# EFFECT OF PHENETHYL ALCOHOL ON INDUCTION OF ALKALINE PHOSPHATASE IN ESCHERICHIA COLI

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Received September 4, 1970

SUMMARY: Phenethyl alcohol, at concentration of 0.15 to 0.2%, specifically inhibits induced formation of alkaline phosphatase activity, but not that of $\beta$-galactosidase in Escherichia coli. This inhibition is reversible. Evidence has been obtained which suggests that this chemical prevents conversion of inactive monomer sub-units to active alkaline phosphatase dimer rather than inhibiting de novo synthesis of sub-units themselves.

Alkaline phosphatase is one of several hydrolytic enzymes in E. coli that seem to be localised in the space between cell membrane and cell wall (1, 2). The enzyme has been shown to be an unusually stable zinc metalloprotein composed of two identical sub-units (3, 4). Recent experiments of Schlesinger (5) and Torriani (6) are suggestive of a mechanism in which inactive monomer sub-units, synthesised in the cytoplasm, diffuse through the cell membrane to a periplasmic region where subsequent dimerisation to active enzyme occurs.

In this communication, we report our studies with phenethyl alcohol (PEA), an agent believed to alter bacterial cell membrane structure (7), which support the mechanism proposed by Schlesinger and Torriani. PEA has been found to inhibit specifically induction of alkaline phosphatase in E. coli, but not that of $\beta$-galactosidase, an internal enzyme (1) formed in the cytoplasm (8). This inhibition appears to result from interference with the dimerisation of monomer sub-units rather than the inhibition of synthesis of sub-units themselves.

## RESULTS AND DISCUSSION

Rosenkranz et al. reported that in E. coli, at levels which were not inhibitory to other cellular processes, PEA inhibited induced formation of β -galactosidase and alkaline phosphatase (9, 10), whereas Prevost and Moses (11) found that this agent simultaneously inhibited alkaline phosphatase induction as well as general synthesis of proteins. It will be seen from Fig.1 that at concentration of 0.25%, PEA inhibits induction of both alkaline phosphatase and β -galactosidase in E. coli $B_{12}$/methionine auxotroph. However, at lower concentrations, i.e. 0.15 and 0.2%, PEA exhibits differential action. At 0.15% PEA, induction of alkaline

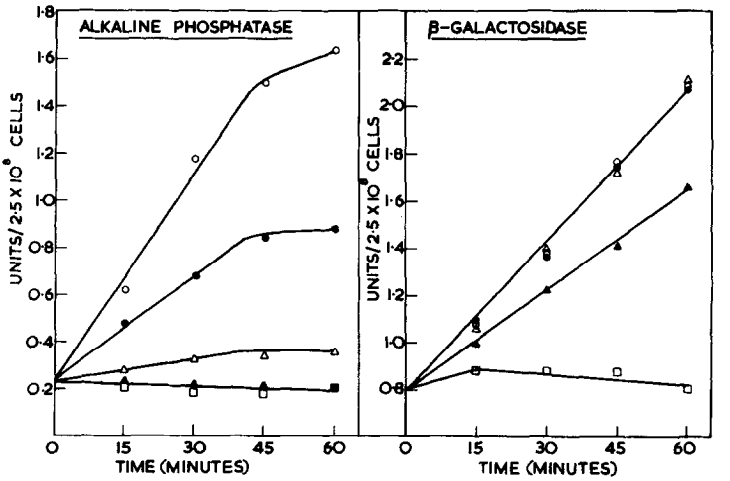


Fig. 1 Effect of PEA on induction of β-galactosidase and alkaline phosphatase in E. coli $B_{12}$/methionine auxotroph 113-3. Cells grown for 18 hr in nutrient broth were washed and suspended in phosphate-free medium ( (A-Pi) medium used by Torriani (6) ) containing isopropyl-thio-β-D-galactopyranoside ($10^{-3}$ M) at the cell density of 5 x $10^8$ cells/ml and incubated at 37°C. At the end of 1½ hr, PEA was added. This is considered as 'zero-time' in the figure. β -galactosidase activity was assayed with o-nitrophenyl-β-D-galactopyranoside as substrate, as described by Pardee et al. (12) and alkaline phosphatase activity with p-nitrophenylphosphate as substrate according to the method of Torriani (13). For both the enzymes, one unit of enzyme activity is defined as that amount which catalyses the hydrolysis of 1 mμ mole of substrate per min under respective conditions of assay. Specific activity is expressed as units of enzyme per 2.5 x $10^8$ cells. The density of cell suspension was determined by measuring absorption at 490 mμ. O——O, No PEA; ●——●, 0.1% PEA; Δ——Δ, 0.15% PEA; ▲——▲, 0.2% PEA; □——□, 0.25% PEA.

phosphatase is suppressed by about 90%, but that of β-galactosidase is unaffected. Formation of alkaline phosphatase is completely inhibited in the presence of 0.2% PEA in the medium, while β-galactosidase synthesis is lowered only by about 10%. As seen in Fig.2, this effect of PEA on alkaline phosphatase induction is reversible; the enzyme formation is resumed as soon as the cells are transferred to the medium devoid of PEA.

