Na^+ DEPENDENT ACTIVE TRANSPORT OF α-AMINOISOBUTYRIC ACID INTO CELLS OF A MARINE PSEUDOMONAD

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Previous work has revealed a specific requirement of a number of marine bacteria for Na^+ for growth and metabolism (MacLeod and Onofrey, 1957; MacLeod, et al., 1958; Payne, 1960; Tyler, et al., 1960). Neither the intracellular enzymes investigated (MacLeod and Hori, 1960; Pratt and Happold, 1960) nor the membrane adenosine triphosphatase present (Drapeau and MacLeod, 1963) could be shown to be specifically activated by Na^+. This suggested that Na^+ might be involved in the transport of substrates into the cell. In order to test this possibility, it was necessary to dissociate the uptake of substrates from their subsequent metabolism. For this purpose, non-metabolizable analogues of metabolizable substrates have been used.

The organism studied, marine pseudomonad B-16, has been the subject of a number of previous investigations (cf.MacLeod and Matula, 1962). It is typically marine in that it requires Na^+ specifically for growth and oxidative metabolism and lyses in distilled water. The cells, grown in 1% trypticase, 0.5% galactose dissolved in a solution of appropriate salts were harvested by centrifugation and washed three times by resuspension in and centrifugation from volumes of 0.05M $MgSO_4$ equal to those of the growth medium.

For transport studies cells of the organism were suspended at a level of 200μgm dry wt per ml in a medium containing 0.01M

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KCl, 0.026M $MgSO_4$, Tris(hydroxymethyl)aminomethane phosphate buffer pH 7.2, 0.1M, galactose 0.1%, chloramphenicol 100μg per ml., α-amino-isobutyric acid -1-C^{14}(C^{14}-AIB), 5 x 10^{-5}M(0.22 μcuries per μmole) and varying concentrations of NaCl. The suspensions were contained in erlenmeyer flasks incubated in a waterbath shaker at 25°. At appropriate intervals 1.0 ml aliquots of the suspensions were filtered through Millipore HA filters. The cells on the filter were washed three times with 1 ml portions of the salt solution used for the preparation of the growth medium. The filters and their adhering cells were transferred to vials, air-dried and their radioactivities measured using a Packard Tricarb Liquid Scintillation Spectrometer.

Respirometer studies revealed that under conditions permitting the oxidation of other amino acids by the marine pseudomonad B-16 (Tomlinson and MacLeod, 1957) no oxygen uptake due to the addition of α-aminoisobutyric acid (AIB) occurred. This compound is also not metabolized by animal tissues and has been suggested as a model for the study of amino acid transport (Christensen and Jones, 1962).

Washed cells of the marine pseudomonad when incubated with C^{14}-AIB rapidly accumulated radioactivity in the presence but not in the absence of NaCl (Fig. 1). At the steady state level the intracellular concentration was approximately 3000 times the extracellular. The addition of unlabelled AIB to the cells resulted in the displacement of radioactivity, as might be expected if AIB were present in the cells in a chemically unaltered form and the steady state level was the result of a balance between uptake of the compound from the medium and its return to the medium from an intracellular pool (Horecker et al., 1960).

Payne has presented evidence interpreted as indicating a role for Na^+ in inductive enzyme formation in marine bacteria (Payne, 1960;

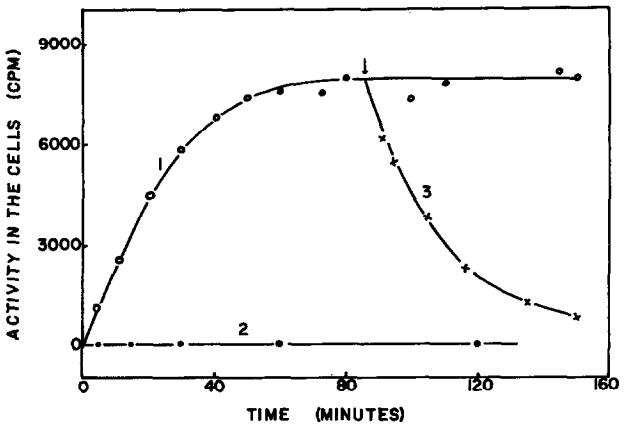

Fig. 1 Requirement for Na^+ for the uptake of C^{14}-∝-aminoisobutyric acid by cells of marine pseudomonad B-16. Curve 1, uptake of radioactivity in the presence of 200mM NaCl; Curve 2, uptake in the absence of NaCl; Curve 3, displacement of radioactivity from cells in a portion of the suspension by the addition at the time indicated by the arrow of unlabelled ∝-aminoisobutyric acid to the medium to a final concentration of 10^{-2}M.

Rhodes and Payne, 1962). The possibility that the uptake of AIB shown in Fig. 1 might be due to the preliminary formation of an inductive enzyme is rendered unlikely not only by the fact that the uptake took place without a lag but also because it occurred in the presence of ten times as much chloramphenicol as Payne found to be necessary to prevent inductive enzyme formation in his marine bacteria.

