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STOPPED-FLOW STUDIES OF SALT-INDUCED TURBIDITY CHANGES OF *ESCHERICHIA COLI*

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SUMMARY

1. The kinetics of salt-induced increases in turbidity of suspensions of *Escherichia coli* have been investigated using a GIBSON–MILNES type stopped-flow spectrophotometer (*Biochem. J.*, 91 (1964) 161).

2. The rates of increase in turbidity were dependent on the medium osmotic pressure rather than its molarity or ionic strength.

3. Increases in turbidity were first order with respect to the concentration of the added salt. However, using a given concentration of added salt, the rates of increase in turbidity were shown to be linear, suggesting that the rates were zero order with respect to the intracellular osmotic pressure.

INTRODUCTION

Addition of many electrolytes or non-electrolytes to suspensions of Gram-negative bacteria causes increases in turbidity[1–7,9]. These increases in turbidity are thought to be due to plasmolysis[1–5] or decreases in whole cell volume[6,7]; water moving out of the cell in order to regain osmotic equilibrium between the cytoplasm and the environment. There are exceptions to this situation since increases in media NaCl concentration cause increases in turbidity of a marine Pseudomonad despite penetration of NaCl into the cell[8,9]. In this case decreases in cellular volume are due to ionic interaction of NaCl with the cell envelope[9]. However, in *Escherichia coli* various salts and sucrose cause plasmolysis and increases in turbidity that are dependent on the medium osmotic pressure[5]. In addition electrolytes but not non-electrolytes cause contraction in whole cell volume, presumably due to electrostatic interaction with the cell wall[5,10].

Turbidity changes induced by increases in medium solute concentration are very rapid, and study of the changes with a conventional spectrophotometer only enables measurement of total turbidity increases. To the authors' knowledge very little work has been done on the kinetics of these turbidity changes[3,6]. The work

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reported in this paper examines the kinetics of salt-induced increases in turbidity of *E. coli* using a stopped-flow spectrophotometer and shows their osmotic dependence.

MATERIALS AND METHODS

E. coli, strain K-12, was grown aerobically at 37° to the mid-log phase on a glycerol–casamino acids medium.[11] Cells were harvested by centrifugation for 10 min at 10000 × *g* at 4°. They were then washed in 10 mM Tris–maleate (pH 7.0) recentrifuged and resuspended in 10 mM Tris–maleate (pH 7.0) to a final absorbance of approximately 5.0 at 700 nm (1 cm cuvette). The cells were maintained in ice until required (not more than 3 h) and then diluted immediately before the experiment with 10 mM Tris–maleate (pH 5.0) to give an absorbance of approx. 0.5 at 700 nm.

The kinetics of salt-induced turbidity changes were measured in a modified GIBSON–MILNES stopped-flow spectrophotometer[12,13]. The instrument was maintained at 37° by circulating thermostatted water around the flow unit. The apparatus was operated in the transmission mode with a 2-cm path-length observation tube. Changes in transmittance at 700 nm occurring after mixing a suspension of *E. coli* with an equal volume of salt solution (in 10 mM Tris–maleate, pH 5.0) were displayed as the vertical deflection of a trace on a storage oscilloscope screen. Time was displayed as the horizontal co-ordinate, the timescale being determined by the oscilloscope sweep-rate. Data were collected by photography of the oscilloscope traces. The photographs were then enlarged onto graph paper and the *Y*-deflections converted from transmittance to absorbance, using the Beer–Lambert law.

RESULTS

Addition of salts to lightly buffered suspensions of *E. coli* causes rapid increases in turbidity (Phase I effects)[2,4]. Once maximal turbidity increases have been obtained, the turbidity is either maintained or decreases (Phase II effects)[4]. Preliminary experiments showed that Phase II decreases in turbidity were K^+ dependent in this strain of *E. coli* and optimal rates occurred at pH 5.0 and 37°. Phase I increases in turbidity were therefore measured at pH 5.0 and 37°.

