ADENOSINE TRIPHOSPHATE AND CATABOLITE REPRESSION OF β-GALACTOSIDASE IN ESCHERICHIA COLI

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SUMMARY: Addition of ATP to spheroplasts, induced for β-galactosidase synthesis, either in the presence of glycerol or in the absence of any carbon source, was found to reduce the differential rate of the enzyme formation. The effect of ADP, AMP, GTP, UTP and CTP was much weaker than that of ATP. Since the effect of ATP was reversed by cyclic AMP, it was concluded that ATP exerted its effect in the mechanism of catabolite repression.

Several observations, reported in recent years, have suggested that cyclic 3';5' adenosine monophosphate (cyclic AMP) might play a role in the synthesis of β-galactosidase, and that both catabolite repression and transient repression are, presumably, the result of a depletion of the intracellular cyclic AMP (1-6). Since no metabolism of the sugar, present in the medium, has appeared to be necessary for the manifestation of the transient repression (5,6), it seems that the depletion of the intracellular cyclic AMP depends merely on the penetration of the sugar into the cell. On the other hand, the permanent catabolite repression has been found to depend on both, the efficiency of the catabolism of the carbon source and on its consumption by anabolic processes (7). This might lead to the conclusion that the extent of the permanent repression depends, not only on the level of the intracellular cyclic AMP, but also on the level of some catabolite, which presumably antagonizes the action of cyclic AMP.

Several indications that ATP is the catabolite involved in the manifestation of catabolite repression have been reported in early communications (7-11). However, none of them was based on a direct estimation of the effect of this compound on the synthesis

of catabolite repressible enzymes. This estimation was the aim of the present study. Since normal cells of E. coli are impermeable to ATP, spheroplasts of this organism were used. Spheroplasts prepared from E. coli by EDTA-lysosyme treatment have been found to be permeable to materials unable to penetrate normal cells of E. coli, as actinomycin D (12). Moreover, according to the observations of Neu & Heppel (13,14) such spheroplasts seem to lose the surface localized ATPase, which could, otherwise, interfere with the penetration of ATP as an intact molecule.

MATERIALS AND METHODS: Lysosyme was purchased from Calbiochem and deoxyribonuclease (DNase) from Wortington. All other materials were described elsewhere (15).

E. coli K-12 Hfr.H requiring thiamine was grown up to the logarithmic phase in a medium composed of a salts solution described previously (15), to which 1 mg/ml casein hydrolysate and 0.2% (w/v) glycerol were added.

The cells were harvested by centrifugation, washed once with the salts solution, from which Mg^{++} ions were omitted, and then resuspended in a Tris-HCl buffer (30 mM, pH 8.0) containing 20% (w/v) sucrose. The density of the suspension was adjusted so that in a dilution of 1:10 it was equivalent to about 0.2 O.D. units. To this suspension 4 mM EDTA, 50 μg/ml lysosyme and 5 μg/ml DNase were added. This was allowed to stand at room temperature for 10-15 min with occasional gentle mixing. The formation of spheroplasts by this treatment was checked microscopically and confirmed by a complete lysis of a sample of the suspension when diluted 1:10 in cold distilled water. The suspension was centrifuged for 20 min at 3000 x g in cold, and the pellet was carefully suspended in the salts solution to which 10 mM $MgSO_4$ or 20% (w/v) sucrose were added as stabilizers. The density of the suspension was adjusted to 0.3 O.D. units. When sucrose served as stabilizer, 2 mg/ml of casein hydrolysate was required for detecting significant β-galactosidase formation and ^{14}C-leucine incorporation.

Induction of β-galactosidase synthesis and the assay of its activity were as previously described (15).

Total protein synthesis was followed by the incorporation of ^{14}C-leucine (0.05 μCi/ml added together with 4 μg/ml non-radioactive leucine). This was done as described elsewhere (16).

