

¹³C-NMR AND ²³Na-NMR STUDIES ON A HALOPHILIC EUBACTERIUM
H. Gilboa*, R. Regev*, I. Pery* and Y. Avi-Dor*

Departments of Chemistry* and Biology+
Technion - Israel Institute of Technology
Haifa 32000, ISRAEL

ABSTRACT

Carbon-13 nmr measurements were carried out in order to determine the compatible solutes in the cytosol of the halophylic bacterium Ba₁ at different salt concentrations of the growth medium. On a defined growth medium the main organic solute accumulated in the cytosol was ectoine. If glycine betaine was added to the growth medium, betaine was the accumulated solute. Sodium-23 nmr spectrum shows that the intracellular "free" sodium concentration is about 0.1M NaCl when the Sodium concentration of the growth medium was 1M.

Osmotic adaptation by halophilic eubacteria is achieved by partial exclusion of salts and their replacement by organic solutes of low molecular weight which have the double role of osmoregulators and osmoprotectors(1). Difficulties in identifying the organic solutes were resolved by the application of ¹³C-nmr techniques. The estimation of the intracellular salt concentration, and especially that of Na⁺, yielded contradictory results, mainly because it required separation of the extracellular and intracellular compartments. Recently, by using ²³Na-nmr spectroscopy in the presence of paramagnetic shift reagents, the intracellular sodium concentrations were estimated(2,3).

In the present work, a halophilic eubacterium, Ba₁(4) was used which is known to accumulate, in response to osmotic stress, glycine-betaine from the medium. It is, however, not known whether betaine also plays a role in osmoregulation when the cells are compelled to synthesize the latter from a

simple carbon source. There is also little information on the intracellular Sodium concentration in Ba₁ under various conditions of growth.

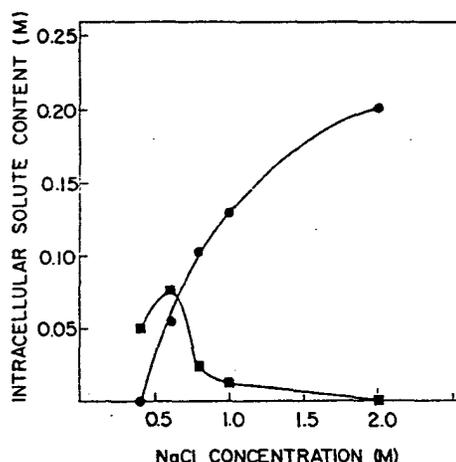


Fig.1 Effect of NaCl concentration in Defined Glucose Medium on the intracellular solute content of cells of Ba₁. (●), Ectoine; (■), Trehalose.

In cells grown on a defined glucose medium, at external NaCl concentrations over 0.4M, ¹³C nmr shows that the accumulated low molecular weight organic solutes were trehalose and ectoine. The concentrations of the solutes depend on the external salt concentration. Figure 1 shows the concentration of these solutes in the cytosol as a function of the NaCl concentration in the medium. It is clear from Fig.1 that trehalose could be

assigned as a "weak" compatible solute while ectoine is a "strong" one. Ectoine is a novel cyclic amino acid that was discovered in a halophilic phototrophic bacterium by Galinski et al.(5). When betaine was added to the medium it accumulated in the cells and replaced ectoine.

The concentration of the cell-associated sodium was determined by ^{23}Na nmr using the shift reagent $\text{Dy}(\text{TPP})_2^{-7}$ (Fig.2). At 1M NaCl of the growth medium the inner "free" sodium was about 0.1M. This may give a total inner concentration of 0.25M if only 40% of the sodium was detected.

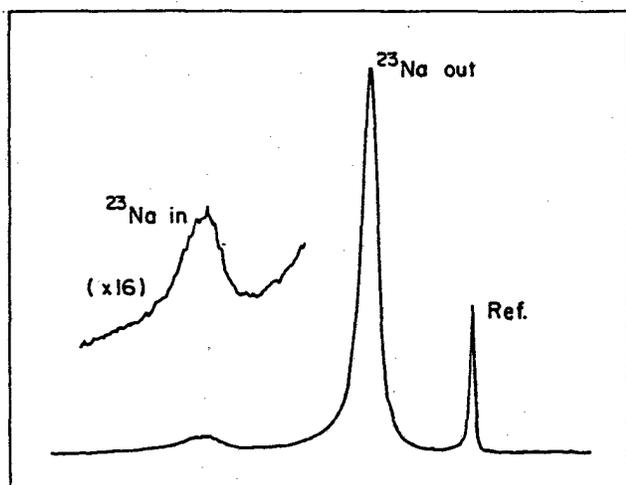


Fig.2 ^{23}Na nmr spectrum of Ba₁ grown on 1M NaCl.

Studies on an unrelated microorganism vibrio costicola show similar results to these for Ba₁.

REFERENCES

1. Imhoff, J.F. (1986) FEMS Microbiol. Rev. 57-66.
2. Castle, A.M., Macnab, R.M. and Shulman, R.G. (1986), J. Biol. Chem. 261, 3288-3294.
3. Rotman, A. Gilboa, H. Schechter, Y. and Silver B.L. (1986) Proceedings of XXIII congress AMPERE, Roma, Italy, pp 532-533.
4. Rafaeli-Eshkol, D. (1968), Biochem. J. 109, 679-685.
5. Galinski, E.A., Pfeiffer, H.P. and Truper, H.G. (1985), Eur. J. Biochem. 149, 135-139.