Binding of Aluminium Ions by *Staphylococcus aureus* 893

The kinetics of aluminium ion binding from aqueous solution by *Staphylococcus aureus* strain 893 (a wound isolate, University of Strathclyde) have been shown to be rapid, a surface phenomenon and dependant on the hydrogen ion concentration of the solution[1].

The nature of this binding has now been investigated in the pH range 2.0–6.0. 2 mechanisms of binding have been found.

Experimental procedures. Nutrient agar cultures of *S. aureus* 893 were grown as previously described[1]. Washed cells were suspended in distilled water ($D_{660\,nm}$ of 1.0 ≡ 354 µg/ml dry weight at 105 °C) and used to prepare isolated cell walls (SALTON[2]).

The uptake of aluminium by cells from solutions containing 10–20 mg/l at pH values from 2.0–6.0 were determined by the method of GILES and MCKAY[3]. Solution concentrations of aluminium were determined by JONES and THURMAN[4] method.

The binding of hydrogen ion by cell walls was determined by the method of KENCHINGTON[5] in 0.05*M* KCl suspensions with 0.02*N* HCl using a Radiometer TTTlc pH meter and recording unit as described by A. D. BROWN[6].

Results and discussion. The adsorption isotherm (20 °C, pH 6.0) of aluminium ions by cells of *S. aureus* 893 is shown in Figure 1 where 1180 µmoles/g dry weight is bound.

The influence of hydrogen ion concentration on the binding of aluminium is shown in the Table where maximum values are stated. The pH shift to more acid values show the association of aluminium ions with acid groups on the cell. At pH values of 4.0–4.4 the amount of aluminium bound has fallen to 240 µmoles/g, and at pH value of 3.9 and below no detectable binding of aluminium could be determined. Also no significant fall in the pH of the solution was noticed at pH values below 4.0 indicating no hydrogen ion-aluminium ion exchange.

The most abundant aluminium ion in solutions of low concentrations of aluminium at pH values about 4.0 is the mononucleate hydroxide $[Al(OH)_2]^+$[7,8], and aluminium is attached as this ionic species to the mobile layer of counter ions associated with the dissociable groups of the staphylococcal cell surface[9].

With rising hydrogen ion concentration of the medium 3 factors operate to effectively reduce the adsorption of aluminium by the cells. (1) More cations competing for the exchangeable sites on the cell surface; (2) the increase in the electropositivity of the hydrated aluminium ions $[Al(OH)_2]^+ \rightarrow [Al(OH)]^{++} \rightarrow [Al]^{3+}H_2O$; (3) the number of dissociable groups on the cell surface is reduced.

The desorption isotherm for bound aluminium from *S. aureus* 893 (20 °C pH 3.0) is a straight line relationship, indicating the replacement of an $[Al(OH)_2]^+$ ion by a H^+. Extrapolation of this line to zero concentration of free aluminium ions yields an intercept of 220 µmoles bound aluminium/g dry weight. This residual aluminium (which corresponds to the amount of aluminium adsorbed from

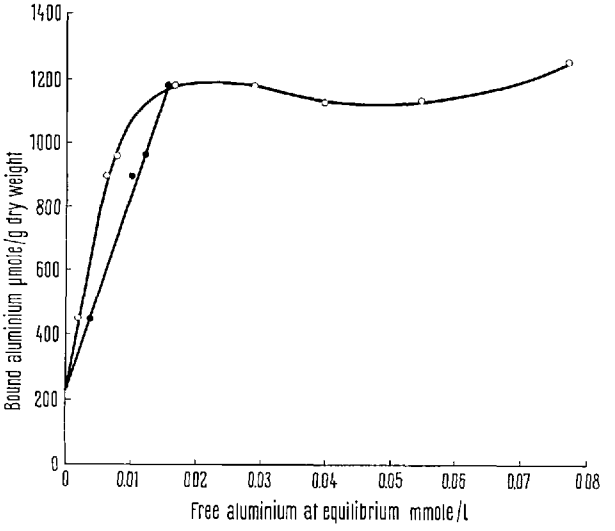

Fig. 1. o——o Adsorption isotherm of aluminium by *S. aureus* 893 at 20 °C and pH 6.0. •——• Desorption isotherm of aluminium by *S. aureus* 893 at 20 °C and pH 3.0.

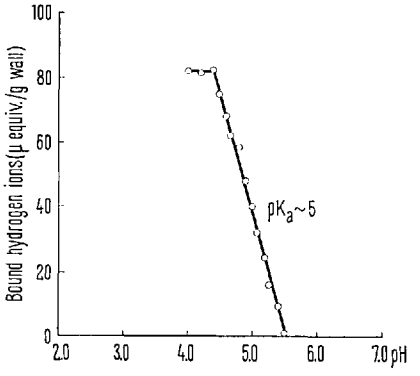

Fig. 2. Hydrogen ion binding of isolated walls of *S. aureus* 893.

