) followed by heating at 105 °C for 5 min. In addition, [1,2-¹⁴C]taurine plus unlabelled carrier taurine was used as a standard and was detected by counting radioactivity. The solvent systems for paper chromatography included: butan-1-ol/acetic acid/water (3:1:1, by vol.), propan-2-ol/formic acid/water (40:5:5, by vol.), and propan-2-ol/95% ethanol/water (50:50:15, by vol.). TLC solvent systems included: pyridine/ethyl acetate/acetic acid/water (5:5:1:3, by vol.), butan-1-ol/acetic acid/water (6:1:2, by vol.), and 95% ethanol/water (67:33, v/v).

After chromatography, the dried chromatograms were cut into strips 25 mm wide surrounding the origin and extending in 12.5 mm increments. The strips were placed in scintillation vials, moistened with 0.1 ml distilled water and left at room temperature overnight to dissolve material on the chromatogram. Scintillation cocktail was then added and radioactivity determined.

Table 1. *Uptake of [1,2-¹⁴C]taurine by S. aureus strains*

The values represent the average of two experiments with strains M, NS58D and SA222 and are the results of one experiment with the other strains.

Strain	10 ⁻⁶ × Radioactivity (c.p.m.)			Percentage uptake calculated using values for:	
	Uninoculated medium	Spent medium	Harvested cells	Spent medium	Harvested cells
M (encapsulated)	2.28	1.56	0.34	31	15
M variant (unencapsulated)	2.31	1.37	0.45	41	19
H (unencapsulated)	2.31	1.89	0.19	18	8
T (microencapsulated)	2.08	1.68	0.38	19	18
Smith diffuse (encapsulated)	2.18	0.98	0.69	55	32
NS58D (encapsulated)	2.64	0.47	1.00	82	38
11587 (encapsulated)	2.40	0.41	0.19	83	8
SA222 (encapsulated)	2.32	1.86	0.11	20	5

RESULTS

Uptake of [1,2-¹⁴C]taurine by various S. aureus strains

The organisms were grown overnight in PYK medium in the presence of [1,2-¹⁴C]taurine and radioactivity taken up was determined by (i) the difference between uninoculated and spent medium, and (ii) direct counting of harvested cells resuspended in water (Table 1). Both the encapsulated and unencapsulated strains took up radiolabelled taurine from the growth medium. Uptake varied from 18–83% of the taurine supplied amongst the different strains, according to method (i). However, the percentage uptake of radiolabelled taurine appeared to be less when the harvested cells were counted [method (ii)]. The difference in uptake measured by the different methods did not appear to be due to different degrees of quenching between the samples since the H-numbers of all samples were similar. [The H-number is a means of quench correction available on Beckman scintillation counters which works by measuring the Compton edge of photoelectrons generated by an external ¹³⁷Cs source and comparing the position of that edge between quenched and unquenched samples (Anonymous, 1978).] This raises the possibility that some of the taurine had been metabolized to CO₂. No experiments were carried out to confirm or deny this possibility.

By method (i) 30% or more of the radiolabelled taurine supplied was taken up by the M and M variant strains. Similar results were obtained when uptake of taurine was studied in these strains grown in BHI broth. In a separate experiment, 19.6 and 23.2 nmol taurine were taken up per mg dry weight of M and M variant strains, respectively, after overnight growth in PYK medium, as determined by counting cell-associated radioactivity.

Distribution of [1,2-¹⁴C]taurine taken up among cellular fractions

The distribution of the radioactivity taken up by the strains is shown in Table 2. In the M strain the radioactivity was confined mainly to two fractions: the cold TCA-soluble fraction (86%) and the hot TCA-soluble fraction (9%). The distribution of radioactivity was virtually identical whether the M strain had been grown in PYK or BHI media (data not shown). The M variant strain showed a very similar distribution pattern except that a much lower amount of the radioactivity (3%) was present in the hot TCA-soluble fraction. Thus, perhaps somewhat surprisingly, most of the radioactivity taken up by the encapsulated M and unencapsulated M variant strains remained in the pool metabolites fraction.

Table 2. *Distribution of [1,2-¹⁴C]taurine taken up by various S. aureus strains among cellular fractions*

The values represent the average of two experiments with strains M, NS58D and SA222 and are the results of one experiment with the other strains. For starting amounts of radioactivity see Table 1, harvested cells column.

