THE SITE OF ACTION OF ADENOSINE-5'-TRIPHOSPHATE ON β-GALACTOSIDE TRANSPORT IN *ESCHERISCHIA COLI*

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The active transport and accumulation of β-galactosides by *Escherischia coli* seems to differ in several ways from the transport of many other carbohydrates including hexoses, sugar alcohols and glucosides. These latter carbohydrates accumulate as phosphorylated derivatives; their transport seems to depend on a phosphoenolpyruvate-requiring phosphorylation system and is sensitive to fluoride [1–3]. In contrast, lactose is not accumulated as lactose phosphate, and its accumulation is inhibited by azide but not by fluoride [3]. Furthermore, it has recently been suggested by Scarborough, Rumley and Kennedy [4] that adenosine triphosphate (ATP) but not phosphoenolpyruvate, is involved in the transport of β-galactosides. In their experiments they removed the soluble contents of the cells, including the nucleotides, by washing with ice-cold tris buffer. The cells were then given a mild osmotic shock in the presence or absence of ATP by rapid three-fold dilution from 150 mM tris, and the rate of *o*-nitrophenyl-β-galactoside (ONPG) hydrolysis determined in the presence and absence of ATP. After this treatment ATP caused a six-fold increase in the rate of hydrolysis compared with the control, where ATP was omitted from both the dilution and the assay. Scarborough et al. concluded that ATP acts on the outside of the membrane, and suggested that it might convert a carrier from a low-affinity to a high-affinity form.

That ATP should act on the outside of the membrane seems surprising for several reasons. In normal circumstances the membrane is rather impermeable to nucleotide triphosphates, while the cell wall is readily permeable [5]. Thus nucleotides synthesised inside the cell would not be available while those in the periplasmic space would diffuse away. Though the preliminary experiments of Schachter, Johnson and Kirkpatrick [6], using thiodigalactoside to protect against N-ethylmaleamide inhibition, suggested that the energy for active transport is applied to the outside of the membrane to cause an increase in the affinity of the carrier for its substrate, a more extended investigation [7] points to the opposite conclusion – that energy coupling causes a reduction of carrier affinity on the inside of the membrane. This is in accord with the kinetic experiments of Koch [8] and Winkler and Wilson [9].

The results of Scarborough, Rumley and Kennedy have been confirmed in this laboratory, but it is suggested that ATP penetrates into the cells during the unusual conditions of the rapid dilution, and is probably stimulating ONPG transport by acting inside the membrane. Some evidence for this is presented.

The bacterial strain (ML 308), the culture methods and experimental procedures of Scarborough, Rumley and Kennedy were followed, except that 1 mM dithiothreitol replaced 1 mM 2-mercaptoethanol as sulfhydryl protector. The ONPG hydrolysis rate of ATP-treated cells assayed in the presence of ATP is several-fold greater than that of control cells assayed in the absence of ATP. But the stimulation caused by ATP present during the assay is less than that caused by the pretreatment with ATP (table 1). That observation is not easily explained by the hypothesis of Scarborough et al. Qualitatively the same results were obtained when the two washes in tris buffer were of 5, 7 and 10 min duration, but the longer washes gave lower control rates and more stimulation by ATP.

It is not clear what happens during the sudden

Table 1

Cells harvested at 2°, suspended twice in ice-cold tris-HCl buffer (100 mM, pH 7.7, containing 1 mM dithiothreitol) for 7 min, resuspended in 5.0 ml of "buffer A" (150 mM tris-HCl, pH 7.7, 15 mM Na_2PO_4, 15 mM $MgSO_4$, 1 mM dithiothreitol) and warmed to room temperature. Aliquots (1.0 ml) of this were rapidly diluted into 2.0 ml of 1 mM dithiothreitol (control cells), or dithiothreitol containing 7.15 mM ATP (disodium tris salt at pH 7.4) (ATP-treated cells).Assay mixture contained 2.1 ml of buffer (50 mM tris HCl, pH 7.7, 30 mM NaN_3) 0.3 ml of the diluted cell suspension, and 0.6 ml of 30 mM ONPG in buffer. When required the buffer also contained 15 μmoles ATP (disodium tris salt). Blanks contained, in addition, 30 mM formaldehyde. Hydrolysis was stopped after 10 min by addition of 2.0 ml 2 M Na_2CO_3.

