# SEVERAL NUCLEOSIDE-3′ AND/OR 5′-POLYPHOSPHATES STIMULATE β-GALACTOSIDASE INDUCTION IN *ESCHERICHIA COLI*

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## 1. Introduction

*Streptomyces* nucleotide 3′-pyrophosphokinase catalyzes the transfer of 5′-β-γ-pyrophosphoryl group of either ATP, dATP or pppApp to 3′-OH of acceptor nucleotides leading to the formation of an unusual variety of nucleoside polyphosphates [1,2].

Among the unusual nucleotides, ppGpp has received much attention. It is thought to be a signal molecule in bacterial metabolism [3] and to act both as a positive and a negative effector of several specific operons [4–8]. ppGpp inhibits the key enzymes in several metabolic pathways [9,10]. Moreover, (p)ppApp, (p)ppGpp and some other highly phosphorylated nucleotides may be the regulators in bacterial differentiation or early sporulation [11–13].

Little is known about the physiological role(s) of other 3′-pyrophosphorylated purine and pyrimidine ribo- or deoxyribonucleotides, not even the natural occurrence of the pyrimidine derivatives and also of pppdApp. NAD- and FAD-3′pyrophosphates are completely devoid of respective cofactor or inhibitory activities [14]. In [15] tryptophanase and β-galactosidase syntheses were reported stimulated by purine nucleoside polyphosphates; this study was mainly concerned with tryptophanase rather than β-galactosidase induction. We report here that various purine and pyrimidine nucleoside polyphosphates namely pppdApp, Gpp, ppGpp, ppCpp, ppUpp as well as cAMP and cGMP stimulate β-galactosidase induction at the cellular level. We presuppose the natural occurrence of pppdApp, ppCpp and ppUpp in addition to the already known natural occurrence of (p)ppGpp [16,17], (p)ppApp [17] and ppGp [18]. Guanosine-3′-diphosphate (Gpp) was included here as it is known to be a natural amplification factor of glucocorticoid-induced operon transcriptive action in rat liver system [19].

*Abbreviations:* pppdApp, deoxyadenosine-5′-triphosphate-3′-diphosphate; (p)ppApp, adenosine-5′-diphosphate(triphosphate)-3′-diphosphate; (p)ppGpp, guanosine-5′-diphosphate-(triphosphate)-3′-diphosphate; ppGp, guanosine-5′-diphosphate-3′-monophosphate

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## 2. Materials and methods

### 2.1. *Strains and media*

*Escherichia coli* strain HF 4704S, *thy, rel*$^+$ was obtained from Dr T. Komano [20]. Nutrient agar medium contained per liter: yeast extract 2 g, polypeptone 5 g, meat extract 1 g, NaCl 4g, thymine 5 mg, thiamine–HCl 5 mg and agar 15 g, pH adjusted to 7.0 with 1 N NaOH. Culture medium was as in [15]. Cells were grown aerobically at 37°C and this was followed spectrophotometrically at 660 nm.

### 2.2. *Chemicals*

Isopropyl-β-thiogalactopyranoside (IPTG), *o*-nitrophenyl-β-galactopyranoside (ONPG), chloramphenicol, actinomycin D and rifampicin were purchased from Sigma. Purine and pyrimidine nucleoside polyphosphates as lithium salts were prepared in our laboratory [1,2]. Guanosine-3′-diphosphate (Gpp) was synthesized from UpG and dATP or ATP followed by ribonuclease A cleavage [21].

### 2.3. *Cell permeabilization*

Bacterial cells at mid-log phase (3–4 h) were

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harvested by centrifugation at 10 000 × *g* for 10 min at 15°C followed by washing with cold 0.1 M potassium phosphate buffer (pH 7.0) containing 0.15 M NaCl. The cells were then subjected to permeabilization as in [22] except that incubation period was 5 min. Absorbance of the permeabilized cell suspension after dilution (10-fold) with the prewarmed medium was ~0.55 at 660 nm.

### 2.4. *Enzyme induction and assay*

β-Galactosidase induction and assay were done as in [15]. One unit of β-galactosidase catalyzes the hydrolysis of 1 nmol ONPG/ml . min at 37°C. The units of the enzyme in the sample were calculated from the fact that 1 nmol *o*-nitrophenol has an absorbance of 0.0043 under the experimental conditions (using 10 mm light-path).

## 3. Results and discussion

For higher induction of β-galactosidase, the cells were grown in a poor medium (inositol as the carbon source) and suspended in the same. The effects of pppdApp, Gpp, ppGpp, ppCpp and ppUpp as well as of cAMP and cGMP at their increasing concentrations on the IPTG-induced β-galactosidase synthesis in *E. coli* are shown in fig.1. The optimal concentration of each of ppGpp, ppCpp and ppUpp was 0.4 mM and those of cAMP, ppdApp and Gpp were 0.6 mM, 0.2 mM and 0.02 mM, respectively. Cyclic GMP was relatively ineffective. At or above 0.1 mM, Gpp was inhibitory. Of all the nucleoside polyphosphates, pppdApp exhibited unexpectedly the lowest positive effect (27%) and Gpp a sharp optimum. Cyclic AMP demonstrated the highest stimulatory effect (~100%) and ppCpp the next (56%). Normal nucleotides dATP, 5′-GDP, 5′-CDP and 5′-UDP up to 0.4 mM were found to be inactive as stimulators.

