The effects of rapid increases in osmotic pressure on *Escherichia coli*

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Actively growing *Escherichia coli* cells respond to increased osmotic pressure by increasing their internal pools of K⁺ (Epstein & Schultz, 1965) and of the amino acids glutamate, proline and α-amino butyric acid (Britten & McClure, 1962; Measures, 1975). The change in amino acid pools may be brought about either by increased rates of their synthesis or by stimulation of the rate of their uptake. The present study was initiated to discover how the transport rates of amino acids were controlled by osmotic pressure. In particular the role of K⁺ in the control mechanism was investigated because of the role of this ion in stimulation of amino acid synthesis (Measures, 1975; Gould & Measures, 1977).

The effect of osmotic pressure on transport of amino acids into endogenously respiring *E. coli* cells was investigated by using concentrated solutions of sorbitol (0.5 M) and lactose (0.4 M). In control experiments with *E. coli* strain 7 (Hayashi et al., 1969), neither sorbitol nor lactose were transported; however, in subsequent experiments it became apparent that, at high concentrations of sorbitol, some metabolism occurs (see below). In experiments in which the osmotic pressure was rapidly increased 3-fold, only aspartate and proline transport systems were stimulated in both the presence and absence of K⁺. Glutamate transport only occurred in the presence of K⁺, but under these conditions was stimulated 4-fold by a 3-fold increase in osmotic pressure. The transport systems for alanine, lysine and isoleucine were all inhibited by increases in osmotic pressure.

Amino acid uptake was studied over a range of osmotic pressure to determine the relationship between the magnitude of the increase in osmotic pressure and transport activity. The results of proline-transport assays in media of increasing osmotic pressure are shown in Figs. 1(a) and 1(b) similar results were obtained with aspartate (not shown). It is apparent that lactose and sorbitol were not equivalent as agents to increase the osmotic pressure. Lactose-mediated pressure increases resulted in a gradual 4-fold increase in the rate of transport, and this effect showed no K⁺-dependence (Fig. 1a). In the absence of K⁺, sorbitol gave qualitatively similar results to lactose (Fig. 1b). However, in the presence of K⁺, addition of only 10 mM-sorbitol (compared with 0.4 M lactose) caused a 5-fold increase in the rate of uptake of proline (Fig. 1b). The sorbitol effects could be simulated by lactose in the presence of 0.5 mM-glucose (Fig. 1a), suggesting that the effects of sorbitol were influenced by metabolism of this compound.

Subsequently it was observed that sorbitol at 10 mM caused an increase in the respiration rate of *E. coli* cells from 54.4 to 89 mmol of O₂ consumed/min per mg of cells. Osmotic pressure increases themselves were found not to have any effect on the rate of respiration. K⁺ appears to play a role in coupling sorbitol or glucose metabolism to proline uptake in *E. coli*. It was observed that there was no direct correlation between the respiration rate and the rate of proline uptake. Proline uptake is energized by the protonmotive force generated by respiration (Hamilton, 1975). Thus in the presence of glucose and independent of the presence of K⁺, the respiration rate of the cells was increased over 5-fold. Proline transport, however, was only significantly stimulated by glucose in the presence of K⁺ (Fig. 1a). Therefore there appears to be a direct effect of external K⁺ on proline uptake, which is dependent upon the presence externally of a metabolizable carbon source. This effect requires further investigation.

In conclusion, it is apparent that external K⁺ has no regulatory role in the response of amino acid transport to osmotic pressure, but does have other effects on energy coupling.


![Fig. 1. Response of the proline transport system to osmotic shock](image)

*E. coli* strain 7 was incubated in media containing increasing concentrations of (a) lactose and (b) sorbitol to increase the osmotic pressure. The [U-¹⁴C]proline concentration was 5 μM (specific radioactivity 5 Ci/mmol). Incubations were carried out in the presence ((), (.) and absence ((), (.) of K⁺ (1 mM). In addition, glucose and K⁺ (△) were added to some incubations with lactose. Chloramphenicol (50 μg/ml) was present in all incubations.