Pretreatment of cells	ATP concentration during assay (mM)	ONPG hydrolysed in 10 min after subtracting formaldehyde blanks (mμmoles/mg dry wt)	As % control
Control	0	103.1	100
	5	221.5	215
ATP-treated	0.5	306.5	297
	5.5	433.0	420

Table 2

As described in the text. In the two experiments specific activities and external ATP concentrations were different (3.81 mM and 4.38 mM respectively).

	Time of [^{14}C] ATP addition	Cpm per mg dry wt cells	ATP (mμmoles) per mg dry wt	per μl cell H_2O
Expt. 1	Before dilution	809	9.58	3.55
	2 min after dilution	587	6.94	2.57
Expt. 2	Before dilution	300	10.19	3.77
	$\frac{1}{2}$ min after dilution	288	9.75	3.61

dilution, but it seems that ATP might penetrate cells more easily during that treatment than at other times. Thus Britten and McClure [10] found an increased sulphate uptake together with a release of the soluble contents of the cell during a similar dilution. To test this possibility cells were washed in ice-cold tris buffer and suspended in "buffer A" as before, at a cell density of 23 mg/ml. A 1 ml aliquot was added to each of two tubes, one of which contained 1.8 ml of 1 mM dithiothreitol and 0.2 ml of [^{14}C] ATP (50 mM), while the other contained only the dithiothreitol. To this second tube the [^{14}C] ATP was added after the cells. The cells were centrifuged down, washed with 10 ml of mineral medium, and triplicate samples taken for radioactive counting. More radioactivity was associated with the cells when the [^{14}C] ATP was present during the dilution, than when it was added afterwards (table 2), which would be expected if ATP entered more readily during that treatment. When the [^{14}C] ATP was added immediately after dilution (table 2, expt. 2) there was less difference. It is likely that the cells take a definite time to seal up; a phenomenon which has been well studied in erythrocytes [11]. If the volume of cell water is taken as 2.7 μl/mg dry wt, following Winkler and Wilson [9], it can be seen that the internal concentration approaches that of the external solution.

Even when the ATP was added after dilution 7 mμmoles/mg dry wt was associated with the cells. Some of this ATP may have been absorbed outside the cells, or trapped in the cell wall and periplasmic space. However, it was firmly bound, for only half the radioactivity was lost after prolonged washing (50 min) in mineral medium, and it seems very likely that some was inside the cells.

These results throw doubt on the conclusion of Scarborough et al., that ATP stimulates ONPG hydrolysis by acting at the outside of the cell membrane.

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References

[1] S.Tanaka, D.G.Fraenkel and E.C.C.Lin, Biochem. Biophys. Res. Commun. 27 (1967) 63.
[2] W.Kundig, F.D.Kundig, B.E.Anderson and S.Roseman, J. Biol. Chem. 241 (1966) 3243.
[3] E.P.Kennedy and G.A.Scarborough, Proc. U.S. Natl. Acad. Sci. 58 (1967) 225.
[4] G.A.Scarborough, M.K.Rumley and E.P.Kennedy, Proc. U.S.Natl. Acad. Sci. 60 (1968) 951.
[5] R.W.Brockman and L.A.Heppel, Biochem. 7 (1968) 2554.
[6] D.Schachter, N.Johnson and M.A,Kirkpatrick, Biochem. Biophys. Res. Commun. 25 (1966) 603.
[7] D.Schachter and A.J.Mindlin, J. Biol. Chem. 244 (1969) 1808.
[8] A.Koch, Biochem. Biophys. Acta 79 (1964) 177.
[9] H.H.Winkler and T.H.Wilson, J. Biol. Chem. 241 (1966) 2200.
[10] R.J.Britten and F.T.McClure, Bact. Rev. 26 (1962) 292.
[11] P.Seeman, J. Cell Biol. 32 (1967) 55.