AMINO ACID-DEPENDENT CONTROL OF THE TRANSPORT OF α-METHYL GLUCOSIDE IN E. COLI

Yoshihiro Sokawa and Yoshito Kaziro

Department of Chemistry, Institute of Medical Science,
University of Tokyo, Takanawa, Minatoku, Tokyo.

Received December 2, 1968

RNA synthesis in E. coli is dependent on the presence of a full complement of amino acids and the locus "RC" on its chromosome is responsible for the control of RNA synthesis (Borek, Ryan and Rockenbach, 1955; Pardee and Prestidge, 1956; Stent and Brenner, 1961). However, we have recently demonstrated that the incorporation of ^{14}C-acetate into lipid fraction is subject to amino acid control in stringent strains of E. coli (Sokawa, Nakao and Kaziro, 1968). Moreover, the synthesis of carbohydrate was shown to be also dependent on the presence of amino acids (Sokawa, Nakao and Kaziro, unpublished). Consequently, it was suggested that the control by RC gene is not limited to RNA synthesis, and many other biosynthetic reactions might be regulated by RC gene.

In this paper we wish to report our recent observation that the transport of α-methyl D-glucoside (α-MG), a non-metabolizable analog of glucose permeation system (Kepes and Cohen, 1962), is also under amino acid control in E. coli. The stringent strains fail to accumulate ^{14}C-α-MG during amino acid starvation whereas the relaxed strains continue to uptake ^{14}C-α-MG in the absence of required amino acids. As in the case of RNA and lipid synthesis, addition of chloramphenicol(CM) restores the transport of α-MG under conditions of amino acid starvation in stringent strains.

MATERIALS AND METHODS

The strains and culture medium used in the present study were described

previously (Sokawa et al., 1968). The stringent strain of E. coli W677 requires leucine and threonine as well as thiamine for growth. The strain 58-161 is a relaxed one requiring methionine.

The cells were grown in M9 medium supplemented with necessary requirements, harvested by centrifugation and washed twice with M9 medium without glucose. The washed cells were resuspended in the above medium to a density of 3-5 x 10^8 cells/ml.

The uptake of ^{14}C-α-MG was measured as follows: To 0.85 ml of the above cell suspension were added 1 umole of D-glucose, 0.025 umole of α-methyl D-glucoside (glucose-UL-^{14}C) (2.4 uC/umole) (Mallinchrodt chemical works, Orlando, Florida), and 50 ug each of the required amino acids and 25 ug of CM when specified. The final volume was adjusted to 1.0 ml. After incubation with shaking at 37°, 0.2 ml aliquots were taken at various times, filtered through Millipore HA filters and the cells were washed twice with 2 ml of M9 medium at room temperature. The filters were immediately dried under an infrared lamp and the radioactivities were counted in liquid scintillation spectrometer using a toluene-based counting mixture.

RESULTS

Figure 1 shows the effect of the removal of amino acid and of addition of CM on the uptake of ^{14}C-α-MG by two E. coli auxotrophs W677(Leu$^-$, Thr$^-$, B_1^-, RC^{str}) and 58-161(Met$^-$, RC^{rel}). As indicated in Fig. 1-A, the transport of α-MG into the cells by a stringent strain W677 was almost completely abolished by removal of the required amino acids, leucine and threonine. On the other hand, a relaxed strain 58-161 accumulated considerable amount of α-MG in the absence of methionine (Fig. 1-B). Essentially the same results were obtained with two E. coli strains isogenic except RC locus: CP 78(Leu$^-$, Thr$^-$, Arg$^-$, His$^-$, B_1^-, RC^{str}) and CP 79(Leu$^-$, Thr$^-$, Arg$^-$, His$^-$, B_1^-, RC^{rel}).

It was reported that CM is able to release RNA synthesis in the absence of required amino acids in E. coli stringent strains (Aronson and Spiegelman, 1961). The same stimulatory effect of CM on lipid synthesis was observed in amino acid-