Fraction	Strain ...	Distribution of radioactivity (% in fraction)							
		M	M variant	H	T	Smith diffuse	NS58D	11587	SA222
Harvested cells		100	100	100	100	100	100	100	100
Pool metabolites		86	89	99	97	96	12	94	43
Cold TCA pellet		14	11	1	3	4	88	6	57
Lipid		4	8	1	3	3	1	5	41
Ethanol pellet		10	3	<1	<1	1	87	1	16
Nucleic acid-teichoic acid-capsular polysaccharide		9	3	<1	<1	<1	62	1	10
Hot TCA pellet		1	<1	<1	<1	<1	25	<1	6
Protein		<1	<1	<1	<1	<1	8	<1	6
Trypsin pellet (peptidoglycan)		<1	<1	<1	<1	<1	17	<1	<1

Table 3. *Dialysis of spent medium and cold and hot TCA-soluble fractions from S. aureus M strain grown in the presence of [1,2-¹⁴C]taurine*

Fraction	Radioactivity (c.p.m.)			
	Growth medium	Before dialysis	After dialysis	Percentage dialysable
Spent medium	BHI	2.4×10^5	1.3×10^4	95
	PYK	1.3×10^5	1.5×10^3	99
Cold TCA-soluble	BHI	3.4×10^5	3.5×10^3	99
	PYK	4.4×10^4	3.7×10^3	92
Hot TCA-soluble	BHI	2.4×10^4	2.1×10^4	14
	PYK	2.3×10^3	1.7×10^3	26

In most of the other strains the majority of the radioactivity was present in the cold TCA-soluble fraction. Radioactivity was not incorporated into the hot TCA-soluble fraction of the H, T or Smith diffuse strains. The T and Smith diffuse strains have capsular polysaccharides with similar but distinct structures to that of the M strain (Haskell & Hanessian, 1964; Morse, 1963; Wu & Park, 1971). Two of the strains had distinctly different distribution patterns. Only a low amount of radioactivity was present in the cold TCA-soluble fraction of strain NS58D (12%), and appreciable amounts of label were present in the nucleic acid-teichoic acid-capsular polysaccharide, protein and peptidoglycan fractions. Strain SA222 appeared to incorporate substantial amounts of radioactivity into the lipid and nucleic acid-teichoic acid-capsular polysaccharide fractions. The fact that this organism yielded very 'sloppy' pellets led to some concern about the accuracy of results with this strain.

Identity of cell-associated radioactivity and radioactivity in spent growth medium

Dialysis of fractions. The spent growth medium and the cold and hot TCA-soluble fractions from the M strain were first dialysed to see whether the radioactivity was present predominantly in high or low molecular weight forms in the fractions (Table 3). Irrespective of whether the M strain had been grown in BHI or PYK media, more than 90% of the radioactivity was in a low molecular weight form in both the spent medium and the cold TCA-soluble fraction. This indicates that radioactivity remaining in the spent medium does not represent high molecular

Fig. 1. Chromatography of the cold TCA-soluble fraction from the M strain. The pool metabolites fraction from 25 ml of culture was obtained as described in Methods. The lyophilized material was dissolved in 0.2 ml 20 mM unlabelled taurine and 10 μ l amounts were chromatographed. A parallel culture was grown without labelled taurine. The unlabelled lyophilized pool metabolites fraction was dissolved in 0.2 ml 20 mM-taurine containing 0.1 μ Ci $[1,2-^{14}\text{C}]$ taurine, and 15 μ l amounts were chromatographed. In all cases the left-hand axis pertains to chromatography of the pool metabolites fraction of M strain grown in the presence of $[1,2-^{14}\text{C}]$ taurine and the right-hand axis to $[1,2-^{14}\text{C}]$ taurine added to unlabelled pool metabolites fraction. (a) TLC, solvent 95% ethanol/water (67:33, v/v); (b) paper chromatography, solvent butan-1-ol/acetic acid/water (3:1:1, by vol.); (c) paper chromatography, solvent propan-2-ol/95% ethanol/water (50:50:15, by vol.).

weight capsular polysaccharide released by the organism. Most of the radioactivity in the hot TCA-soluble fraction was non-dialysable, as would be expected if this fraction represents primarily taurine in high molecular weight capsular polysaccharide. The amount of this fraction that was dialysable may represent capsular polysaccharide partially hydrolysed by the hot TCA. Results were similar irrespective of whether the strain had been grown in BHI or PYK media.

Chromatographic analysis. The radioactivity in both the spent medium and acid hydrolysate of the hot TCA-soluble fraction from the M strain chromatographed as one spot with the same R_F value as unlabelled or $[1,2-^{14}\text{C}]$ taurine in four different chromatography systems. Thus it would

seem that the likely identity of radioactivity in spent medium is unincorporated and/or excreted taurine, while radioactivity in the hot TCA-soluble fraction may represent taurine incorporated into capsular polysaccharide.

Radioactivity in the cold TCA-fraction did not co-chromatograph with authentic taurine. Results with three different chromatography systems are shown in Fig. 1. Similar patterns were seen upon chromatography of the cold TCA-soluble fractions of the H and NS58D strains which contained greatly differing amounts of label in this fraction (99% and 12% of total cell-associated radioactivity, respectively (see Table 2). Thus it would appear that the majority of the radioactivity in the cold TCA-soluble fraction was not present as taurine but had been converted to another metabolite(s). This result has been confirmed by chromatography of the cold TCA-soluble radioactivity taken up by cell suspensions of the M strain during short-term incubation with [1,2-¹⁴C]taurine in buffer (E. Bieber & B. J. Wilkinson, unpublished observations).

