Anaerobic Transport of Serine and 2-Aminoisobutyric Acid by *Staphylococcus epidermidis*

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A membrane-bound ATPase detected in extracts of anaerobically grown *Staphylococcus epidermidis* was inhibited by a variety of compounds which inhibit ATPases in other organisms. Serine and 2-aminoisobutyric acid (AIB) were shown to enter the organism via the same transport system. The transport of AIB, the membrane potential and the transmembrane pH gradient were partially or completely abolished by the same inhibitors and also by uncoupling agents and lipid-soluble ions. It is proposed therefore that this ATPase generates and maintains an electrochemical gradient of protons across the cytoplasmic membrane of *S. epidermidis* capable of driving AIB uptake. Studies of AIB-induced proton movements suggested that AIB enters via a proton symport mechanism.

INTRODUCTION

A growing body of evidence suggests that the transport of nutrients into anaerobic bacteria depends upon the operation of a membrane-bound ATPase which is responsible for the generation and maintenance of an electrochemical gradient of protons (proton-motive force) across the cytoplasmic membrane (Hamilton, 1976; Harold, 1974; Booth & Morris, 1975). Such a gradient is postulated to act as the major driving force for the operation of a series of secondary active transport systems (proton symport) for the uptake of nutrients.

The facultative anaerobe *Staphylococcus epidermidis* under anaerobic conditions displays certain biochemical similarities, including the fermentation of serine, to the obligate anaerobe *Peptococcus prvotii*, an organism we have previously studied (Montague & Dawes, 1974; Bentley & Dawes, 1974; Reece *et al.*, 1976). As part of an investigation of the metabolism of *S. epidermidis* we have therefore examined anaerobic serine transport using 2-amino[1-¹⁴C]isobutyric acid (AIB) which we found enters the organism via the same transport system. A membrane-bound ATPase and the transport of AIB have been characterized with respect to their interaction with a series of inhibitors.

METHODS

Abbreviations. The following non-standard abbreviations are used throughout this paper: DCCD, *N,N'*-dicyclohexylcarbodiimide; DICD, diisopropylcarbodiimide; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; DDA⁺, dibenzyltrimethylammonium; TPMP⁺, triphenylmethylphosphonium; chlorhexidine, 1,6-di-4'-chlorophenyldiguanidohexane; NBD-Cl, 4-chloro-7-nitrobenzo-2-oxo-1,3-diazole; DMO, 5,5'-dimethyloxazolidine-2,4-dione.

Growth of organism. *Staphylococcus epidermidis*, isolated in our laboratory, was grown anaerobically at 37 °C in static culture in medium containing (g l⁻¹): KH₂PO₄, 10; NaCl, 5; NaOH, 2; glucose, 5; Difco Bactopeptone, 10; Lab M yeast extract, 10; cysteine hydrochloride, 0.5. The pH was adjusted to 7.2 with NaOH. Resazurin (0.8 mg l⁻¹) was added as an indicator of anaerobiosis. Fully grown cultures yielded approximately 1.2 mg dry wt organisms ml⁻¹. The organism was subcultured every 2 or 3 d in 50 ml round, flat-bottomed flasks with 1 cm diam. necks which were filled to the neck with liquid medium. The stock

organism was held as stab-cultures in solid medium composed of growth medium supplemented with Lab M no. 1 agar (15 g l⁻¹).

Measurement of bacterial density. The density of cultures and bacterial suspensions was measured turbidimetrically with a Pye Unicam SP600 spectrophotometer at 570 nm.