Fig. 1 shows the results of a typical experiment. Addition of $MgCl_2$ to a suspension of *E. coli* to give a final $MgCl_2$ concentration of 0.4 M resulted in an immediate decrease in transmittance to a minimal value after about 80 msec. When cells were mixed with an isotonic solution of buffer there was little or no noise or artifactual increases or decreases in transmittance of the type reported by BLUM AND FORSTER[14] for erythrocytes and *E. coli* and LOVETT[6] for *Serratia marcescens*. These workers assumed that the artefacts they obtained were due to flow-dependent alignment of the non-spherical cells in the observation chamber. The artifact could be reduced, but not eliminated, by use of baffles designed to induce turbulence. Since we did not observe such an artefact with the present apparatus it would appear that the critical velocity of flow was always exceeded, resulting in complete turbulence.

When the results are replotted in terms of absorbance (Fig. 2) it can be seen that increases in turbidity were linear for most of the rise period. When differing concentrations of a given salt were used and the initial linear rates of absorbance increase plotted against salt concentration, a straight line graph is obtained (Fig. 3a).

Thus the rate of increase in absorbance is proportional to the medium salt concentration (up to an initial concentration of at least 0.5 M $MgCl_2$, the highest concentration used). Furthermore, if two different salts were used the rates of increase in absorbance could be seen to be dependent on the osmotic pressure exerted by the applied salt (Fig. 3b) and not its molarity (Fig. 3a) or its ionic strength. Similar results were obtained with $MgCl_2$, $CaCl_2$, NaCl, RbCl, KCl, KBr, KI and KNO_3.

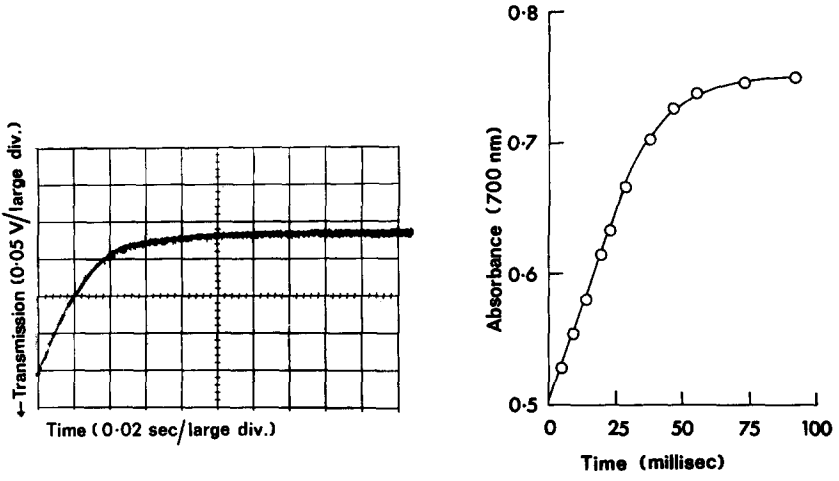

Fig. 1. Oscilloscope trace of the change in transmission at 700 nm on addition of $MgCl_2$ to *E. coli*. $MgCl_2$ in 10 mM Tris–maleate (pH 5.0) was mixed with an equal volume of *E. coli* suspended in 10 mM Tris–maleate (pH 5.0) in a stopped-flow spectrophotometer as described in METHODS. The final $MgCl_2$ concentration was 0.4 M and the final *E. coli* concentration was 0.27 mg dry wt. per ml. Experimental temperature was 37°.

Fig. 2. The change in absorbance on addition of $MgCl_2$ to *E. coli*. The data given in Fig. 1 are redrawn in terms of absorbance, derived from transmission using the Beer–Lambert law.

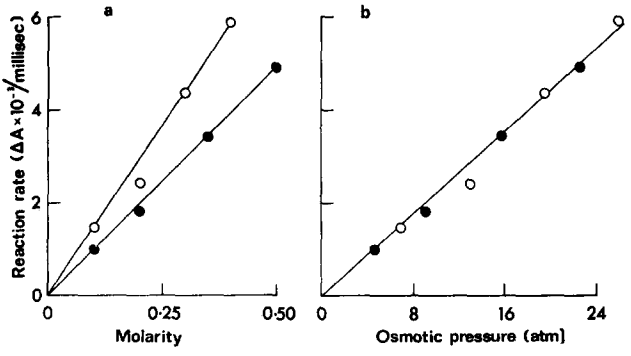

Fig. 3. The rate of increase in turbidity of *E. coli* at different salt concentrations. *E. coli* suspended in 10 mM Tris–maleate (pH 5.0) was mixed with equal volumes of NaCl (● —●) or $MgCl_2$ (○—○) in 10 mM Tris–maleate (pH 5.0) to give the desired final salt concentrations and the increases in absorbance measured in a stopped-flow spectrophotometer at 700 nm. The rates of increase in turbidity are expressed in terms of (a) molarity, and (b) osmotic pressure of the added salts. Experimental temperature was 37°.