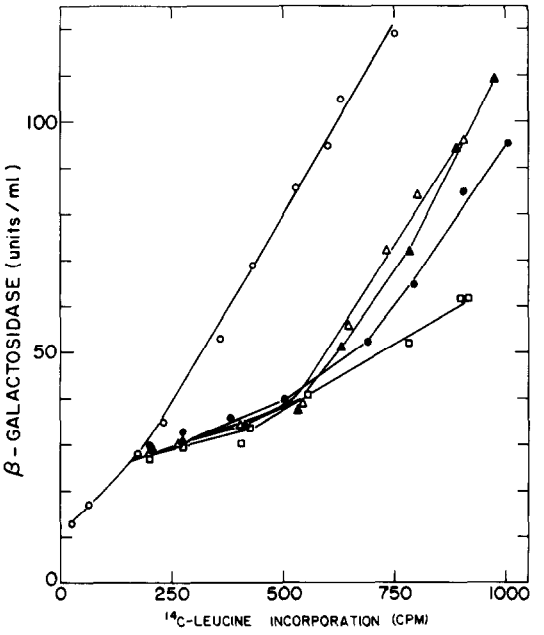

Fig. 1. The effect of ATP, ADP and AMP in the presence of 0.05% glycerol. Spheroplasts, suspended in salts solution containing 10 mM $MgSO_4$ as a stabilizer, were incubated with 0.2 mM IPTG, 0.05 μCi/ml ^{14}C-leucine and 4 μg/ml nonradioactive leucine. After 20 minutes the suspension was divided into 5 equal portions. One of them, serving as a control, was incubated with no additions (○). The rest received 0.02% (w/v) glycerol and were incubated with: (△) no further additions, (□) 1 mM ATP, (●) 1 mM ADP and (▲) 1 mM AMP. Samples for the assay of β-galactosidase formation and ^{14}C-leucine incorporation were taken, up to 20 min in intervals of 10 min, and then in intervals of 15 min. The differential rate of the enzyme synthesis was described by plotting the enzyme activity against the incorporation of radioactivity.

RESULTS AND DISCUSSION: Fig. 1 demonstrates the manifestation of catabolite repression of β-galactosidase, caused by 0.05% (w/v) glycerol, in spheroplasts suspended in salts solution containing 10 mM $MgSO_4$ as stabilizer. It is seen that after a sever repression the enzyme formation almost completely recovered. The duration of the sever repression varied from one experiment to another, most probably because of the difficulty to obtain

percise standard conditions with such a system. Though this was not checked directly, the recovery in the enzyme formation seemed to result from the depletion of glycerol from the medium, due to its consumtion by the spheroplasts, because as can be seen from Fig. 2, no recovery occured when the concentration of glycerol was increased to 0.2% (w/v). Addition of 1 mM ATP increased the repression caused by both concentrations of glycerol. When 0.05% glycerol was used ATP prevented the recovery of the enzyme formation, whereas with 0.2% it increased immediately the extent of the repression. The effect of ADP was much weaker and that of AMP was almost insignificant (see Fig. 1).

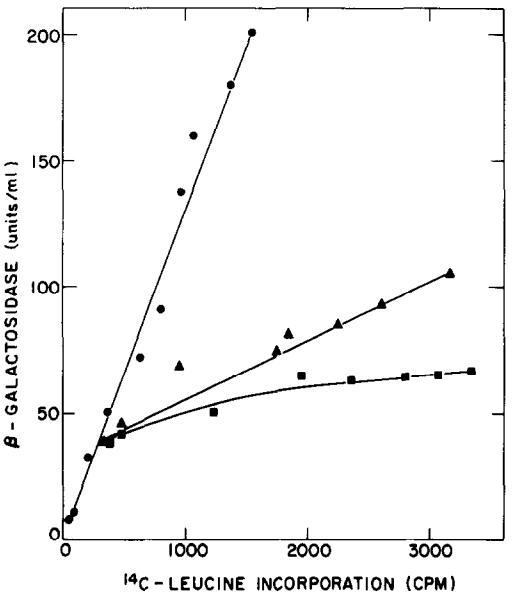

Fig. 2. The effect of ATP in the presence of 0.2% glycerol. Experimental details are similar to those described in Fig. 1 except that the concentration of glycerol was 0.2% (w/v) and that only the effect of ATP was tested. Symbols are: (●) no additions, (▲) glycerol, (■) glycerol + ATP.