Preparation of particulate fraction for ATPase assay. Cultures (5 l) in late exponential phase, grown at 37 °C in 5 l aspirators, were harvested at 28000 g and 37 °C on a Sorvall RC-5 centrifuge fitted with a Szent-Györgyi and Blum type KSB-R continuous flow system operating at 120 ml min⁻¹. The organisms were washed three times with O₂-free 0.8 M-Tris/HCl buffer pH 7.6 containing 50 mM-mercaptoethanol and recovered by centrifuging at 12000 g for 10 min at 37 °C. The cell pellet was then resuspended in 20 ml 0.5 M-glycylglycine buffer pH 6.8 containing 50 mM-mercaptoethanol, 10 mM-EDTA (Na⁺ salt) and lysozyme at 0.5 mg ml⁻¹. Two methods were routinely employed for cell breakage. (1) Bacterial suspension (10 ml, about 20 mg dry wt ml⁻¹) was homogenized with gas chromatography beads according to the method of Bentley & Dawes (1974). The resulting preparation was centrifuged at 12000 g for 10 min at 0 °C. This process was repeated three times to remove intact organisms. The cloudy-yellow supernatant was centrifuged at 100000 g for 1 h at 0 °C to sediment the particulate fractions. The grey-brown pellet was resuspended in 4 ml 90 mM-Tris/HCl buffer pH 6.5, with a hand-held Teflon homogenizer, and stored at 4 °C. (2) This method was based on that of Kaback (1972). The bacterial suspension (20 ml, about 300 mg dry wt ml⁻¹) was incubated for 45 min at 37 °C. Lysis often occurred during this stage; if it did not, the bacterial suspension was centrifuged at 48000 g for 15 min at 37 °C, and the cell pellet was resuspended in 20 ml 90 mM-Tris/HCl buffer pH 7.6 containing 5 mM-MgCl₂ and incubated at 37 °C for about 30 min until lysis had occurred, as judged by an increase in viscosity. A spatula-tip of DNAase was then added to lower the viscosity and the lysate was treated as described for the disrupted cell suspension.

Assay of ATPase. ATPase was assayed by a modification of the method of Muñoz *et al.* (1968). The reaction mixture in 10 ml glass vials contained (in 0.5 ml): 7 µmol Tris/HCl buffer pH 7.6, 4 µmol MgCl₂, 5 µmol ATP (pH 7.1, adjusted with NaOH) and 100 to 200 µg extract protein in 0.1 ml. Incubation was at 37 °C. The reaction was stopped at intervals by adding 0.5 ml ice-cold 10% (w/v) trichloroacetic acid. After centrifuging for 5 min at 5000 g and 20 °C to remove precipitated protein, samples (0.12 ml) were removed and assayed for P_i. Trichloroacetic acid was added to the control prior to the enzyme preparation. All assays were in duplicate. One unit of ATPase activity is defined as that amount of enzyme which liberates 1 µmol P_i from ATP in 10 min at 37 °C under the assay conditions.

Preparation of bacterial suspensions for transport studies. Bacterial suspensions (about 18 to 22 mg dry wt ml⁻¹) were prepared from cultures (500 ml) in the mid-exponential phase of growth, according to the procedure of Bentley & Dawes (1974), and held under O₂-free N₂ at 37 °C.

Anaerobic transport assay. Samples (1 ml) of bacterial suspension were transferred, with anaerobic precautions and immediate mixing, to water-jacketed tubes at 37 °C, continuously flushed with O₂-free N₂, via a hypodermic needle through a Suba-seal cap. The tubes contained (in 5 ml) 68 mM-NaH₂PO₄ buffer pH 6.8 and appropriate concentrations of radiochemical and inhibitor. Inhibitors were added as methanolic solutions; controls containing methanol were always used. During incubation at 37 °C, samples (0.3 or 0.5 ml) were removed at intervals with a syringe through the Suba-seal cap and filtered through Oxoid membrane filters (0.45 µm pore size, 2.5 cm diam.), using the apparatus described by Midgley & Dawes (1973). The bacteria were washed twice with 2 ml buffer at ambient temperature, the whole operation taking approximately 20 s. There was no evidence for loss of radiochemical due to temperature shock. The efficiency of washing was checked by a control containing organisms inactivated by 2% (v/v) formaldehyde; this also served as a zero-time reading. The filters were then placed in vials containing 10 ml scintillation fluid (Bray, 1960) and their radioactivity was measured in a Beckman LS-233 scintillation spectrometer.

