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THE BUFFER VALUE AND TRANSMEMBRANE POTENTIAL OF *ESCHERICHIA COLI*

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SUMMARY

1. Suspensions of intact and sonically disrupted *Escherichia coli* have been titrated with strong acid and strong base in order to determine the specific buffer value of intact (B_0) and disrupted (B_1) organisms. In deionized water, $B_0 = 0.1097 \pm 0.0143$ mMole/(g·pH) and $B_1 = 0.1875 \pm 0.0172$ mMole/(g·pH). These values are unaffected by the presence of 0.1 M KCl or 0.1 M NaCl. B_1 is assumed to represent the specific buffer value of the cytoplasm.

2. Under the conditions of the experiments, the average ambient internal pH (pH_1) of the organisms was 6.55 ± 0.17 while the average ambient external pH (pH_0) was 7.00 ± 0.36. In the presence of 0.1 M KCl or 0.1 M NaCl, $pH_1 \simeq 5.20$ and $pH_0 \simeq 5.40$ regardless of which salt was used.

3. The data are found to be consistent with the existence of a transmembrane potential (E_m) and outwardly directed active transport mechanism for H^+ across the cell membrane. The minimum E_m is calculated to be —26 mV.

INTRODUCTION

The control of acid–base balance is an important but relatively unstudied element in the homeostasis of bacteria. Unlike vertebrate cells which exist in an extracellular fluid whose pH is closely regulated by the lungs and kidneys, bacteria are frequently subjected to rather severe acid or base challenges. To resist these challenges, bacteria (and vertebrate cells[2]) have three mechanisms for controlling their internal pH: (1) cytoplasmic buffers, (2) control of the flux of H^+ across the cell boundary, and (3) rate of production of H^+ by metabolic processes. KASHKET AND WONG[2] have shown that *Escherichia coli* do in fact regulate their internal pH. By measuring internal pH (pH_1) as a function of external pH (pH_0), they found a linear relationship with a slope (S) less than one. Similar experiments and findings have been reported by ADLER *et al.*[3] for rat diaphragm and by IZUTSU[4] for frog toe muscle.

We have made direct measurements of the buffer value of intact and disrupted *E. coli* in order to examine further the acid–base balance of bacteria. The results are consistent with the existence of an electrochemical gradient and an outwardly

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directed active transport mechanism for H^+ across the cell membranes. The internal buffer capacity is found to be very close to values reported for vertebrate muscle cells. The results permit a lower limit to be placed on the transmembrane potential of *E. coli*.

The pH_i of *E. coli* can be determined by measuring the pH of a suspension of organisms disrupted by sonication[2]. The pH_i determined in this way agrees with values determined by the distribution of the weak acid 5,5-dimethyl-2,4-oxazolidine dione (DMO) across the cell membranes. Experiments of the same type have been performed by ECKEL *et al.*[5] on rat muscle and by a number of other investigators on erythrocytes (see review by WADDELL AND BATES[6]). It is assumed in the present paper that the pH_i determined *via* sonication accurately reflects the internal pH of *E. coli*.

The buffer value of suspensions of disrupted organisms is determined by titration with HCl and NaOH and is taken to represent the internal buffer value (B_i). Titrations of intact organisms yield the external buffer value (B_0). Direct measurement of cellular buffer capacity is not a new idea. BATE SMITH[7] reported such measurements on vertebrate muscles in rigor in 1938. More recently, GILBY AND FEW[8] examined the binding of H^+ to *Micrococcus lysodeiktius* and ECKEL *et al.*[5] determined the buffer capacity of homogenized rat muscle.

METHODS

E. coli B were grown in a defined medium designed by Mr. W. B. Mercer (personal communication) consisting of 5 g glucose, 2 g NH_4Cl, 3 g NaCl, 0.88 g $MgCl_2 \cdot 6H_2O$, 0.115 g Na_2SO_4, 0.004 g $Fe_2(SO_4)_3$, 7.35 g KH_2PO_4, 18.4 g $Na_2HPO_4 \cdot 7H_2O$, and deionized metal distilled water to make 1 l of solution (pH approx. 7.0). All chemicals were A.C.S. Analytical Reagent Grade. The medium was sterilized by filtration through a 0.22-μm Millipore Corp. (Bedford, Mass.) filter. After inoculation with 5 ml of a 6.5-h subculture, the culture flasks were shaken vigorously on a rotary shaker for 17 h at 37°. The organisms were harvested by centrifugation at 16300 × *g* for 10 min, washed once in deionized water, and the pellet stored at 4° for 18 h before use. The usual yield was a 5 g wet pellet per l of medium.