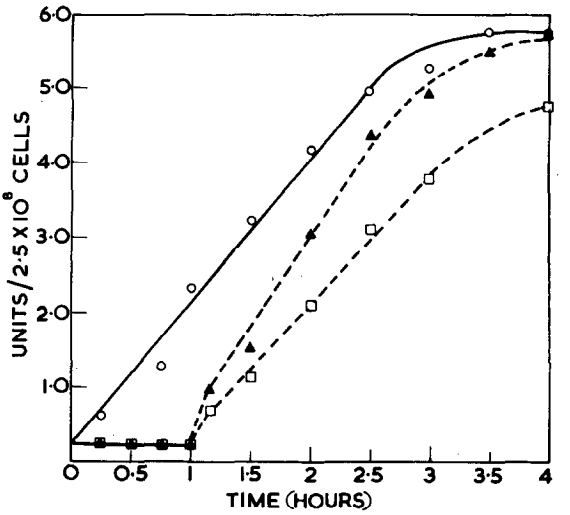


Fig.2 Reversal of inhibition by PEA of alkaline phosphatase induction. E. coli cells were incubated at 37°C in (A-Pi) medium at the initial cell density of 5 x $10^8$ cells/ml. At the end of 1½ hr, PEA was added. After further 1 hr, the cells were collected on Millipore filters (HAWP), washed and transferred to fresh (A-Pi) medium and incubated at 37°C. Other details as in Fig.1. O——O, No PEA; ▲——▲, 0.2% PEA; □——□, 0.25% PEA; ▲- - -▲, incubated for 1 hr in 0.2% PEA and then in (A-Pi) medium; □- - -□, incubated for 1 hr in 0.25% PEA and then in (A-Pi) medium.

The differential effect of PEA on formation of these two enzymes was also observed with E. coli 15 T⁻U⁻ at the same concentration range. It is possible, however, that PEA may exert this effect at somewhat different concentration ranges in other strains of E. coli and under different growth conditions.

The action of PEA on alkaline phosphatase formation could either be due to specific inhibition of synthesis of the enzyme protein or

due to interference in the conversion of inactive monomer sub-units to active phosphatase dimer. Silver and Wendt (7), from their studies on permeability to acriflavin and efflux of intracellular potassium in E. coli cells treated with PEA, have inferred that PEA affects cell membrane structure. These authors have suggested that observed inhibitory effects of this chemical on processes such as initiation of DNA replication (14, 15), conjugation (16, 17), and sporulation (18) are secondary consequences of the changes in cell membrane conformation. Recent studies indicate that monomers of alkaline phosphatase are accumulated in a periplasmic region (5, 6) where dimerisation to active enzyme takes place, possibly mediated by the cell membrane (19). It, therefore, seems more likely that action of PEA on alkaline phosphatase formation results from interference in the dimerisation rather than inhibition of the synthesis of sub-units themselves.

It has been shown that under conditions of growth in 'low $Zn^{++}$'-containing medium, the synthesis of active alkaline phosphatase in E. coli is suppressed while monomer sub-units accumulate (6). In order to ascertain whether incubation of cells in PEA-containing medium also leads to a similar accumulation of the sub-units, the following experiment was carried out. Cells were incubated in phosphate-free medium in the presence of 0.2% PEA at 37°C for 1 hr, after which they were collected on Millipore filters and subsequently incubated in phosphate-free medium devoid of PEA but containing chloramphenicol, which ensured no further synthesis of alkaline phosphatase protein. It is apparent from Table 1 that the enzyme activity of cells, previously incubated with 0.2% PEA, was increased even in the presence of chloramphenicol. In the control experiment in which de novo synthesis of the enzyme protein was suppressed by chloramphenicol during incubation of cells with 0.2% PEA, no such increase in enzyme activity was observed. This lends support to the presumption that PEA allows synthesis of inactive monomers but precludes their conversion to active enzyme dimer. The increase in alkaline phosphatase activity during incubation in the presence of chlora-