Extraction and identification of accumulated material. Bacterial suspensions (2 ml, 60 mg dry wt ml⁻¹) were incubated with [¹⁴C]AIB (2 mM, 0.5 µCi µmol⁻¹) under anaerobic conditions at 37 °C for 20 min in the presence of 10 mM-glucose, after which the bacteria were recovered by centrifuging at 12000 g for 10 min at 37 °C. The organisms were washed twice with O₂-free 68 mM-NaH₂PO₄ buffer pH 6.8 and resuspended in isopropanol/water (50:50, v/v; 2 ml) for 1 h. The suspension was then centrifuged at 12000 g for 10 min at 37 °C and the supernatant was concentrated to dryness by rotary evaporation under reduced pressure. The residue was taken up in 100 µl water for examination by electrophoresis. This treatment extracted 98% of the accumulated radioactive material.

Determination of the transmembrane pH gradient (ΔpH). The flow dialysis technique of Colowick & Womack (1969) as applied by Ramos *et al.* (1976) was adapted for anaerobic use. The upper chamber (diam. 2 cm, operational vol. 2 ml) of a jacketed flow cell, held at 37 °C, was sealed with a Suba-seal cap and continuously gassed with O₂-free N₂ via a hypodermic needle through the cap. The lower chamber was of the same diameter and volume as the upper; they were separated by Visking tubing (500 to 1000 molecular weight cut-off), prepared by boiling in EDTA (10 mM) and stored in distilled water. Both chambers were continuously stirred with magnetic bars. Bacterial suspension (0.5 ml, 80 to 100 mg dry wt ml⁻¹) was trans-

ferred with anaerobic precautions to the upper chamber, which contained O₂-free 68 mM-NaH₂PO₄ buffer (0.4 ml) at a given pH. The same O₂-free buffer containing distilled water (9:1, v/v) was pumped through the lower chamber by a flow inducer (Watson-Marlow, Falmouth, Cornwall) at 4 ml min⁻¹. 5,5'-Dimethyl [2-¹⁴C]oxazolidine-2,4-dione (DMO; 1 µCi, specific activity 59 µCi µmol⁻¹) was added to the upper chamber 1 min after the flow inducer was started. Fractions (4 ml) were collected on a Gilson fraction collector and samples (2 ml) were added to 10 ml scintillation fluid (Bray, 1960) and assayed for radioactivity by liquid scintillation spectrometry.

Calculation of results. Concentration gradients for solutes taken up by the organism were calculated using the value for intracellular water of 1.5 µl (mg dry wt organisms)⁻¹, obtained by the method of Stephenson *et al.* (1978). The membrane potential ($\Delta\psi$) was calculated from the Nernst equation,

$$\Delta\psi = -2.303 \frac{RT}{F} \log \frac{[^{137}\text{Cs}^+]_{\text{in}}}{[^{137}\text{Cs}^+]_{\text{out}}}$$

using steady-state concentration values obtained from ¹³⁷Cs⁺ uptake experiments in the presence of valinomycin (e.g. Fig. 2), or from [³H]TPMP⁺ uptake experiments (Table 2). Internal pH was calculated from the equation (Waddell & Butler, 1959):

$$\frac{[\text{DMO}]_{\text{in}}}{[\text{DMO}]_{\text{out}}} = \frac{1/K_a + 1/\text{H}^+_{\text{in}}}{1/K_a + 1/\text{H}^+_{\text{out}}}$$

ΔpH was determined by difference.

Measurement of proton movement. The method of Collins *et al.* (1976) was used. Bacterial suspensions in 67 mM-NaK phosphate were starved for 4 h at 37 °C under N₂ and centrifuged at 12000 *g* for 10 min at 37 °C. The pellet was resuspended in O₂-free 0.5 mM-Hepes/Tris buffer pH 6.8 (20 to 30 mg dry wt organisms ml⁻¹) and 4 ml portions were incubated at 37 °C in a water-jacketed glass vessel, continuously flushed with O₂-free N₂ and stirred magnetically. The pH in the reaction vessel was measured with an EIL micro-combination electrode (part no. 140 200) inserted through a Suba-seal cap in the top of the vessel, and an EIL Vibret pH meter (model 3920). The pH was recorded continuously with a Servoscribe multichannel pen recorder (model 1S) set at 50 mV to give a full-scale deflection (20 cm) of 0.08 pH unit.