Expressed mathematically the results are first order, and

$$\frac{dA}{dt} = k\pi$$

where A is absorbance, t is time from addition of the salt, π is the medium osmotic pressure and k is the rate constant. In the experiment shown in Fig. 3 the rate constant (k) was $0.825 \cdot 10^{-3}$ increase in absorbance per msec per mg dry wt. of bacteria per ml for an applied medium osmotic pressure of one atmosphere. Consistent values of k were obtained with separate samples of bacteria ($0.74 \cdot 10^{-3}$ to $0.91 \cdot 10^{-3}$ in 5 different experiments). KOCH[15] has shown that changes in intracellular volume of *E. coli* are almost inversely proportional to changes in absorbance and therefore the rates of volume decrease must be almost proportional to the increases in medium osmolarity.

The turbidity increases did not appear to be under metabolic control since, using 0.2 M KCl to induce the turbidity changes, the rates were independent of pH (pH 5.0, 7.0 and 9.0) and were unaffected by the metabolic inhibitors 2,4-dinitrophenol ($2 \cdot 10^{-4}$ M), carbonyl cyanide *m*-chlorophenyl hydrazone ($2 \cdot 10^{-5}$ M), cyanide ($1 \cdot 10^{-2}$ M), iodoacetate ($2 \cdot 10^{-3}$ M) and *p*-chloromercuribenzoate ($4 \cdot 10^{-5}$ M).

DISCUSSION

The osmotic dependence of the salt-induced turbidity changes of *E. coli* suggest that increases in medium osmotic pressure are compensated by a movement of water from the cell, thus reducing the intracellular volume, in order to regain osmotic equilibrium between the cell and its environment. For a given increase in extracellular osmotic pressure it would be expected that the net rate of water flow from the cell would be dependent on the difference in osmotic pressure between the inside and the outside of the cell. The rate of increase in absorbance on addition of a salt to a suspension of *E. coli* should therefore be exponential, since as water flows from the cell the intracellular osmotic pressure rises to that of the medium. The osmotic pressure of the medium would be unaffected by this movement of water, as the total intracellular volume of the dilute suspension is negligible compared to that of the medium.

Fig. 2 shows that such an exponential increase in turbidity did not occur and that the increase was linear for most of the rise period. The results therefore indicate that the increases in turbidity are first order with respect to the medium osmotic pressure but zero order with respect to the intracellular osmotic pressure. The reasons for the latter observation are at present unknown, but the interpretation of water movements as given above may be an oversimplification, as it does not take into account the mechanical strength of the wall and the turgor pressure that it will exert at low medium osmotic pressures[16]. Furthermore volume changes are not exactly inversely proportional to absorbance changes[15]. An alternative interpretation of these results could be based on SCHOLANDER'S[17] theory of volume changes due to the external solute pressure of the medium.

Our results agree with observations on salt-induced total turbidity changes of bacteria in that they are dependent on the medium osmotic pressure[5]. This is an osmotic effect, therefore, rather than an ionic contraction of the wall as has

been shown for the effect of NaCl on a marine Pseudomonad[8,9], though this does not exclude the possibility of such an interaction simultaneously occurring[5]. The kinetics of salt-induced turbidity increases of *E. coli* are rather different to those of *Pasteurella tularensis*[3]. In the latter organism the rates of increase of turbidity are about four orders of magnitude slower than those of *E. coli* and are exponential (first order) for a given concentration of added salt. Furthermore the rate constant varies with the concentration of the added salt and depends on whether the added salt is mono- or divalent[3]. It may be that *P. tularensis* is atypical in its responses: certainly the magnitude of the total turbidity changes are much larger in this organism than is found with other Gram-negative bacteria, as well as the rates of increase of turbidity being much slower than is usual.

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