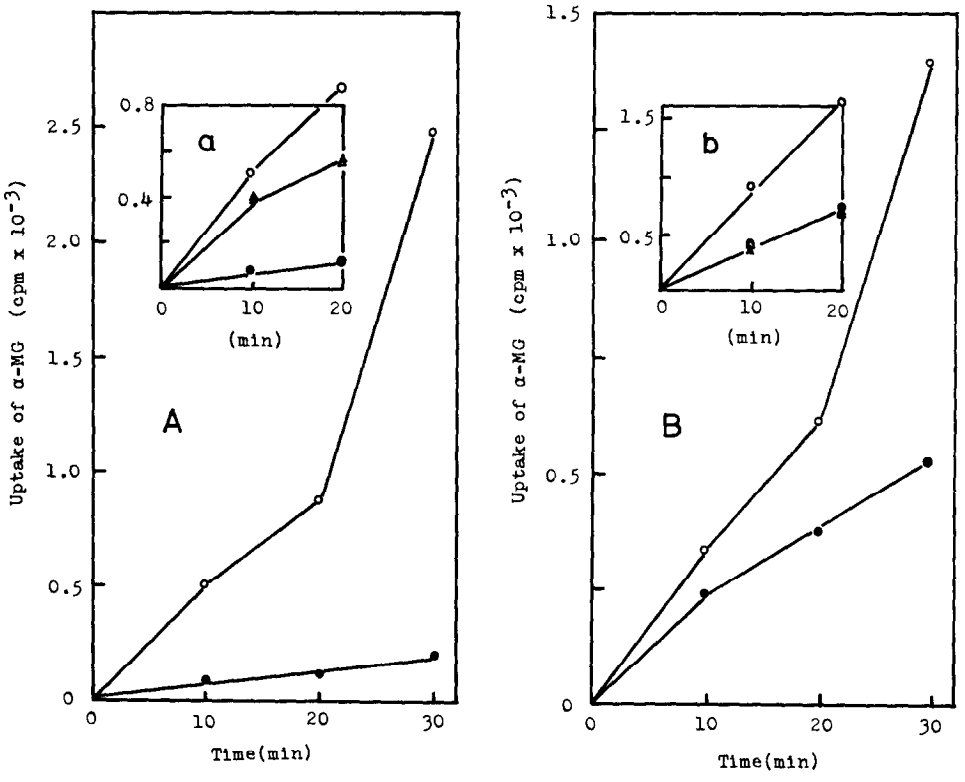

Fig. 1. Effect of removal of amino acid and addition of chloramphenicol (CM) on α-methyl D-glucoside(α-MG) uptake by the stringent and relaxed strains of E. coli. (A) and (a): W677 (stringent strain), requiring leucine and threonine; (B) and (b): 58-161 (relaxed strain), requiring methionine. For details see text.

○——○ in the presence of amino acids

●——● in the absence of amino acids

△——△ addition of CM in the presence of amino acids

▲——▲ addition of CM in the absence of amino acids

---

starved culture of stringent strains (Sokawa et al., 1968). The effect of CM on the uptake of α-MG is illustrated in Figs. 1-a and 1-b. In the absence of required amino acids the uptake of α-MG by stringent strain W677 was elevated by the addition of CM to the same level as when CM was added to the control culture supplemented with required amino acids (Fig. 1-a). In the case of relaxed strain 58-161 (Fig. 1-b), the uptake of α-MG continued in the absence of methionine at about half of the maximum rate, and the addition of CM did not result in the further increase of the rate.

DISCUSSION

In a previous report, it was shown that when a stringent strain of E. coli is starved of amino acids, lipid synthesis is extremely reduced (Sokawa et al., 1968). The addition of CM elevated the activity of lipid synthesis to the same level as when CM is added to the culture supplemented with required amino acids. The present findings demonstrate that the transport of α-MG and therefore of glucose is also under amino acid control. Here again CM displayed the same stimulatory effect on the transport of α-MG as on RNA and lipid synthesis. These results, together with an unpublished observation that the synthesis of carbohydrate is also under the same control, indicate that stringency of the control is not restricted to the synthesis of RNA.

The fact that most, if not all, biosynthetic reactions in E. coli are under regulation by RC gene, can be explained by assuming that the entry of extracellular substrates is inhibited in the absence of amino acids. Since in the medium used in the present study, glucose serves as an energy source as well as a sole carbon source for macromolecular synthesis, the impairment of the transport of glucose would necessarily result in the inhibition of all the biosynthetic reactions in the cells. Although we have so far measured only the transport of α-MG, the recent report by Nierlich (1968) that the entry of RNA precursors into cellular nucleotide pool is blocked by deprivation of amino acid suggests that the transport system other than glucose are also under RC gene control.

The possibility, however, remains to be examined that the decrease in ATP during starvation of amino acids is the cause rather than the result of the failure of the transport of glucose and leads to the general impairment of all the biosynthetic reactions.

SUMMARY

The transport of α-MG was examined in the stringent and relaxed strains of E. coli. The stringent strain failed to accumulate α-MG during amino acid starvation whereas the relaxed strain continued to uptake α-MG in the absence

of the required amino acid. Furthermore, the addition of CM restored the transport of α-MG under conditions of amino acid starvation in the stringent strain. These results indicate that the transport of α-MG is under the rule of RC gene in E. coli.

REFERENCES

Aronson, A.I., and Spiegelman, S., Biochim. Biophys. Acta, 53, 70 (1961).
Borek, E., Ryan, A., and Rockenbach, J., J. Bacteriol., 69, 460 (1955).
Kepes, A., and Cohen, G.N., in The Bacteria, ed. I.C. Gunsalus and R.Y. Stanier, Vol.4, p.179-222 (1962), New York and London, Academic Press.
Nierlich, D.P., Proc. Natl. Acad. Sci. U.S., 60, 1345 (1968).
Pardee, A.B., and Prestidge, L.S., J. Bacteriol., 71, 677 (1956).
Sokawa, Y., Nakao, E., and Kaziro, Y., Biochem. Biophys. Res. Commun., 33, 108 (1968).
Stent, G.S., and Brenner, S., Proc. Natl. Acad. Sci. U.S., 47, 2005 (1961).