The specificity of the requirement for Na^+ for the uptake of AIB is shown in Table 1. In the presence of NaCl, a one-hundred-fold increase in radioactivity occurred. None of the other additives had any appreciable effect on the uptake of the compound by the cells.

The presence of an oxidizable substrate (in this case galactose) in the suspending medium could be shown to stimulate the uptake of AIB. That an oxidizable substrate appeared not to be an absolute requirement for the process was probably due to the high endogenous activity of the cells. The question then arose as to whether the requirement for uptake was a direct one concerned with transport

TABLE 1

Specificity of the requirement of marine pseudomonad B-16 for Na^+ for the uptake of α-aminoisobutyric acid

Addition to suspending medium*	C^{14} activity of cells c.p.m.
0	66
NaCl	6467
KCl	4
RbCl	22
NH_4Cl	27
LiCl	29
sucrose	44

* at a concentration of 200mM. Incubation time: 45 min.

or an indirect one involving the metabolism of the oxidizable substrate. To distinguish between these possibilities, the concentration of Na^+ required for optimum rate of uptake of AIB was determined. The results, Fig. 2, show that 200 mM Na^+ was needed. Previous studies (MacLeod et al., 1958) have shown that for optimum rate of oxidation of the oxidizable substrate present (galactose) only 50mM Na^+ is required. This has been confirmed under the conditions used in the experiments reported here. There is thus a role for Na^+ in the uptake of AIB which is separate from any other possible role of Na^+ in oxidative metabolism.

Separate experiments have shown that the uptake of D-fucose, a non-oxidizable analogue of galactose is also Na^+ dependent in these cells.

The results obtained support the conclusion that the primary function of Na^+ in marine bacteria may be to permit the transport of substrates into the cell. Previously observed differences in the quantitative requirements for Na^+ for the oxidation of various substrates by cells of this marine bacterium (MacLeod et al., 1958) can now be explained if one assumes a number of different permeases in the cell membrane with quantitatively different requirements for Na^+ for activation. This extends to a bacterial species a role for Na^+ observed in certain

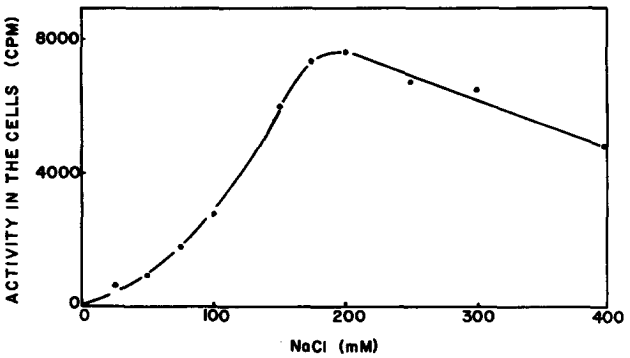

Fig. 2 Quantitative requirements of marine pseudomonad B-16 for Na^+ for the uptake of ∝-aminoisobutyric acid. Incubation time: 70 min.

animal tissues. The absorption of sugars by the intestinal tissues of various animals (Riklis and Quastel, 1958; Csàky, 1962; Bihler and Crane, 1962) and the incorporation of amino acids and other compounds into isolated thymus nuclei (Allfrey et al., 1961) have been shown to involve the operation of Na^+ specific transport mechanisms.

REFERENCES

Allfrey, V.G., Meudt, R., Hopkins, J.W. and Mirsky, A.E., Proc. Nat. Acad. Sci. (U.S.) 47, 907 (1961).
Bihler, I. and Crane, R.K., Biochim. et Biophys. Acta 59, 78 (1962).
Christensen, H.N. and Jones, J.C., J. Biol. Chem. 237, 1203 (1962).
Csàky, T.Z., Federation Proc., 22, 3 (1962).
Drapeau, G.R. and MacLeod, R.A., J. Bacteriol. in press (1963).
Horecker, B.L., Thomas, J. and Monod, J., J. Biol. Chem. 235, 1580(1960).
MacLeod, R.A. and Onofrey,E., J. Cellular Comp. Physiol.,50, 389(1957).
MacLeod, R.A., Claridge, C.A., Hori,A. and Murray,J.F., J. Biol. Chem. 232, 829 (1958).
MacLeod, R.A. and Hori,A., J. Bacteriol., 80, 464 (1960).
MacLeod, R.A. and Matula, T., Can. J. Microbiol. 8, 883 (1962).
Payne, W.J., J. Bacteriol. 80, 696 (1960).
Pratt, D. and Happold, F.C., J. Bacteriol., 80, 232 (1960).
Rhodes, M.E. and Payne, J.W.,Antonie van Leeuwenhoek 28, 302 (1962).
Riklis, E., and Quastel, J.H., Can. J. Biochem. and Physiol., 36, 347 (1958).
Tomlinson, N. and MacLeod, R.A., Arch. Biochem. Biophys. 70, 477 (1957).
Tyler, M.E., Bielling, M.C. and Pratt, D.B., J. gen. Microbiol. 23, 153 (1960).