Competitive binding of aluminium ions by *S. aureus* 893 cells at increasing concentrations of hydrogen ion

pH of Al solutions	Initial pH of *S. aureus* 893 + aluminium, mixture	pH of reaction mixture at equilibrium	Δ pH of Al mixture	Aluminium bound µmoles/g
4.35	6.00	5.40	0.60	1180
4.35	4.55	4.50	0.05	240
3.80	3.92	3.90	0.02	nil, or too low to be determined

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[6] A. D. BROWN, J. molec. Biol. *12*, 491 (1965).
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[9] F. GALDIERO, M. LEMBO and M. A. TUFANO, Experientia *24*, 34 (1968).

solutions of pH 4.00–4.40), could not be displaced by solutions of high hydrogen ion concentration e.g. pH 2.0 and 1.0. Failure to dislodge this aluminium by H^+ suggest that it must be rigidly bound in a high affinity linkage[9], e.g. covalent linkage, and that groups on the cell surface dissociating in the pH region 4.0–4.4 are the most important factor in the interaction of aluminium with *S. aureus* 893.

Thus 2 types of binding occur: (1) aluminium is bound firmly in a complex with groups on the cell surface; then (2) subsequent binding is as exchangeable cations in the mobile layer of counterions. It has been shown that the binding of aluminium has lethal effects (BRADLEY, FISH and PARKER)[1]. Of the 2 types of binding described complex formation is potentially the more lethal.

The isolated cell walls of *S. aureus* 893 bound a maximum of 82 μ equiv. H^+/g at pH values 4.00–4.40. Figure 2 (compare GALDIERO)[10]; cell walls also bound 130 μmoles Al/g and cell walls with complexed aluminium utilized 94 μ equiv. H^+/g at pH 4.00 with a maximum utilization at pH 3.80 of 138 μ equiv. H^+/g.

From this preliminary data it is evident that aluminium is bound in a high affinity linkage by surface groups of $pK_a \sim 5$ on *S. aureus* 893.

Résumé. Deux types de liaisons ont lieu quand des ions d'aluminium réagissent avec les parois cellulaires isolées de *Straphylococcus aureus* espèce 893. La première interaction a comme résultat la formation d'un complexe stable qui affecte des groupes occupant le mur de la cellule avec un $pK_a \sim 5$. On suppose l'existence d'un composant de surface constitué par de la protéine combinée à de l'acide teichoique. La deuxième liaison consiste en ions interchangeables dans la couche mobile de cations recouvrant la paroi.

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A Preliminary Assessment of the Techniques for Measuring Primary Production in Macrophytic Marine Algae

Attempts to assess the productivity of aquatic plants under natural conditions have produced considerable controversy over the methods employed[1–3]. In this investigation of the rates of primary production of the marine alga, *Caulerpa prolifera* (Forsk.) Lamour, in Canary Waters, oxygen production and ^{14}C incorporation were measured and compared under varying conditions.

Fronds of the alga were collected from a depth of 5 m by divers and immediately on reaching shore they were rinsed in seawater and transferred to incubation bottles. At no time during this transfer were they taken out of water or exposed to direct sunlight. The sealed bottles were attached to wire frames underwater at appropriate depths. The frames were secured to ropes buoyed at the surface, and buffeting of the buoys produced sufficient agitation in the bottles to ensure an even mixing of dissolved gases and nutrients around the tissue. Photosynthetic oxygen production was determined using the light/dark bottle technique[4], oxygen concentration changes in the seawater being measured with a polyethylene-lead-silver electrode system[5]. Estimates of organic production were derived from oxygen figures assuming a photosynthetic quotient of 1.0. ^{14}Carbon incorporation measurements were based on the method of STEEMAN-NIELSEN[6]. A 1 mC ampoule (Radiochemical Centre, Amersham, England) of $Na_2{}^{14}CO_3$ (56 mC/m*M*) was diluted to 500 ml, giving a final activity of 2 μC/ml, and 2.5 ml of this stock solution was added to 197.5 ml of freshly drawn seawater. After incubation (see Tables) the algal fronds were washed quickly, then transferred to 80% ethanol for transport to Britain. Radioactivity was measured in a Packard Tricarb Scintillation Counter at 53% efficiency.

Preliminary experiments with varying tissue/incubation volume ratios indicated no apparent nutrient deficiency effects under the conditions used in the 24-h studies with *Caulerpa*. Similarly, no apparent nutrient deficiency effects were noted in concurrent experiments with algae photosynthesizing at much higher rates, if ratios of 0.3 g dry weight tissue/l seawater or lower were used.

From Table I, it can be seen that with the oxygen method direct 24-h and short-term measurements gave similar estimates for daily production. However, if the

Table I. A comparison of net daily production estimates for *Caulerpa prolifera*, established by oxygen and ^{14}C methods

Method	Daily production mgC/g dry weight
Oxygen method[a]	
Direct 24-h	4.52[c]
Calculated from 3-h[b]	4.56
^{14}Carbon method	
Direct 24-h	4.60
Calculated from 3-h[d]	5.18

[a] Carried out in incubation bottles of 1 l capacity; tissue dry weight approximately 0.2 g. [b] Based on 40 incubations at different 3-h periods throughout the daylight period. [c] This value is remarkably close to an estimate based on a recalculation of the oxygen production figures obtained for *C. prolifera* in the Mediterranean, i.e. 4.19 mgC/g dry weight[7]. [d] This figure is based on 2 sets of incubations under conditions of bright sun between 11.00–14.00. A 12-h daylength period was assumed, i.e. net daily production = $12x - 12y$, where x is ^{14}C incorporation/h in light and y is the respiratory loss of carbon/h calculated from oxygen measurements.

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