ACCEPTOR FOR THIOMETHYL GALACTOSIDE IN ESCHERICHIA COLI K 12 +

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A regulatory molecule (effector), entering into a bacterial cell, should be bound by a specific acceptor in order to initiate its regulatory action on the synthesis of definite enzymes. In the case of ß-galactosidase induction (Jacob and Monod, 1961) the synthesis of three enzymes coded in the lac operon can be started by methylthio-ß-D-galactoside (TMG). The inducing substance itself has been demonstrated not to be metabolized ("gratuitous" inducer, Monod and Cohn, 1952). In experiments with labelled TMG the radioactivity is bound faster by i^+-strains and can be washed out more easily from distinct i^--mutants, in which ß-galactosidase is not induced (Wacker, unpublished results).

Chromatography of a crude extract of E. coli K 12 after induction with ^{3}H-TMG on DEAE cellulose has shown that the bulk of radioactivity can be eluted with the first buffer fractions as unbound TMG. With increasing salt concentration, at about 0,1 M NaCl, a second sharp peak of radioactivity appears, representing TMG bound to a protein fraction (Lodemann et al., 1967). In this communication experiments on isolation and first characterization of a TMG acceptor are presented. By labelling with ^{32}P-phosphate as well as with ^{14}C-adenine and ^{14}C-uracil and disc gel electrophoresis, we were able to suggest that the acceptor is a nucleoprotein.

As has been shown in our laboratory, the TMG acceptor complex did also appear in chromatogramms after "in vitro induction", performed in the following manner: E. coli K 12 was cultivated in 400 ml glycerol medium with vigorous shaking to the end of logarithmic phase, as described (Lodemann et al., 1967). The cells were harvested, washed twice with TM-buffer (10^{-2} M Tris-HCl, 10^{-4} M magnesium acetate, pH 7,4) and subjected to ultrasonic disruption in 6 ml TM-buffer. After centrifugation (30 min., 105 000 · g) 120 µg

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^{3}H-TMG (28 mC/mMol) per ml extract were added and incubated for 5 minutes at 37°C. The extract then was applied to a DEAE cellulose column (1,5 x 21 cm), equilibrated with TM-buffer containing 10^{-4} M unlabelled TMG. After washing the column with 150 ml of this buffer, a concave gradient was employed, made up by filling two chambers of a VARIGRAD each with 133 ml of buffer and the third with 133 ml of 1 M NaCl in the same buffer. Aliquots of the eluate fractions were mixed with dioxane scintillation solvent and counted in a TRICARB liquid scintillation spectrometer. For labelling with ^{32}P the cells were grown in the presence of 1,65 mC ^{32}P-orthophosphate per liter medium.

At a concentration of about 0,1 M NaCl a fraction emerges from the column which contains ^{3}H- and ^{32}P-activity as well. The maximum of ^{32}P distribution is shifted to a somewhat higher salt concentration because small ^{32}P-nucleotides too are eluted in this region (Fig. 1). After dialysis (4 hours, 0°C) of the combined fractions 47 to 50 against TM-buffer, about 75 % of the ^{32}P-activity and nearly all of the ^{3}H-activity could be detected outside the dialysis tube. ^{3}H-TMG was likewise completely dialyzed out of this fraction against TM-buffer, containing 10^{-4} M unlabelled TMG, thus indicating a rather

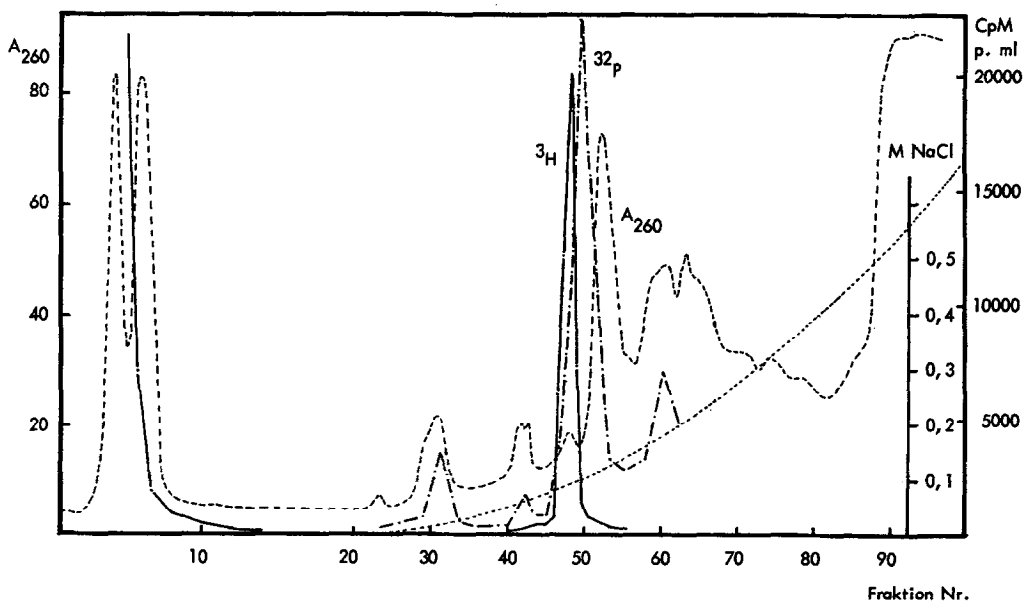

Fig. 1. DEAE cellulose chromatography (1,5 x 21 cm) of a cell-free extract of E. coli K 12, grown in 400 ml glycerol medium, containing 0,66 mC ^{32}P-orthophosphate. The extract (6 ml) was incubated with 720 µg (0,1 mC) ^{3}H-TMG (5 min., 37°C). Elution with a gradient (400 ml) from 0 to 1 M NaCl in TM-buffer, containing 10^{-4} M unlabelled TMG. Fractions 20 to 90 containing 3,8 ml each. (– – – – –) absorbance at 260 mµ; (————) ^{3}H-activity; (—·—·—·—) ^{32}P-activity; (----------) NaCl concentration.

