Inorganica Chimica Acta, 46 (1980) L33–L34

Intracellular Potassium Level: Possible Trigger for Bacterial Logarithmic Growth

WUNG-WAI TSO* and WAI-PING FUNG

Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

Received November 8, 1979

Some recently synthesized macrocyclic polyethers (crown ethers) [1], being able to form stable complexes with alkaline metal ions and to incorporate in the lipid fraction of the cell membrane, have aroused considerable research interest in the field of biochemistry [2–4]. 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) and 2,3,11,12-dicyclohexyl-1,4,7,10,13,16-hexaoxacyclooctadecane (dicyclohexyl-18-crown-6), two representatives of the group, have been shown to be highly toxic to mammals [1, 5]. The toxicity, however, when tested in prokaryotes, appears to be less severe and at *sublethal* concentrations crown ethers produce a growth lag and reduce the population size at the initial stationary phase [6]. Both effects are crown ether concentration-dependent. In brief, the crown ethers exhibit ionophoretic properties and behave very similarly to the natural ionophores, gramicidin, valinomycin, nigericin and monactin, by influencing the transport of monovalent cations across the cell membrane [3, 7–10]. In this letter, we report on our finding that the toxic effect of 18-crown-6 on *Escherichia coli* growth can be *specifically* reversed by potassium ion.

Experimental

Bacterial strain AW405, a derivative from *Escherichia coli* K12, was used [11]. 18-crown-6 was purchased from Sigma and was used without further purification. Other chemicals used were of analytical grade. All glasswares were rinsed twice with glass distilled water and sterilized where necessary.

An amount of twenty millilitres of sterile 1% tryptone broth (Difco) containing either (i) 18-crown-6 alone or (ii) 18-crown-6 plus sodium chloride (at two final sodium ion concentrations: 2×10^{-1} M and 4×10^{-1} *M*) or (iii) 18-crown-6 plus potassium chloride (also at final concentrations equivalent to their sodium counterparts) or (iv) no extra chemical addition, was aseptically dispersed into a series of optically matched, sterile Klett flasks

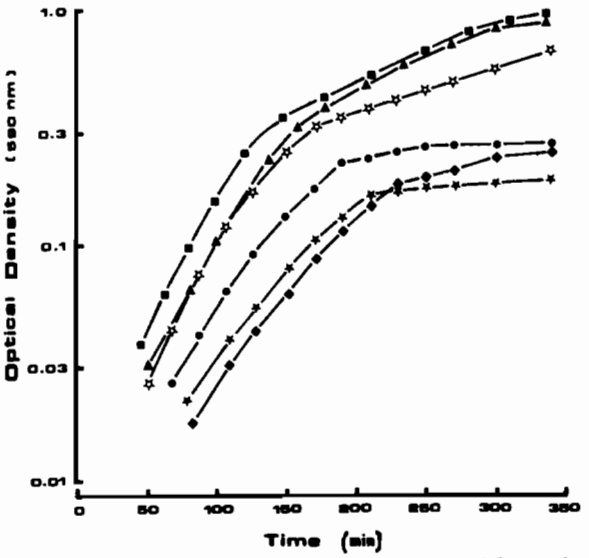

Fig. 1. Effect of 18-crown-6 and cations on bacterial growth. Growth curves of *E. coli* in (☆) tryptone broth alone, and in tryptone broth supplemented with (★) 18-crown-6, (●) 18-crown-6 plus 0.2 *M* NaCl, (◆) 18-crown-6 plus 0.4 *M* NaCl, (■) 18-crown-6 plus 0.2 *M* KCl and (▲) 18-crown-6 plus 0.4 *M* KCl.

(screw-capped flask with side arm attachment for spectrophotometric measurement). The concentration of 18-crown-6 used was 10^{-2} *M*. To each flask, approximately 0.1 ml aliquot of a tryptone broth containing healthily grown, early logarithmic phase culture of *Escherichia coli* was added so that the inoculated media contained about 10^6 viable cells per ml. Usually the initial optical density reading at 590 nm read below 0.02. All flasks were then incubated at 37 °C in a gyrotory water bath shaker (model G76, New Brunswick Scientific). Absorbances were measured at intervals of 20 minutes using a Spectronic-70 (Bausch and Lomb) colorimeter. Sterile tryptone broth served as the blank.

Results

The presence of sublethal level of crown ether in the growth medium affected the three phases in the growth curve: an appearance of a lag period, a decrease in the slope at the logarithmic phase and an early development of the initial stationary phase at a lower microbial population as shown in Fig. 1. Of these changes, the effect on the logarithmic phase slope is in general less severe and was found to be insignificant at slightly lower 18-crown-6 concentrations [6]. Since tryptone broth at the culture concentration contained 7.5×10^{-4} *M* potassium and $1.2 \times$

*Author to whom all correspondence should be addressed.