Ten g of pellet which had been washed 2 more times in deionized water (or for some experiments 0.1 M KCl or 0.1 M NaCl), was resuspended in 250 ml of deionized water (pH approx. 5.5) or salt solution (pH approx. 5.6–6.4). Ten-ml aliquots of the suspensions were transferred to 11 centrifuge tubes and the weights of the aliquots determined. The remainder of the suspension was broken into 15-ml aliquots and sonicated on a Branson Sonifier (Model W185D, Heat Systems-Ultrasonics, Plainview, N.Y.) using two 60-sec bursts at setting 6. Ten-ml aliquots of the disrupted suspensions were then transferred to another 11 centrifuge tubes and the weights determined. Acid (0.1 M HCl) or base (0.1 M NaOH) was then added to each tube in varying amounts (0.05–0.15 ml) and the tubes shaken vigorously for a few seconds. The tubes were centrifuged at 36500 × *g* for 10 min and the pH of each supernatant determined using a Model 12 pH meter (Corning Scientific Instruments, Corning, N.Y.). The dry weight of organisms in each tube was determined from triplicate dry weight determinations on 10-ml aliquots of suspensions placed in glass weighing bottles and oven dried until constant weight was reached. Typically, a 10-ml suspen-

sion had a dry weight of 0.09 g. When salt was present, the dry weights were corrected for the additional weight of the salt.

The buffer value of a solution is in general given by the number of mmoles of base added per l of solution divided by the resulting pH change. The basic unit is therefore the mmoles/(l·pH) which has been defined by WOODBURY[1] as 1 Slyke. In the present experiments the buffer value depends upon the concentration of organisms so a more meaningful measure is the specific buffer value defined as the buffer value of a 1-l solution containing 1 g (dry wt.) of organisms. Consequently, the specific buffer value (B) for the intact (B_0) and disrupted (B_1) organisms was determined by plotting the number of mmoles of base (or acid) added per g dry wt. against the pH of the supernatants (pH range: 5–9). The slopes of the curves were determined from a least squares fit of the data using a Wang Laboratories (Tewksbury, Conn.) Model 700 A Programmable calculator. The specific buffer value (B_1 or B_0) was taken as this slope with units mmoles/(g·pH) = Slykes/g per l.

RESULTS

The results of a single experiment on suspensions in deionized water are shown in Fig. 1. A difference in specific buffer value between the intact and disrupted organisms is clearly seen. In this particular case the intact (external) specific buffer value (B_0) is 0.103 mmole/(g·pH) while the disrupted (internal) specific buffer value (B_1) is 0.184 mmole/(g·pH). The figure also shows that the ambient (no added acid or base) $pH_0 = 7.02$ with $pH_1 = 6.57$ indicating that the cytoplasm is slightly more acid than the environment which is usually the case for vertebrate cells[1,4]. When about 0.1 mmole of acid per g dry wt. is added to a suspension, $pH_1 = pH_0 \simeq 6.0$ (iso-pH point). KASHKET AND WONG[2] obtained an iso-pH of 7.31 using a slightly

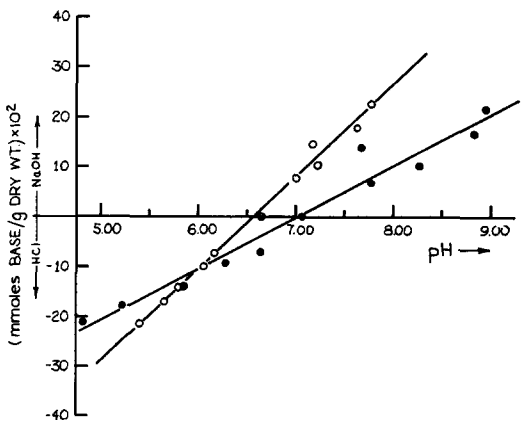

Fig. 1. Titration curves of suspensions of intact (●) and disrupted (○) *E. coli* in distilled water. The curves are obtained by measuring the pH of the supernatants after incremental additions of strong acid (HCl) or strong base (NaOH). The linear curves were obtained by the method of least squares. The slope of a curve gives the specific buffer capacity (B). In this single experiment, the intact value (B_0) was 0.1030 mmole/(g·pH) while the disrupted value (B_1) was 0.1840 mmole/(g·pH). The buffer curves intersect at pH 6.00 which is called the iso-pH point. The ambient pH (*i.e.* no added acid or base) of the intact suspensions is 7.02 while the disrupted ambient value is 6.57.