Table 1. Increase in alkaline phosphatase activity in presence of chloramphenicol in E. coli cells previously incubated in phosphate-free medium containing 0.2% PEA.

| | Sp. activity units/$2.5 \times 10^8$ cells | Increase in sp. activity over initial level | Relative increase % of control |
|---|---|---|---|
| 1. Zero time | 0.21 | | |
| 2. 1 hr in (A-Pi) | 2.00 | 1.79 | 100 |
| 3. 1 hr in (A-Pi) plus PEA | 0.21 | 0 | 0 |
| 4. 1 hr in (A-Pi) plus CM | 0.21 | 0 | 0 |
| 5. Cells from 3 in '(A-Pi) plus CM' for further 1 hr | 0.55 | 0.24 | 13.4 |
| 6. 1 hr in '(A-Pi) plus PEA plus CM' | 0.21 | 0 | 0 |
| 7. Cells from 6 in '(A-Pi) plus CM' for further 1 hr | 0.21 | 0 | 0 |

Cells ($6.5 \times 10^8$/ml) were incubated in (A-Pi) medium at 37°C. At the end of 1½ hr, PEA (0.2% final concentration) and chloramphenicol (CM) (100 µg/ml final concentration) were added, wherever indicated. This is considered as zero time. The incubations were continued for another hour and aliquots were assayed for enzyme activity. At this time interval, cells from expt. 3 and 6 were collected on Millipore filters and transferred to (A-Pi) medium containing CM (100 µg/ml). After incubation for additional 1 hr aliquots were assayed for enzyme activities.

mphenicol was only about 13% of control and incubation for extended periods did not result in any further rise in activity. The reason for this is not known. It has been shown that monomer sub-units are unstable (20) in contrast to the active dimer enzyme. This may restrict capacity of cells to accumulate sub-units.

Dimerisation of alkaline phosphatase sub-units is known to be dependent on $Zn^{++}$ (6, 21). The action of PEA on the dimerisation cannot be due to chelation of $Zn^{++}$ since $Zn^{++}$, at the final concentration of $10^{-3}$ M included in the PEA-containing medium, did not reverse the PEA effect.

Whether PEA directly affects dimer formation, or not, was tested in an in vitro system. It is seen from Fig. 3 that 0.2% PEA partially suppresses this reaction. Since, however, the appearance of enzyme activity

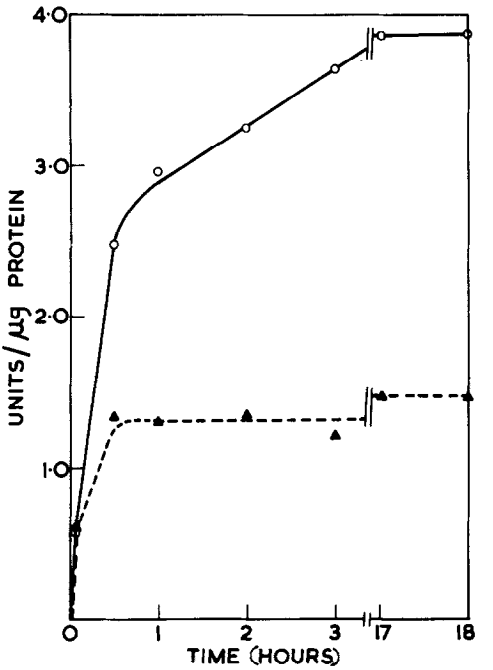


Fig. 3 Reactivation of alkaline phosphatase sub-units in the presence of PEA. Alkaline phosphatase was purified from E. coli $B_{12}$/methionine auxotroph 113-3 by the method of Torriani (22) and sub-units from this enzyme were obtained by acid inactivation according to the procedure of Schlesinger and Barrett (21). The monomer sub-units (69.6 μg/ml) were incubated at 37°C in the presence of 0.1 M Tris-Cl (pH 7.4), 0.4 mM $ZnSO_4$, 1 mM $MgCl_2$. PEA was added at the final concentration of 0.2%. Aliquots were removed and assayed at the indicated times. O——O, No PEA; ▲- - - -▲, 0.2% PEA. Definition of enzyme unit, as in Fig.1.

is totally prevented at this concentration of PEA in intact cells, it is unlikely that this agent interferes with the dimerisation per se. Rather, it seems plausible that the PEA effect stems from its primary action on the cell membrane.

The present findings reveal yet another subtle effect of phenethyl alcohol which should prove useful in studying regulatory aspects of alkaline phosphatase formation in relation to cell structure.

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