weak bond between TMG and its acceptor. On the other hand, the TMG acceptor complex can be restored by incubation (5 min., 37°C) in the presence of 120 µg ^{3}H-TMG per ml. Following rechromatography of this mixture on DEAE cellulose, the radioactivity of both ^{3}H and ^{32}P was eluted in exact the same fraction at a concentration of about 0,1 M NaCl.

Incubation of the cell-free extract with trypsin (15 min., 37°C) destroyed its acceptor activity, as was shown by subsequent chromatography. With RNase and DNase we were not able to detect any effect on the binding capacity. This indicates a protein being the essential factor for binding of TMG (Lodemann et al., 1967).

In an other experiment the pooled TMG acceptor complex was dialyzed, freeze dried, then incubated as before with ^{3}H-TMG and applied to analytical disc gel electrophoresis, using a length of 10 mm for the spacer gel and of 75 mm for the small pore gel. After separation the gel column was cut into segments of 4 mm length and radioactivity was estimated after soaking each segment with 0,5 ml water over night. The pattern of radioactivity showed a peak at the position of the bromphenol blue marker, containing both ^{3}H- and ^{32}P-label (Fig. 2). This would suggest that the TMG acceptor consists of a protein, containing phosphorus, perhaps as a polynucleotide.

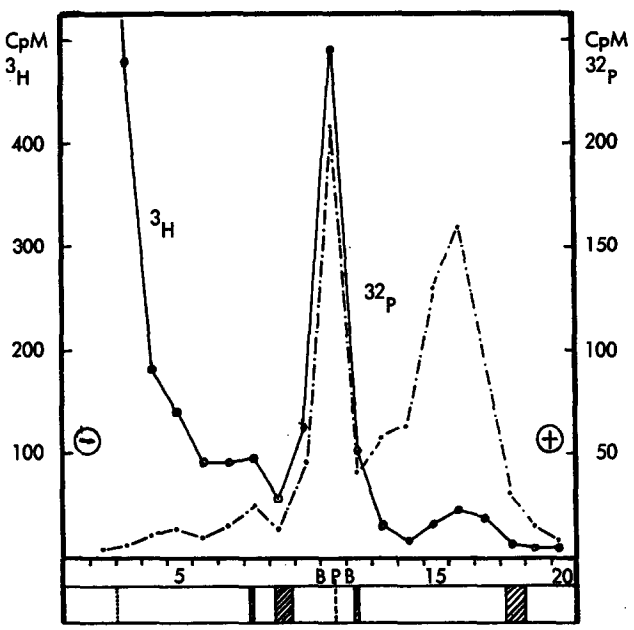

Fig. 2. Analytical disc gel electrophoresis (400 V, 35 min.) of the TMG acceptor peak (Fig. 1). The column was cut into segments of 4 mm length, the radioactivity was counted in a liquid scintillation spectrometer. A second column was stained with amido schwarz (lower part). (———) ^{3}H-activity; (— · — · —) ^{32}P-activity; BPB, bromphenol blue marker.

In order to proove this assumption, a cell culture was supplemented with uracil-[2-^{14}C] and adenine-[8-^{14}C] (40 mC each per liter) and induced with 50 µg ^{3}H-TMG (3hours, 37°C). DEAE cellulose chromatography of the cell-free extract yielded a radioactive peak, containing both ^{3}H- and ^{14}C-activity at the same gradient concentration of about 0,1 M NaCl. Dialysis (1 hour, 0°C) caused a loss of 37 % of the ^{3}H-label, while there was no significant change in the ^{14}C-activity. The dialyzed solution was freeze dried and subjected to disc gel electrophoresis as described above. Again a sharp peak of ^{3}H- and ^{14}C-radioactivity appeared together with the bromphenol blue marker, similar as shown in Fig. 2.

These results lead us to conclude that the acceptor for TMG in E. coli K 12 might be a nucleoprotein.

It is discussed that the repressor could be composed of a protein and a nucleic acid component in addition (Sadler and Novick, 1965). This would explain its specificity for a distinct nucleotide sequence (Miller and Sobell, 1966). At the present time we are not able to decide, whether the TMG acceptor here described is identical with the repressor for the lac operon. Further efforts shall be made in order to answer this question.

References

Jacob, F. and J. Monod, J. Mol. Biol., 3, 318 (1961).

Lodemann, E., D. Drahovský, R. Flöhl and A. Wacker, Z. Naturforschg., in press (1967).

Miller, J.H. and H.M. Sobell, Proc. Natl. Acad. Sci., 55, 1201 (1966).

Monod, J. and M. Cohn, Adv. Enzymol., 13, 67 (1952).

Sadler, J.R. and A. Novick, J. Mol. Biol., 12, 305 (1965).