different titration procedure. This undoubtedly reflects a difference in growth conditions, *E. coli* strains, and methods of harvesting.

The measurements of pH_i and pH_0 represent steady-state values since the measured pH values were constant from 10 min (the earliest we could make the determination) to over 4 h after the addition of acid or base.

The averaged results of 14 experiments in both deionized water and 0.1 M salt solutions are summarized in Table I. Using Student's *t*-test, there was no significant difference in the B_i or B° between the three cases (water, 0.1 M KCl, or 0.1 M NaCl). Thus, added salts have no effect on the specific buffer value of the intact or disrupted organisms. There is, however, a significant difference in the ambient pH's between water and 0.1 M salt solutions. In deionized water $pH_0 = 7.00$ and $pH_i = 6.55$ while in either 0.1 M NaCl or 0.1 M KCl pH_0 is approx. 5.40 and pH_i is approx. 5.20. At the same time, the iso-pH shifts from 5.87 to 5.00 (Table II). The cause of these salt effects is not known but it may indicate an exchange of H^+ for Na^+ or K^+. It is somewhat surprising that Na^+ and K^+ have equal effects since in *E. coli* they are not metabolically equivalent[9,10]. In this regard it is important to note that the organisms' metabolic activity in the present experiments is very low because of the absence of substrate and the conditions of harvesting.

The averaged data may be compared to those of KASHKET AND WONG[2] by

TABLE I

SUMMARY OF DATA: AMBIENT pH AND BUFFER VALUES OF *E. coli* IN VARIOUS SOLUTIONS

S.D., standard deviation. Specific buffer capacity units: mmoles/(g·pH). In the text, intact buffer value = B° and disrupted buffer value = B_i.

Solution	*State of organism*	*Average ambient pH ± S.D.*	*Average specific buffer value ± S.D. × 10²*	*Number of experiments*	*Average linear regression coefficient*
Water	Intact	7.00 ± 0.36	10.97 ± 1.43	7	0.970
	Disrupted	6.55 ± 0.17	18.75 ± 1.72		0.993
0.1 M KCl	Intact	5.40 ± 0.43	9.07 ± 1.79	4	0.983
	Disrupted	5.20 ± 0.17	17.55 ± 3.35		0.983
0.1 M NaCl	Intact	5.45 ± 0.44	8.23 ± 1.41	3	0.984
	Disrupted	5.19 ± 0.20	19.36 ± 2.27		0.996

TABLE II

SUMMARY OF DATA: ISO-pH AND SLOPE (S) OF pH_i *vs.* pH_0 FOR *E. coli* IN VARIOUS AQUEOUS SOLUTIONS

Iso-pH: pH_0 when $pH_0 = pH_i$; $S = B_0/B_i$, see Table I and text. S.D., standard deviation obtained by propagation of errors.

Solution	*Iso-pH*	$S \pm S.D.$
Water	5.87	0.601 ± 0.096
0.1 M KCl	4.99	0.517 ± 0.142
0.1 M NaCl	5.00	0.425 ± 0.088

plotting pH_i against pH_0 using pairs of points obtained from equal additions of acid or base. For deionized water, this results in a straight line whose slope S is 0.60 with an iso-pH of 5.87. The averaged results for all cases are summarized in Table II. The data suggest that $S_{H_2O} > S_{KCl} > S_{NaCl}$. However, Student's t-test does not indicate a statistically significant difference in the values of S. Since both pH_i and pH_0 are linear in the amount of acid or base added per g dry wt., $S \equiv B_0/B_i$.