The synergistic effects of increasing concentrations of pppdApp and ppCpp added independently in presence of optimum (0.6 mM) or sub-optimum (0.15 mM) levels of cAMP on β-galactosidase induction were studied (fig.2). Each nucleoside polyphosphate and cAMP was added together to the cells. Both pppdApp and ppCpp were found to exert additional stimulatory effects over either level of cAMP. Under these conditions the saturating concentration of pppdApp dropped to a lower level of 0.1 mM in comparison with that obtained in fig.1. However, over 0.1 mM a

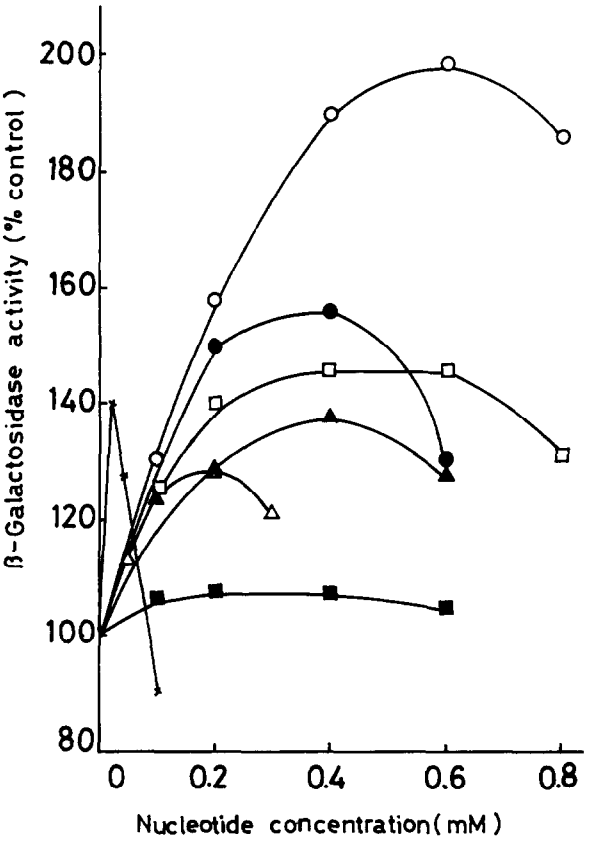


Fig.1. Effects of various concentrations of pppdApp, Gpp, ppGpp, ppCpp, ppUpp, cAMP and cGMP on β-galactosidase induction. β-Galactosidase activity was assayed 20 min after IPTG addition: pppdApp (△——△); Gpp (×——×); ppGpp (□——□); ppCpp (●——●); ppUpp (▲——▲); cAMP (○——○); cGMP (■——■).

sharp fall in the stimulatory effect was observed followed by inhibition at or above 0.2 mM. Similar results were also obtained when combined effects of cAMP and ppGpp were studied as above and the maximum stimulation occurred at 0.1 mM ppGpp (not shown). On the other hand the saturating concentration of ppCpp remained the same as in fig.1. The data thus apparently indicate the different mechanism of function of purine and pyrimidine nucleoside polyphosphates. The results also suggest that this unusual variety of nucleotides and cAMP each stimulate β-galactosidase induction in an independent fashion.

The medium for induction contained $Mg^{2+}$ (1.1 mM) and it was postulated that a higher concentration of $Mg^{2+}$ might be favourable for stronger induc-

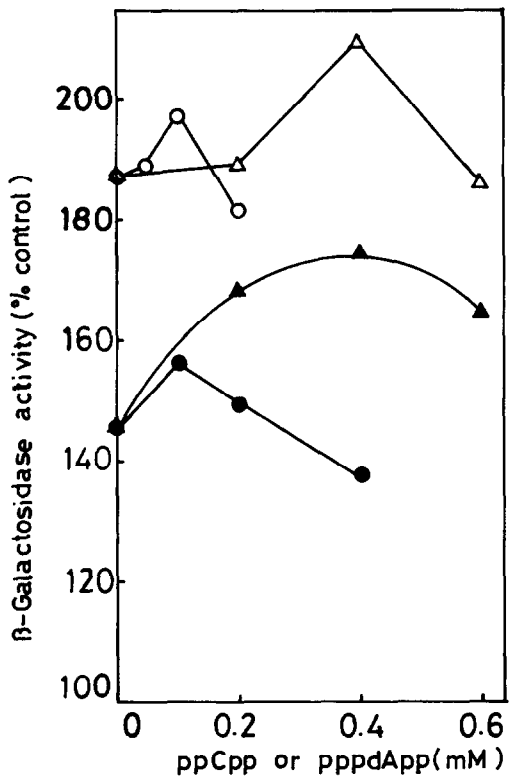


Fig.2. Effects of various concentrations of ppCpp and pppdApp at different cAMP levels. β-Galactosidase activity was assayed 20 min after IPTG addition:

| cAMP (0.15 mM) | cAMP (0.6 mM) |
|---|---|
| + ppCpp (▲——▲) | + ppCpp (△——△) |
| + pppdApp (●——●) | + pppdAPP (○——○) |

tion of the enzyme. However, we could not detect any increase in effect even at 10 mM $Mg^{2+}$ (not shown). Furthermore, we tested the effects of $Li^+$ on the enzyme induction as the nucleotides were lithium salts. This ion at ~3 mM exhibited a negligible effect and inhibited the induction at ≳7 mM (not shown).

The finding [23] that $NH_4^+$ was the most active and $Li^+$ was ineffective in preserving the peptidyl transferase activity of 50 S ribosome in vitro tempted us to prepare ppGpp–$NH_4$ salts to see if there is any striking stimulation of β-galactosidase induction by these nucleotides. $Li^+$-free $NH_4$-salt of ppGpp was prepared by Sephadex G-15 column chromatography (not shown). This nucleotide was then tested for its effect on β-galactosidase synthesis in comparison with

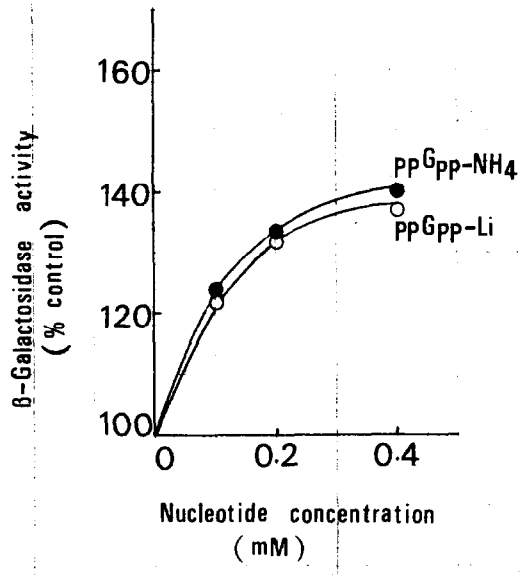


Fig.3. Effects of lithium- and ammonium-salts of ppGpp on β-galactosidase induction. Assay conditions were as in fig.1.

its Li-form. Induction medium also contained ~20 mM $NH_4Cl$. No significant difference between the stimulatory activities of these 2 forms of ppGpp was observed (fig.3). So the effects of nucleoside polyphosphates in this in vivo system did not depend on whether these were in $NH_4$-form or Li-form under the experimental conditions. Data also indicate that $Li^+$ at its low concentration (<2.5 mM) brought by the nucleotides in our experimental system was not inhibitory, rather it can be said to be inert.

These data reveal that the nucleoside polyphosphates namely pppdApp, Gpp, ppGpp, ppCpp and ppUpp stimulate β-galactosidase induction in addition to the already known positive effect of ppGpp and ppApp. In *E. coli* cAMP regulates the expression of some genes by activating a cAMP-binding protein (CAP) that stimulates the transcription of defined subset of genes by directly interacting with the transcriptional complex [24]. Cyclic GMP normally is known to antagonize this effect of cAMP [25] which explains, at least in part, the observed inertness of cGMP in stimulating β-galactosidase induction. The higher stimulatory effect of nucleotides observed in cells grown on poor energy source is possibly because of the fact that this type of medium causes a relatively small decrease of intracellular cAMP level [22]. This further suggests that the nucleoside polyphosphates

act, in part, by increasing the cellular cAMP concentration.

The regulatory effects of ppGpp have been shown to take place not on *lac* mRNA elongation, degradation or translation but on transcription initiation and furthermore this ppGpp function is known to be directed by a protein factor [5]. These findings suggest that, like ppGpp, other pyrophosphorylated nucleotides may have acted to promote *lac* transcription initiation.

An important feature of these results that invites further attention is the lack of remarkable difference in effects as well as of specificity in stimulating β-galactosidase induction by nucleoside polyphosphates at their optimum concentrations. Similar results indicating insignificant difference in stimulatory effects of (p)ppGpp and (p)ppApp on both tryptophanase and β-galactosidase inductions were obtained in [15]. This may indicate the presence of responsive regulatory factor or factors inactivated or removed during permeabilization or induction. Another possibility is that ppGpp-binding protein, if it has broad specificity like *Streptomyces* nucleotide 3′-pyrophosphokinase, may be interacting with other 3′-pyrophosphonucleotides in a similar fashion. The mechanisms by which these nucleoside polyphosphates act to give uniformity in stimulatory function remain to be elucidated.

Since a protein factor is reported to be responsible for ppGpp function, use of protease inhibitor or stabilizer in the induction system would be expected to concede more effective stimulation of induction.

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