Hydrogen Ion Binding of Bacterial Cell Wall

In previous investigations it was shown that the cell wall isolated from *Staphylococcus aureus*[1] can bind sodium, potassium, calcium and magnesium ions. Since this binding capacity depends on the hydrogen ion concentration of the medium, the bacterial cell wall hydrogen ion binding capacity has been investigated.

Experimental procedures. The cell wall from *S. aureus* (strain 22 Istituto Sieroterapico Italiano) was isolated by SALTON's scheme[2] and further treated with 0.1 *M* ethylene diaminetetra-acetic acid (pH 7.5) as described in previous papers[1]. Hydrogen ion binding capacity was studied by suspending 50 mg of lyophilized cell wall in 10 ml of HCl or NaOH at 0.001–0.000001 *N*. After 5 h incubation with shaking, at 25 °C in an atmosphere of nitrogen, the hydrogen ion concentration was measured with a Beckman Expandomatic pH-meter. The amount of bound hydrogen ions was calculated from the difference in the hydrogen ion concentration of HCl solutions with and without the cell wall. The activity coefficient in the presence of cell wall was assumed equal to that of pure HCl solutions, as the error due to small amounts of cell wall is negligible[3]. Phosphates were determined by FISKE and SUBBAROW[4] method on hydrolyzed cell wall.

Results and discussion. In working out the data to determine the dissociation coefficient and binding capacity, the mass law equation was used[5,6]. The cell wall may be regarded, for this purpose, as polymer chains in three-dimensional arrays with independent, mutually non-competitive binding sites. The cell wall titration curve for *S. aureus* is given in Figure 1. The binding-sites were estimated by SCATCHARD plot[7] (Figure 2), which SANUI et al.[6] had already used to calculate the ion-binding capacity of rat liver microsomes and human erythrocyte ghosts. SCATCHARD's plot of our data yields a curve which is concave towards the top suggesting the presence of more than one species of binding site. Assuming that in the cell wall there are 2 major classes of binding site in the range below pH 6.00, the curve shown in Figure 2, according to SANUI et al.[6], may be derived from:

$$\frac{HX}{f_h(H^+)} = \left[\frac{(X^-_{1t})}{K_1} + \frac{(X^-_{2t})}{K_2}\right] - \frac{(HX_1)}{K_1} - \frac{(HX_2)}{K_2}$$

where f_h is the activity coefficient, HX the number of bound hydrogen ions, K_1 and K_2 the dissociation constants, X^-_{1t} and X^-_{2t} the maximum binding capacities of the 2 different classes of sites, X^-_1 and X^-_2 binding sites in free state and HX_1 and HX_2 binding sites in bound state. If the approximations are made that at low hydrogen ion concentration HX_2 is negligible, and at higher concentrations HX_1 is essentially constant, then plots of $(HX)/f_h(H^+)$ versus (HX_1) and (HX_2) should yield 2 straight lines of which the slopes and intercepts allow us to calculate the constants required (Table). The electrostatic correction is neglected in this treatment[8] as it is obviously difficult to estimate the cell wall charge and the parameter W in DEBYE-HÜCKEL's theory. *S. aureus* cell wall, therefore, presents dissociable groups between pH 2 and 6 with pK 3.19 and 4.70. In the alkaline range about 0.8% carboxyl equivalent is titrated with a pK 7.15.

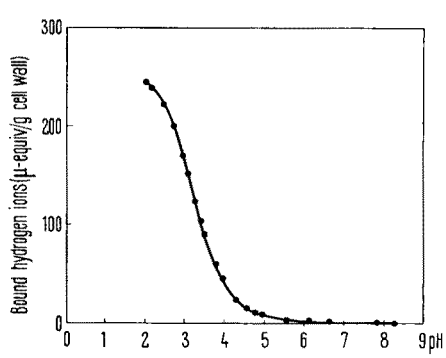

Fig. 1. Hydrogen ion binding of *S. aureus* cell wall.

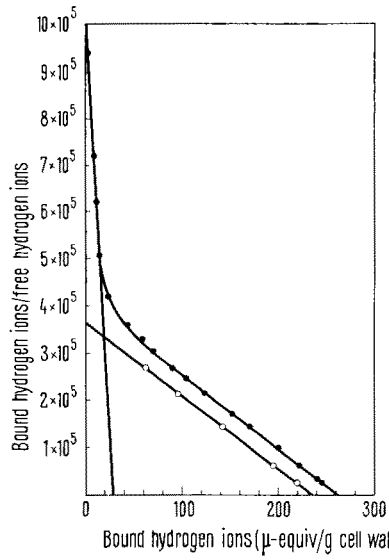

Fig. 2. SCATCHARD plot of the titration data for *S. aureus* cell wall. The curve is resolved into 2 components according to mass law equation 1 and the approximations which follow (see text).