Analyses. P_i was determined by the method of Clarke & Morris (1976). Protein was determined by the method of Lowry *et al.* (1951) after boiling portions (1 ml) of the particulate preparations, and bovine serum albumin as protein standard, with 1 M-NaOH (4 ml) in stoppered tubes for 10 min to solubilize all membrane protein. High-voltage electrophoresis was performed at 2.5 kV for 45 min on Whatman no. 1 chromatography paper, using acetic acid/formic acid (100%)/water (150:50:1800, by vol.) buffer pH 1.9. Standards were localized by spraying with ninhydrin/cadmium acetate solution (Heathcote *et al.*, 1973). Radioactivity was detected by cutting up the sample lane into sections (1 × 3 cm) and counting the radioactivity of these immersed in scintillation fluid (Bray, 1960).

Chemicals. Analytical grade chemicals were used whenever possible. Radiochemicals were purchased from The Radiochemical Centre, Amersham. Valinomycin, mercaptoethanol, *p*-nitrophenyl phosphate and bovine serum albumin were obtained from Sigma and DNAase, ATP, GTP, CTP, ADP and AMP from BDH. Inhibitors were obtained from a variety of sources as listed by Stephenson *et al.* (1978).

RESULTS

[¹⁴C]Serine and [¹⁴C]AIB accumulation by *S. epidermidis*. Under anaerobic conditions L-[3-¹⁴C]serine was accumulated by washed suspensions of *S. epidermidis*. The variation in the initial rate of serine uptake in response to varying the external serine concentration over the range 0.1 to 1.0 mM demonstrated the involvement of a component having a *K_m* value of 0.12 mM. As serine is fermented rapidly by the organism to yield acetate, lactate and CO₂ as the principal products (R. Sivakanesan & E. A. Dawes, unpublished observations), an assay for serine transport avoiding the drawbacks inherent in the use of metabolizable substrates for such studies was sought. Thus the transport of AIB was investigated and found, from conventional double-reciprocal plots, to enter *S. epidermidis* by a process having a *K_m* of 0.38 mM. The substrate range employed was 0.1 to 5.0 mM. Serine competitively inhibited AIB transport with a *K_i* of 0.5 mM, suggesting that serine and AIB were both substrates for the same transport system in this organism. Intracellular material, accumulated when washed suspensions were exposed to AIB, was extracted and examined as described in Methods. Approximately 96% of the accumulated radioactivity behaved as

Table 1. *Concentration ranges of various inhibitors of AIB uptake, membrane-bound ATPase and membrane potential ($\Delta\psi$) required to give partial or maximum inhibition*

Inhibitor	Concentration range*		
	AIB uptake†	Membrane-bound ATPase‡	$\Delta\psi$ §
Chlorhexidine	20–200 $\mu\text{g ml}^{-1}$	50–200 $\mu\text{g ml}^{-1}$	30–125 $\mu\text{g ml}^{-1}$
DCCD	200–600 μM	100–300 μM	200–600 μM
NBD-Cl	150–450 μM	100–300 μM	30–300 μM
Azide	10–60 mM	15–80 mM	20–100 mM
DICD	3–18 mM	2–12 mM	4–16 mM
CCCP	30–60 μM	—	3–30 μM
DNP	2–8 mM	—	1–4 mM
DDA ⁺	0.5–7 mM	—	4–12 mM
TPMP ⁺	0.5–7 mM	—	1–8 mM

* The first value in each column is the concentration of inhibitor causing 20% inhibition while the second is that causing maximum inhibition, generally > 90%.

† Determined by filtration assay using [¹⁴C]AIB (0.3 mM, specific activity 0.3 $\mu\text{Ci } \mu\text{mol}^{-1}$).