10^{-2} *M* sodium, the addition of monovalent cation in the range of 10^{-1} *M* will overwhelm the background cationic effect. As shown in the figure, the growth curves of cultures containing 18-crown-6 supplemented with potassium and sodium salts respectively fell into two groups. Those with potassium resembled the original non-crown ether growth profile and those with soidum still left the crown ether toxic effect unshadowed. The effect of two doses of sodium on the lag further indicated that higher sodium content does not reduce the lag. To avoid complication due to high osmolarity the highest ion concentration attempted was limited to 0.4 *M*.

Discussion

In a simple synthetic system, crown ether distributes mainly in the organic phase [12]. It has also been shown by solvent extraction studies that both free and complex forms essentially stay in the organic phase [12, 13]. In bacterial culture, it is most likely that the crown ethers are trapped predominantly in the membranous fraction of the bacteria, either in the free or in the complex form. An addition of potassium will not remove the trapped crown ether from the membrane and that reduction of toxicity by potassium ion should not be interpreted as a dissociation of the crown ether from the membrane by complexing with potassium. Apparently, this effect of potassium in releasing the 18-crown-6 toxicity can be stemmed from either that the toxic, free crown ether has been converted to its harmless complex form, or that the addition of potassium has replenished the intracellular potassium content that had leaked out due to the incorporation of ionophoretic 18-crown-6.

Crown ether has high complexation affinity for monovalent cations and in 18-crown-6, the complexation affinity is more specific for potassium [4, 13]. According to this nature, unless the two cationic complexes are toxicologically different, otherwise we should expect that a higher sodium ion concentration will give a growth profile similar to that resulted from potassium addition. The observation that higher sodium content (0.4 *M*) neither shortens the lag nor raises the optical density at the initial stationary growth phase indicates that the cationic complexation is not the main factor in dictating crown ether toxicity. A bacterium in the lag phase has high potassium but low sodium content intracellularly [14]. Since the incorporation of antibiotic ionophores led to a rapid loss of the monovalent cation gradient by exchanging with the external medium [9], the potassium specific effect thus observed leads to the possibility that a critical minimal intracellular potassium concentration has to be established prior to the logarithmic phase growth. In *Streptococcus faecalis*, an inhibition of growth by natural antibiotics has been shown to be reversed by excess potassium addition [8, 9]. In *E. coli* cultures containing *sublethal* level of crown ether and insufficient potassium ion, a process to compensate for the ionophoretic effect, such as an activation of the potassium active transport system, is needed for growth. As stationary grown *E. coli* contains lower potassium content than those in the logarithmic phase [14], a potassium concentration shift parallel to a shift in the growth phase may then suggest a possible role played by this ion in the triggering of logarithmic growth. Further work towards the understanding of this mechanism is in progress.

Acknowledgement

The authors wish to thank K. K. Chan and K. H. Wong for their helpful discussions.

References

1 C. J. Pedersen, *J. Amer. Chem. Soc., 89,* 7017 (1967).
2 H. A. Lardy, *Fed. Proc., 27,* 1278 (1968).
3 B. C. Pressman, *Fed. Proc., 27,* 1283 (1968).
4 J. J. Christensen, J. O. Hill and R. M. Izatt, *Science, 174,* 459 (1971).
5 K. Takayama, S. Hasegawa, S. Sasagawa, N. Namber and T. Ngagai, *Chem. Pharm. Bull., 25,* 3125 (1977).
6 W.-W. Tso and W.-P. Fung, to be published.
7 D. C. Tosteson, *Fed. Proc., 27,* 1269 (1968).
8 F. M. Harold and J. R. Baarda, *J. Bacteriol., 94,* 53 (1967).
9 F. M. Harold and J. R. Baarda, *J. Bacteriol., 95,* 816 (1968).
10 G. Eisenman, S. M. Ciani and G. Szabo, *Fed. Proc., 27,* 1289 (1968).
11 R. Mesibor and J. Adler, *J. Bacteriol., 112,* 351 (1972).
12 K. H. Wong, K. Yagi and J. Smid, *J. Membrane Biol., 18,* 379 (1974).
13 C. J. Pedersen, *Fed. Proc., 27,* 1305 (1968).
14 S. G. Schultz, N. L. Wilson and W. Epstein, *J. Gen. Physiol., 46,* 343 (1968).