Constants for hydrogen ion binding by cell wall of *Staphylococcus aureus* as determined from a mass law treatment of the average data from 3 titrations below pH 9.0

Site	Maximum binding capacity μ-equiv./g (dry weight)	Apparent dissociation constant equiv./l	pK
1	2	7.0×10^{-8}	7.15
2	28	2.0×10^{-5}	4.70
3	232	6.5×10^{-4}	3.19

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Since the composition of the cell wall is highly complex, it is very difficult to try and identify the chemical nature of the dissociable groups and their apparent dissociation constant. However, it may be recalled that, within pH 2 and 6, biologically important chemical groups such as carboxylic and phosphate dissociate.

In *S. aureus* cell wall 262 μ-equiv./g of dissociable groups have been found. Certainly this figure is less than the real number assuming that the terminal carboxylic groups and the side-carboxyl ones were free. Out of the 262 μ-equiv./g found, 28 have pK 4.70 and are certainly identifiable with the carboxylic groups. In fact pK 4.70 is very close to the acetic acid pK and the dissociation pK of carboxylic groups titrated in various proteins (e.g. ribonuclease[9]). Of the remaining 232 μ-equiv./g, it is difficult to state exactly what chemical groups are dissociable with pK 3.19. They may be either terminal α-carboxyl groups (whereas those with pK 4.56 would be side-chain-carboxyl ones[9]) or the phosphate groups of teichoic acid. The highly negative polyribitol phosphate chain certainly acts as the major cell wall polyelectrolyte. Most of the dissociable groups with pK 3.19 are probably phosphate. STROMINGER[10] states that the special structures in some strains of *S. aureus* account for about 20% of the cell wall. In this figure are included glycine polypeptides. Thus, as the *S. aureus* cell wall contains about 12% teichoic acid and, according to the structure reported by BADDILEY et al.[11], the molecular weight of the polymer structural unit is about 490, 1 g of cell wall contains about 245 μ-moles of polymer structural unit. Since each unit contains a dissociable phosphate group, this number agrees well enough with the μ-equiv./g of hydrogen ions bound with pK 3.19. On the other hand, 59.50 μ-moles of total phosphorus were measured on 1 g of *S. aureus* cell wall (strain 22 ISI). When pH is above 6.00 NH_2 groups probably dissociate ($-NH_3^+ \rightarrow NH_2 + H^+$); the pK 7.15 found is very close to the pK 7.8 of NH_2 groups for small molecules[9].

Riassunto. Il cell-wall di *Staphylococcus aureus* è capace di legare cationi. Poichè la capacità legante ioni dipende dal pH del mezzo, si è studiata la capacità legante idrogenioni di cell-wall liofilizzato isolato da *S. aureus*. Titolazioni al di sotto di pH 9.0 hanno dimostrato la presenza di 3 siti leganti con pK rispettivamente di 7,15, 4,70 e 3,19 e capacità massima legante di 2, 28 e 232 μ-equiv./g (peso secco cell-wall). Viene discussa la natura chimica dei gruppi dissociabili.

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Visual and Auditory Evoked Potentials: Specificity of Reticular Formation Modulating Influences

It has become commonplace to consider the brain stem reticular system as exerting diffuse, non-specific influences[1,2]. This conclusion is based largely on studies of lesions and electrical stimulation, both of which are non-selective interventions. The former abolishes all neural conduction in the immediate vicinity, and the latter induces both orthodromic and antidromic impulses in all susceptible synapses and fibres of passage, whether they are facilitatory or inhibitory[3,4]. Chemical stimulation, however, offers a possible solution to these problems since depositing suspected neurotransmitters in the vicinity of synapses may mimic the actions of endogenous neurohumors by activating or inhibiting only those neurons whose postsynaptic membranes are susceptible to that substance[5-7].

A number of investigators have demonstrated modification of sensory evoked potentials by electrical[8-10] and chemical[3,11] stimulation of the reticular formation. In the majority of cases it has been assumed that the reticular influences would have been exerted in like manner over all modalities. The present study was designed to investigate in the same animals the effects of chemical and electrical reticular activation on both visual and auditory evoked potentials.

Methods. Eleven acutely implanted cats were prepared for surgery under ether anesthesia and then maintained on artificial respiration with Flaxedil. Pressure points and incised tissues were topically treated with 2% Xylocaine[12]. Visual and auditory potentials were evoked by single shocks to the optic tract and brachium of the inferior colliculus, respectively. Evoked potentials were recorded from visual cortices I and II, and primary and association auditory cortices[13]. Drugs in solution, adjusted to pH 7.4, consisting of 20 μg of adrenaline bitartrate dissolved in 20 μl of normal saline, and 20 μg of

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