We have observed from many experiments conducted over a period of a year that B_i and B_0 are quite reproducible. However, wide variations in ambient pH and iso-pH have been seen. From the series of experiments described here (carried out over a period of several months) they were fairly reproducible. The cause of the variations could not be determined. They were not related to the pH of the growth medium observed at the time of harvesting.

DISCUSSION

Our value of S (0.60) for *E. coli* in deionized water compares favorably to that calculated from the data of KASHKET AND WONG[2] (0.57) and to that of rat diaphragm[6] (0.6) but is somewhat smaller than 0.8 estimated from the data of IZUTSU[4] for frog toe muscle. The internal buffer capacity in 0.1 M NaCl of 0.194 ± 0.023 mmoles/(g·pH) is about the same as the values obtained by ECKEL *et al.*[5] of 0.195 ± 0.029 and 0.158 ± 0.010 for muscle of rat.

The internal specific buffer value is about twice the external value. This can only mean that the surface membranes are a significant barrier to the diffusion of H^+. If the H^+ was freely admitted, the internal buffers would be completely accessible to the added acid or base and B_i would equal B_0. In all cases we observe that the ambient external H^+ concentration $[H^+]_0$ is smaller than the ambient internal concentration $[H^+]_i$. If only simple chemical gradients were operating, one would expect that in the steady-state $[H^+]_0 = [H^+]_i$. Thus, it is necessary to assume the existence in *E. coli* of mechanisms for maintaining H^+ concentration gradients.

In the steady-state the time rate of change of $[H^+]_i$ ($d[H^+]_i/dt$) must equal zero[1,11]. This means that the net rate of passive diffusion into or out of the cell must balance the rate of production of H^+ by metabolic sources and the loss of gain of H^+ by active or other transport processes. Mathematically,

$$\Phi^{P}_{net} + \Phi^{A} + Q^{M} \cdot \frac{V}{A} = 0 \tag{1}$$

Where Φ^{P}_{net} (moles/cm^2 per sec) is the net passive flux of H^+ due to an electrochemical gradient, Φ^{A} (moles/cm^2 per sec) is the active transport flux, Q^{M} (mmoles/per sec) is the rate of production of H^+ by metabolic processes, and V/A is the volume to area ratio of the organism. Outward fluxes are defined as positive. If it is assumed for a moment that $\Phi^{A} + \Phi^{M} \cdot (V/A) \simeq 0$ so that H^+ must be in electrochemical equilibrium across the membrane, then one can calculate the transmembrane potential E_M (in mV) from the Nernst equation[11] given (at 20°) by

$$E_M = 58 \log_{10} \frac{[H^+]_o}{[H^+]_i} \tag{2}$$

or

$$E_M = 58(pH_i - pH_o) \tag{3}$$

Taking ambient pH values for deionized water from Table I gives a value of E_M = —26 mV (inside with respect to outside). Under the conditions of our experiments Q^M is expected to be minimal and in the first approximation may be taken as zero so that in the steady-state $\Phi^P_{net} = -\Phi^A$. Little is known about the active transport of ions in bacteria but it appears that in many microorganisms that K^+ ions are actively absorbed in exchange for H^+ (ref. 9). It seems reasonable to assume that there may be an outwardly directed active transport of H^+ which would be consistent with the behavior of H^+ in vertebrate cells[1]. If there is an outward active flux of H^+ there must be a net passive inward flux and the calculated E_M would represent a minimum value. That is, the transmembrane potential would need to be more negative than the equilibrium value of —26 mV in order to have a net inward flux to maintain the steady-state.

The ion or ions responsible for establishing the membrane potential are not known. K^+ is a likely candidate since it appears to be actively transported into the cell. If H^+ were distributed in equilibrium in the presence of 0.1 M KCl, then Table I indicates that the ambient $pH_i - pH_0 = -0.2$. Eqn. 3 would give an $E_M = -12$ mV suggesting a depolarization of the membrane in the presence of high K^+ concentrations. This would be expected if the membrane behaved as a K^+ electrode and a K^+ concentration gradient were maintained by an active transport process. However, the situation is not clear-cut since Table I also shows that 0.1 M NaCl has a similar effect on $pH_i - pH_0$. Since *E. coli* thrive in a variety of environments it might be expected that the organism's transmembrane potential depends upon several ions such as Na^+, K^+ and H^+. The exact potential would be determined by the relative permeabilities of the ions and their concentrations[11].

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