If the effect of ATP, observed in the presence of glycerol, was due to elevation of the ATP level inside the cells, this compound, in an appropriate concentration, could be anticipated to repress the enzyme formation even in the absence of any carbon source. In fact, preliminary experiments with this system showed that without glycerol,

the enzyme formation was repressed by 10 mM ATP. But the results were unreproducible, since with this concentration of ATP the spheroplasts tended to lyse very fast. However, we found empirically that the suspension became much more stable, if sucrose (20%, w/v) was used as a stabilizer instead of Mg^{++}.

A suspension of spheroplasts, induced in sucrose containing medium, was divided into two equal portions. One of them received 10 mM ATP. The other, serving as a control, was incubated without ATP. Samples for the assay of β-galactosidase activity and ^{14}C-leucine incorporation were taken each 15 minutes during 2 hours. The differential rate of β-galactosidase formation was estimated for each portion by calculating the average ratio between the enzyme activity and the radioactivity incorporation. In 12 independently repeated experiments, an inhibition of 40 ± 9% was observed. In one experiment, illustrated in Fig. 3, the effects of ADP and AMP, in the same concentration, were

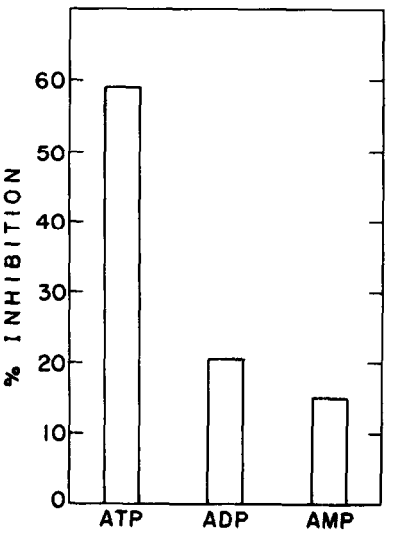

Fig. 3. The effect of ATP, ADP and AMP without carbon source. A suspension of spheroplasts, induced in a medium containing 20% (w/v) sucrose as a stabilizer, was divided into 4 equal portions. One, serving as a control, was incubated with no additions. The others were incubated with 10 mM of ATP, ADP and AMP. Samples for the assay of β-galactosidase formation and ^{14}C-leucine incorporation were taken each 15 min during 2 hr. The differential rate of β-galactosidase formation was estimated for each portion by calculating the average ratio between the enzyme activity and the incorporation of radioactivity. The inhibition by the nucleotides was thus determined, taking the differential rate of the control as 100%.

compared to that of ATP and a clear difference was found. The effects of CTP, UTP and GTP were examined in 5 repeated experiments and inhibitions of 22 ± 11%, 26 ± 12% and 25 ± 15% were found respectively. Statistical analysis of the results (t-test) revealed that the effect of ATP was significantly greater than that of any other nucleotide tested.

Two alternative possibilities can explain these observations: a) The repression of β-galactosidase is not specific only to ATP, but other nucleotides can also mimic its action though in much smaller efficiency. b) Only ATP can repress the enzyme synthesis. The small effects observed with other nucleotides were, apparently, due to their contribution to the ATP pool of the cell either by being converted to ATP (in the case of ADP and AMP) or by providing high-energy-phosphate (in the case of CTP, UTP and GTP).

