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The use of membrane electrodes in potentiometric titrations of living cells

In 1967 (ref. 1) we described a system of coupled ion-exchange membrane electrodes, which we have since been using extensively in the study of biopolymers[2].

This technique proved to be particularly useful in providing us with synthetic *in vitro* models with which to study bioelectrical phenomena displayed by biomembranes[3,4] and biological processes of high specificity and great significance, *i.e.*, the influence of the counterion radius on structural changes of fibrous muscular proteins[5] and their interaction during contraction[6], lipid–protein[7] and drug–biopolymer[8] interactions.

All this could be achieved by the use of a particular device consisting of two ion-exchange permselective membranes arranged to work as electrodes and characterized by sharply different charge densities; this setup has been extensively discussed elsewhere[1].

Bacteria in aqueous saline suspension might be roughly considered as "colloidal macromicelles" whose charge distribution will affect the mean ionic activity of the medium in the usual way. Thus whenever the charge distribution on the bacterial surface is altered by molecules capable of electrostatic interactions, a change in the mean ionic activity of the solution will take place, which is detectable and can be followed.

The idea of reducing a complicated process such as the interaction between drugs and living microorganisms to a phenomenon which could be evaluated by a simple potentiometric measurement was particularly appealing and a challenge. This is an improvement by which living bacteria can be approached and investigated in a nearly equilibrium state. In fact the small amount of time required for carrying out the measurements and the media used allow us to treat microorganisms almost as chemicals.

It is well known that water-soluble synthetic polypeptides of different amino acid compositions and molecular weights display antibacterial activity both *in vivo* and *in vitro*[9–14].

Studies attempting to correlate the acidic, basic and neutral character of this group of compounds with their biological activity showed that antibacterial action is confined to basic polymers, while the neutral and acidic polypeptides in no way affect bacterial growth[12,14]. These classical investigations suggest that one of the possible mechanisms of action of these compounds must be related to electrostatic interactions between the positively charged polypeptides and the negatively charged biomembranes[11].

In the present preliminary investigation we confined our interest to one microorganism, *Escherichia coli*. 20-h-old cultures grown on nutrient agar were used. The organisms were washed twice with the salt solution used in the final experiment and resuspended in the same salt solution to the required concentration ($1 \cdot 10^{12}$ viable cells per ml). The number of viable cells was determined by plating.

The bacterial suspensions, once prepared, were used immediately. Each set of measurements was preceded by a blank titration in order to evaluate the contribution to the electromotive force of the salt added to the bacterial suspension and its effect

on the charges of the bacterial surface (Fig. 1). It is well established[15] that an increase in the total ionic strength of the medium will reduce the electrostatic attraction between ions with opposite charges. Therefore the saline present will diminish the association between the components and will influence the binding of the polyamino acid to the bacterial surface. Consequently we chose a concentration range ($1 \cdot 10^{-4}$–$1 \cdot 10^{-5}$ M KCl) that is the lowest range possible at which the morphological integrity and survival of the microorganism can be ensured while at the same time the negative effect on the components to associate is reduced to a minimum.

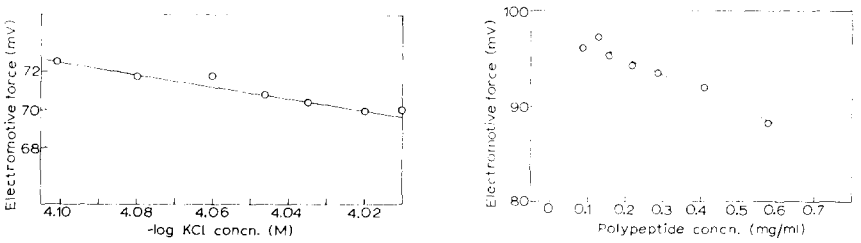

Fig. 1. Electromotive force recorded in a suspension of *E. coli* ($1 \cdot 10^{12}$ viable cells per ml) *plus* $1 \cdot 10^{-4}$ M KCl *vs.* logarithm of KCl concentration when the bacterial suspension is diluted stepwise.

Fig. 2. Electromotive force recorded in solutions of poly-DL-ornithine (n = 4.7) *vs.* the polypeptide concentration.

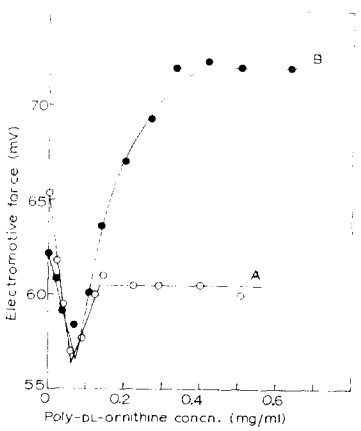

Fig. 3. Electromotive force recorded in a suspension of *E. coli* ($1 \cdot 10^{12}$ viable cells per ml) *plus* $1 \cdot 10^{-4}$ M KCl as a function of the increasing concentration of poly-DL-ornithine (n = 4.7 in Plot A; n = 19.0 in Plot B).

The contribution to the electromotive force of the polymer has been evaluated by means of a calibration curve shown in Fig. 2 and obtained by the stepwise dilution or concentration of a solution of the polypeptide with the electromotive force recorded at each step.

The polyamino acid used was two samples of poly-DL-ornithine with average degrees of polymerization of n = 4.7 and n = 19.0, respectively. They were prepared according to KATCHALSKI AND SPITNIK[16]. The two fractions were separated on Sephadex G-10 and added as an aqueous solution to the bacterial suspension.

In all our experiments the pH values were around 7, mostly 7.2, and no considerable shift from this value was observed throughout the measurements. At this pH value the bacteria and the polymers studied were negatively and positively charged, respectively[11]. All measurements were carried out in a thermostatic bath at 25° ± 0.1° while stirring constantly. The reproducibility of the experimental data obtained by this method is limited only by the necessity of obtaining bacterial suspensions of the same concentrations.

Fig. 3 shows the behavior of a titration of *E. coli* suspension containing $1 \cdot 10^{-4}$ M KCl with a solution of poly-DL-ornithine (4 mg/ml; n = 4.7 in Plot A; n = 19.0 in Plot B).

In agreement with the foreseen electrostatic interaction between components, it might be expected that some changes in the binding of cations in solution should occur. In fact the behavior is different before and after a critical value of the polypeptide concentration. Before this critical concentration, addition of poly-DL-ornithine to the *E. coli* suspension causes the electromotive force to become less positive. After the critical point, any addition of the polymer is responsible for a sharp reversal of this trend. The plots of Fig. 3 show that a new situation occurs at this point where the binding of K^+ to the bacterial surface is at its minimum.

It might be suggested that at this critical point, the association between the polypeptide and the bacteria is at its maximum due to the "dislocation" of K^+ from the binding sites of the bacterial surface indicated by the fall in the measured electromotive force.

Any further addition of polypeptide does not contribute to the potential difference because the constitution of the new system does not differ from a solution of the polymer, containing amounts of a stabilized "complex" between *E. coli* and the polymer itself. It seems to follow that this "complex" binds counterions to the minimum extent at this point.

It appears possible that the elucidation of the electrochemical aspects of this kind of interaction may shed some light on the mechanism of action of natural peptide antibiotics and similar drugs.

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