‡ Release of P_i from ATP by a preparation of ATPase from homogenized *S. epidermidis*.

§ Determined by filtration assay using ¹³⁷Cs⁺ (0.1 mM, specific activity 1 $\mu\text{Ci } \mu\text{mol}^{-1}$) and valinomycin (1 $\mu\text{g ml}^{-1}$).

unchanged AIB indicating that this compound was not metabolized under the assay conditions employed.

The ability of *S. epidermidis* to transport AIB declined steadily after 1 h at 37 °C and was lost completely after 4 h at 37 °C. Hence all experiments were carried out with freshly harvested organisms. Accumulation of AIB was inhibited by the proton-conductors CCCP and DNP, the lipid-soluble ions DDA⁺ and TPMP⁺ and inhibitors of membrane-bound ATPase, namely chlorhexidine, DCCD, NBD-Cl, sodium azide and DICD, over the concentration ranges recorded in Table 1. Typical inhibition curves are shown (Fig. 1). Despite standardization of growth, harvesting and assay procedures, the sensitivity of different batches of organisms to DCCD and DICD was variable.

Characterization of the membrane-bound ATPase of S. epidermidis. ATPase activity detected in the particulate fraction was dependent on Mg²⁺ and enzyme concentrations, and the rate of release of P_i from ATP was linear for at least 10 min under optimum conditions (8 mM-Mg²⁺ and at pH 6.9). The specific activities [units (mg protein)⁻¹] encountered with different preparations varied from 0.66 to 0.98 for homogenized preparations and from 0.50 to 0.78 for lysed preparations. When assayed with a range of substrates (10 mM) the relative activities were: GTP, 133; ATP, 100; CTP, 4; ADP, 0; AMP, 0; *p*-nitrophenyl disodium orthophosphate, 0. The apparent *K_m* (ATP) was 0.63 mM and the apparent *K_m* (GTP) was 2.5 mM as determined over a substrate concentration range of 0.2 to 10 mM. The membrane-bound ATPase was affected by a series of inhibitors at concentrations similar to those shown to inhibit AIB uptake (Table 1). The likely role of this ATPase is to generate and maintain an electrochemical gradient of protons across the cytoplasmic membrane.

Measurement of the membrane potential ($\Delta\psi$) of S. epidermidis. *Staphylococcus epidermidis* was sensitive to the ionophore valinomycin (Fig. 2) which therefore was used to ensure that the organism was permeable to ¹³⁷Cs⁺, thus allowing the distribution of this cation to be used as a measure of $\Delta\psi$ (Ramos *et al.*, 1976). The rate of Cs⁺ uptake was dependent on the concentrations of both valinomycin and Cs⁺. The extent of Cs⁺ uptake was independent of valinomycin concentration over the range 0.2 to 1.6 $\mu\text{g ml}^{-1}$ but linearly dependent on Cs⁺ concentration over the range 0.025 to 0.2 mM. The rate and extent of uptake were enhanced by addition of serine (10 mM) or glucose (10 mM) (Fig. 2). Membrane potentials ranging between –60 and –95 mV were observed with *S. epidermidis*. A

Fig. 1

Fig. 2

Fig. 1. Effect of glucose and various inhibitors on accumulation of [^{14}C]AIB by *S. epidermidis*. Uptake of AIB (0.3 mM, specific activity $0.3 \mu\text{Ci } \mu\text{mol}^{-1}$) was measured as described in Methods. Glucose and inhibitors were added to the bacterial suspension ($7 \text{ mg dry wt ml}^{-1}$) 10 min before [^{14}C]AIB. ●, Control ($10 \mu\text{l}$ methanol present); ○, glucose (10 mM); △, DDA $^{+}$ (4 mM); □, chlorhexidine ($100 \mu\text{g ml}^{-1}$).