DISCUSSION

All *S. aureus* strains examined took up significant amounts of radiolabelled taurine from the growth medium (Table 1). In six out of eight of the strains more than 90% of the cell-associated radioactivity was present in the pool metabolites fraction (Table 2). However, in strain NS58D, radioactivity was present in the nucleic acid-teichoic acid-capsular polysaccharide, protein and peptidoglycan fractions. This suggests that taurine readily enters various metabolic pathways in this strain. In strain SA222 there appeared to be a substantial incorporation of radioactivity into the lipid fraction. Smaller amounts of label were present in this fraction in other strains. A marked discrepancy between taurine uptake measured as depletion from the medium and as radioactivity present in cells (Table 1) also suggested taurine may be degraded to CO₂ in some strains.

Radioactivity remaining in the medium in which the M strain had grown appeared to be present as taurine. Most of the radioactivity in the pool metabolites fraction of the M strain, and other strains examined, did not appear to be present as taurine (Fig. 1). This suggests that staphylococci have a hitherto, we believe, unsuspected capacity to metabolize taurine.

About 10% of the cell-associated radioactivity in the M strain was located in a cellular fraction soluble in hot TCA, which is believed to represent the capsular polysaccharide (Liau *et al.*, 1974; Wilkinson *et al.*, 1979*a*).

The radioactivity appeared to be present as taurine in this fraction, suggesting that exogenously supplied taurine can be used in capsular polysaccharide biosynthesis. The capsular polysaccharide of the *S. aureus* M strain is composed of *N*-acetyl-D-fucosamine, *N*-acetyl-D-aminogalacturonic acid and taurine; taurine is believed to be attached to *N*-acetyl-D-aminogalacturonic acid via an amide bond (Liau & Hash, 1977; Liau *et al.*, 1974). No evidence was found for taurine incorporation into the Smith diffuse and T strain capsular polysaccharides (Table 2), which have similar structures to that of the M strain (Haskell & Hanessian, 1964; Morse, 1963; Wu & Park, 1971). Taurine has been reported to be present in a 'cell surface teichoic acid preparation,' which probably contains capsular polysaccharide, from four serologically distinct encapsulated *S. aureus* strains (Ohtomo *et al.*, 1981). One of these strains, NS58D, incorporated radioactivity from labelled taurine into material soluble in hot TCA, which may represent incorporation into capsular polysaccharide. Similarly, radioactivity from labelled taurine was incorporated into this fraction by encapsulated strain SA222. However, chromatographic analysis of hydrolysed hot TCA-soluble material was not performed. Further precise knowledge about taurine in these capsular polysaccharides is limited since the structures of staphylococcal capsular polysaccharides have been worked out in detail for only a few strains (Wilkinson, 1983).

The observations presented in this paper suggest that both encapsulated and unencapsulated *S. aureus* strains have the capacity to metabolize taurine, and that there is a heterogeneity in that different strains appear to metabolize taurine to varying degrees. Most studies of the bacterial metabolism of taurine have been concerned with taurine degradation in organisms grown on taurine as sole carbon source (Shimamoto & Berk, 1979, 1980), and even as sole nitrogen and

sulphur source (Stapley & Starkey, 1970). To our knowledge, there is currently no information on taurine intermediary metabolism in staphylococci. From studies of taurine degradation in other bacteria we can suggest that taurine may participate in transamination and desulphuration reactions, perhaps leading to sulphite and acetate production in some strains, at which point carbon from taurine may enter a number of metabolic pathways. Sulphite is on the sulphur assimilation pathway in bacteria (Burnell & Whatley, 1980) and taurine may act as a sulphur source for staphylococci. It is not clear whether staphylococci can biosynthesize taurine, although Ohtomo *et al.* (1981) have reported an encapsulated *S. aureus* strain with L-cysteic acid decarboxylase activity which is believed to participate in taurine biosynthesis from cysteine in animal tissue (Meister, 1965).

At this time we do not understand the significance of the staphylococcal capacity for taurine metabolism. In animals, the highest concentrations of taurine are found in muscle, heart, brain, retina, blood plasma, breast milk and urine (Meister, 1965). Thus, staphylococci, whose natural environment is the bodies of warm-blooded animals, are continually exposed to this molecule. Recent work in this laboratory has revealed what appears to be a sodium-dependent active transport system for taurine in *S. aureus* (E. Bieber & B. J. Wilkinson, unpublished observations). In addition to any role taurine might have as a nutrient, it may also be important in the synthesis of highly negatively charged surface molecules which may aid the organisms in resisting host defence systems. The sulphonate group is highly acidic, having a pK_a of 1.5 (Jacobsen & Smith, 1968). Further work will be necessary to appreciate fully the role and significance of taurine metabolism in staphylococci.

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