Cyclic AMP is known to reverse catabolite repression. If the effect of ATP was

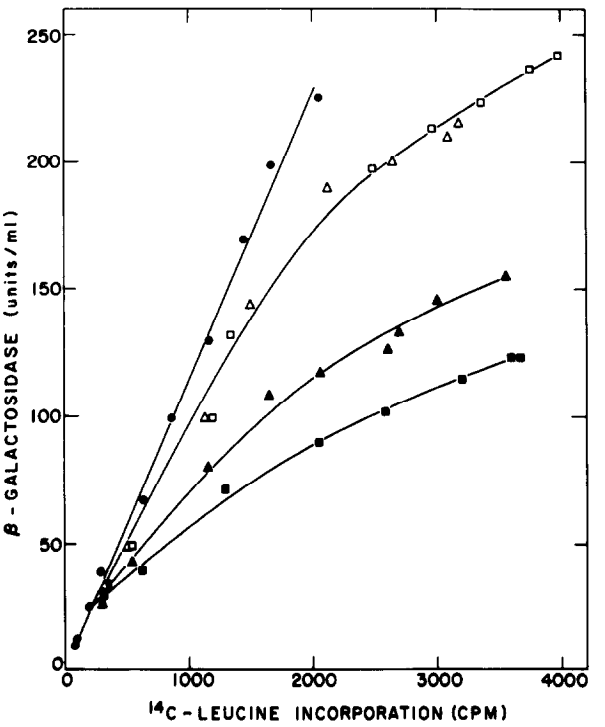

Fig. 4. The reversion of the effect of ATP by cyclic AMP in the presence of glycerol. Experimental details are as in Fig. 1. The symbols are: (●) no additions, (▲) 0.2% glycerol, (■) 0.2% glycerol + 1 mM ATP, (Δ) 0.2% glycerol + 1 mM cyclic AMP, (□) 0.2% glycerol + 1 mM ATP + 1 mM cyclic AMP.

exerted by the mechanism of catabolite repression it should be reversed by cyclic AMP. Fig. 4 shows, indeed, that 1 mM cyclic AMP completely abolished the effect of 1 mM ATP in the presence of 0.2% glycerol. Under these conditions the differential rate of the enzyme formation with cyclic AMP was the same, irrespective of whether ATP was present or not. It is also seen in Fig. 5 that the effect of 10 mM ATP without any carbon source was reduced by 1 mM cyclic AMP.

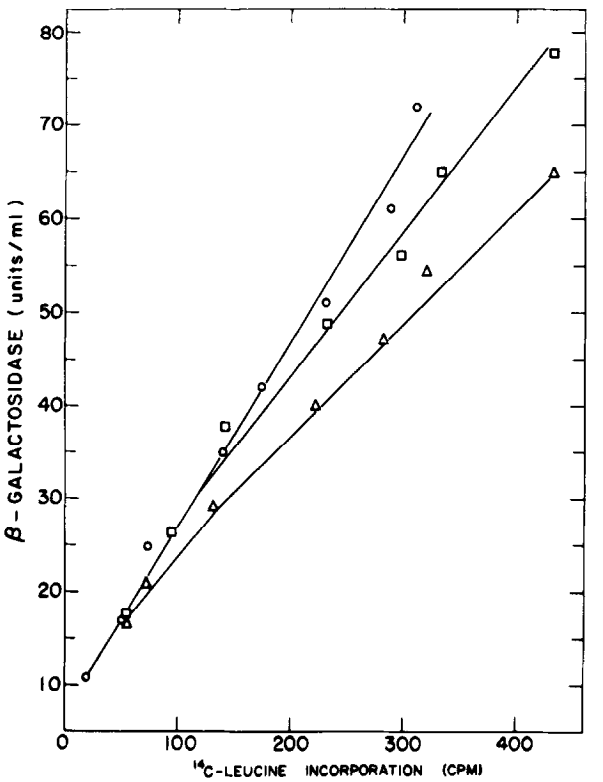

Fig. 5. The reversion of the effect of ATP by cyclic AMP in the absence of carbon source. A suspension of sphroplasts in a sucrose containing medium was divided, after 20 min of induction, into 3 equal portions which were incubated with (○) no additions, (△) 10 mM ATP and (□) 10 mM ATP + 1 mM cyclic AMP. Samples were taken each 20 min.

These observations strongly support the postulation that ATP is involved in producing catabolite repression. However, it is still unclear from this study whether ATP itself represses the enzyme formation or whether it exerts its effect indirectly by activating some other factor.

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