Fig. 2. Effect of glucose, serine and various inhibitors on accumulation of $^{137}\text{Cs}^{+}$ by *S. epidermidis*. Uptake of $^{137}\text{Cs}^{+}$ (0.1 mM , specific activity $1 \mu\text{Ci } \mu\text{mol}^{-1}$) was measured as described in Methods. Glucose and serine were added to the bacterial suspension ($5 \text{ mg dry wt ml}^{-1}$) 5 min before $^{137}\text{Cs}^{+}$, inhibitors were added 10 min before. □, Control ($10 \mu\text{l}$ methanol present); ■, valinomycin ($1 \mu\text{g ml}^{-1}$); ●, serine (10 mM) plus valinomycin ($1 \mu\text{g ml}^{-1}$); ○, glucose (10 mM) plus valinomycin ($1 \mu\text{g ml}^{-1}$); ▲, sodium azide (50 mM) plus valinomycin ($1 \mu\text{g ml}^{-1}$); △, chlorhexidine ($100 \mu\text{g ml}^{-1}$) plus valinomycin ($1 \mu\text{g ml}^{-1}$).

batch of organisms which accumulated AIB 15-fold had a $\Delta\psi$ of -82 mV . This membrane potential was partially or completely abolished by inhibitors of AIB uptake (Table 1); typical inhibition curves are shown in Fig. 2. The value of $\Delta\psi$ (-60 mV) obtained with $^{137}\text{Cs}^{+}$ was checked with a portion of the same bacterial suspension by measuring the uptake of [^3H]TPMP $^{+}$ ($0.05 \mu\text{M}$, 12 mCi mg^{-1}); a value of -65.6 mV was obtained.

Measurement of the ΔpH of *S. epidermidis*. Flow dialysis in the presence of [^{14}C]DMO enabled a ΔpH to be detected with suspensions of *S. epidermidis* at pH values below 6.8 (Fig. 3; Table 2). A value for ΔpH (1.5 units) obtained with [^{14}C]DMO was checked with a portion of the same bacterial suspension by using [$1\text{-}^{14}\text{C}$]acetylsalicylic acid ($1 \mu\text{Ci}$, specific activity 26 mCi mmol^{-1}); the ΔpH was 1 unit. Using portions of the same bacterial suspension, values for ΔpH , $\Delta\psi$ and the amount of AIB uptake were determined. These measurements were repeated with different bacterial suspensions at different external pH values (Table 2). The ΔpH , at an external pH of 5.5, was completely abolished by the inhibitors chlorhexidine ($100 \mu\text{g ml}^{-1}$), DNP (3 mM) and TPMP $^{+}$ (5 mM). In the presence of cetyltrimethylammonium bromide ($20 \mu\text{g ml}^{-1}$) radioactivity was not retained by the organism as compared with a control without bacteria (Fig. 3), showing that the measured ΔpH was abolished when the permeability barrier was destroyed.

Proton movements associated with AIB uptake. Addition of AIB to non-metabolizing

Table 2. *Effect of external pH on $\Delta\psi$, ΔpH and AIB uptake of *S. epidermidis* suspensions*

External pH	Concn gradient of AIB*	$\Delta\psi^\dagger$ (mV)	$\Delta pH^\ddagger$ (units)
5.0	3	40–50	1.0–1.5
5.5	10	50–60	0.6–0.9
6.8	15	80–90	0

* AIB uptake was determined by filtration assay using [14 C]AIB (0.3 mM, specific activity, 0.3 μ Ci μ mol $^{-1}$). Intracellular concentration was calculated from the concentration of AIB accumulated at the plateau, using the value of 1.5 μ l (mg dry wt organisms) $^{-1}$ for intracellular water.

† Determined by filtration assay using 137 Cs $^+$ (0.1 mM, specific activity, 1 μ Ci μ mol $^{-1}$) and valinomycin (1 μ g ml $^{-1}$). These results were confirmed with [3 H]TPMP $^+$ (0.05 μ M; 12 mCi mg $^{-1}$).

‡ Calculated from flow-dialysis experiments carried out with [14 C]DMO (1 μ Ci, specific activity, 59 μ Ci μ mol $^{-1}$).

Fig. 3

Fig. 4

Fig. 3. Uptake of [14 C]DMO by *S. epidermidis* as determined by flow dialysis. The assay was carried out at pH 5.0 as described in Methods with [14 C]DMO (1 μ Ci, specific activity 59 mCi mmol $^{-1}$). Δ , Control, bacteria absent; $\circ$, bacteria (47 mg dry wt ml $^{-1}$) added; $\bullet$, cetyltrimethylammonium bromide (20 μ g ml $^{-1}$) plus bacteria.

Fig. 4. Changes in pH observed on addition of AIB to a washed suspension of non-metabolizing *S. epidermidis* previously starved for 4 h. Bacteria (22 mg dry wt ml $^{-1}$) were allowed to equilibrate to pH 6.6 in 0.5 mM Hepes/Tris buffer pH 6.8 and AIB (final concentration 2.2 mM) was added at the arrowed time. Lower trace, control; upper trace, bacteria pre-incubated with CCCP (20 μ M) for 10 min.

S. epidermidis which had been starved for 4 h at 37 $^{\circ}$ C caused an increase in pH which reached a maximum after 95 s (Fig. 4). This effect was inhibited by the presence of the uncoupler CCCP (20 μ M). Over a range of bacterial concentrations (20 to 30 mg dry wt organisms ml $^{-1}$) and AIB concentrations (2.0 to 5.0 mM), the stoichiometry of the AIB uptake, calculated according to Collins *et al.* (1976), ranged from 0.80 to 1.12 mol H $^+$ /mol AIB with a mean of 0.89. West & Mitchell (1973) claimed that the stoichiometry of the β -galactoside/proton symport is 1:1, and it would appear from this experiment that AIB enters *S. epidermidis* via a proton symport mechanism.

DISCUSSION

Our results on the effects of inhibitors of the membrane-bound ATPase, on the accumulation of AIB, and the maintenance of $\Delta\psi$ and ΔpH (Table 1) afford strong indirect evidence that these phenomena are functionally related by a proton-motive force generated from ATP by a proton-translocating ATPase situated on the cytoplasmic membrane of *S. epidermidis*. This system would give, as a result of proton extrusion, a chemical and electrical potential (inside alkaline and negative) capable of driving AIB uptake, provided AIB enters via a proton symport mechanism. The use of inhibitors for arriving at such conclusions can be criticized, particularly when their specificity is uncertain. For this reason a large number of inhibitors with diverse modes of action were employed and the conclusions drawn were also supported by the results of proton movement studies. The rate and extent of AIB uptake and the magnitude of $\Delta\psi$ depended on the presence of an external energy source; attempts to show that this was also the case for the ΔpH were invalidated by acidification of the upper chamber of the flow cell caused by metabolism of the energy source, and this produced a distribution of DMO which was insensitive to uncouplers and ATPase inhibitors and therefore was not thought to reflect the true value of ΔpH . The $\Delta\psi$ generated by *S. epidermidis* at pH 6.8 was, however, more than sufficient to account for the 15-fold concentration gradient of AIB obtained, again assuming that co-transport of a complex with a single net positive charge was occurring. Although $\Delta\psi$ decreased with decreasing pH, AIB uptake declined more so and, at all pH values tested, $\Delta\psi$ was sufficient to drive uptake. The suggested role of the ΔpH component in amino acid transport is for uptake of acidic amino acids by a proton symport (Hamilton, 1976). The merits and disadvantages of using valinomycin and DMO as probes for $\Delta\psi$ and ΔpH have been discussed (Harold *et al.*, 1970; Gómez-Puyou & Gómez-Lójero, 1977) and it was considered sufficient to confirm the values obtained with TPMP⁺ (for $\Delta\psi$) and with acetylsalicylic acid (for ΔpH), although in both cases the technique is essentially the same. Here we have been concerned solely with anaerobically grown *S. epidermidis* as part of a comparative programme with the obligate anaerobe *P. prevotii*, but since *S. epidermidis* is a facultative organism which, after aerobic growth, possesses a respiratory chain (Jacobs & Conti, 1965), clearly a comparison of the transport properties of the aerobically and anaerobically grown organisms would be of interest.

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