Effect of 5-Fluorodeoxyuridine on the Induction of the *Bacillus subtilis* Phage ϕ 3610

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Many strains of *Bacillus subtilis* are lysogenic and produce phage-like particles that have the property of killing, but of being unable to grow in, sensitive strains of *B. subtilis*. In their study of the PBSX phage particles released from *B. subtilis* 168 after the addition of mitomycin C, Seaman, Tarmy & Marmur (1964) found that the yield of phage was not reduced when the bacteria were treated with mitomycin C in the presence of 5-fluorodeoxyuridine, and they concluded that the phage burst could be obtained in the absence of DNA synthesis. This result is unusual as it is now believed that during the development of a number of viruses expression of 'early' genes and viral DNA replication must occur before the 'late' genes coding for the structural proteins of the virus coat are expressed (Hayes, 1968; Consigli, Minocha & Abo-Ahmed, 1968). It was therefore decided to examine the effect of 5-fluorodeoxyuridine on the development of phage ϕ 3610, a phage-like object similar to phage PBSX, produced after treatment of *B. subtilis* N.C.T.C. 3610 with hydrogen peroxide (Stickler, Tucker & Kay, 1965).

Cultures (10 ml.) of *B. subtilis* 3610 were grown to early exponential phase (10^8 cells/ml.) at 37° with aeration in minimal medium (Davis & Mingioli, 1950) supplemented with 50 mM-glucose, and were then induced with hydrogen peroxide (0.1 mM). The variability in yields of phage with this agent was overcome by simultaneously adding 10 mM-3-amino-1,2,4-triazole, an inhibitor of catalase (Margoliash, Novogrodsky & Schejter, 1960). Induction with hydrogen peroxide in the presence of 3-amino-1,2,4-triazole was performed with and without the addition of 5-fluorodeoxyuridine (0.11 mM) immediately before induction. The turbidity of the cultures was followed and the yield of ϕ 3610 assayed by using the phage-sensitive bacterium *B. subtilis* BS7SR and the 'killing titre' method of Delbrück & Luria (1942). The normally induced culture showed an increase in turbidity for about 90 min., when lysis occurred with the liberation of 1.8×10^9 phage/ml. In the presence of 5-fluorodeoxyuridine there was no increase in turbidity and the yield was 10^8 phage/ml. This result suggests that unlike phage PBSX, phage ϕ 3610 requires DNA synthesis for its development.

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Effect of Sodium Chloride on *Escherichia coli* Alkaline Phosphatase

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A feature of *Escherichia coli* alkaline phosphatase is that the steady-state rate constant, k_0 , includes phosphorylation (k_2) and dephosphorylation (k_3) terms. The maximum activity at low ionic strength can be increased up to twofold in several ways, for instance by high salt concentration, by high substrate concentration (Heppel, Harkness & Hilmoe, 1964) and by tris or ethanolamine (Wilson, Dayan & Cyr, 1964). The last-named two reagents both activate and act as phosphoryl acceptors, which led Wilson *et al.* (1964) to conclude that k_3 is the rate-determining step under these conditions. Although Trentham & Gutfreund (1968) concluded that k_3 was only partly rate-determining, they agreed that tris activated by increasing k_3 , leading to a 1.4-fold increase in k_0 .

We have examined the effect of salt on the enzyme and find that 1M-NaCl produces a twofold activation at alkaline pH, in agreement with Wilson *et al.* (1964). However, under acid conditions there is general inhibition with a maximum tenfold decrease in activity at pH 4. The crossover point between activation and inhibition occurs at pH 7, and this coincides with the change in rate-determining step at low ionic strength (Fernley & Walker, 1966) from k_2 (alkaline) to k_3 (acid).

Stopped-flow observations of the reaction with 4-methylumbelliferyl phosphate in 0.02*M* tris-acetate acid buffers at 20° indicate that at pH 4.8, the addition of 1M-NaCl increases k_2 from 22 sec.⁻¹ to about 60 sec.⁻¹, but decreases k_3 from 0.27 sec.⁻¹ to 0.05 sec.⁻¹. At pH 7.1, k_2 changes from 6 sec.⁻¹ to about 18 sec.⁻¹ and k_3 shows a slight decrease from 6 sec.⁻¹ to 5 sec.⁻¹. Thus NaCl appears to promote phosphorylation and, if anything, to inhibit dephosphorylation.

Comparing the maximum steady-state activations by NaCl and tris at 25° by using *p*-nitrophenyl phosphate gave the following result: 1M-tris-acetic acid plus 1M-NaCl, pH 8.40, $k_0 = 47$ sec.⁻¹; 0.1M-tris-acetic acid plus 2M-NaCl, pH 8.81, $k_0 = 41$ sec.⁻¹. It

is concluded (a) that activation by NaCl can almost match the activation by tris and (b) that the effect is not on k_3 but on k_2 .

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Shay Chloroma Alkaline Phosphatase

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The characterization of normal human and animal tissue alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) has been described (Morton, 1955) and studied in great detail (Moss, Eaton, Smith & Whitby, 1967; Sussman, Small & Cotlove, 1968). Since such studies have not to date been reported for a tumour enzyme we studied the Shay Chloroma, a myeloblastic tumour in the rat (Moloney, Dorr, Dowd & Boschetti, 1962). This tumour contains an average of 8.0 i.u. of alkaline phosphatase/g. wet wt. of tumour. The enzyme was purified by butanol extraction, acetone fractionation, ammonium sulphate fractionation, gel filtration on Sephadex G-200 and chromatography on DEAE-cellulose DE52.

Enzyme activity was eluted in 3 peaks from DEAE-cellulose DE52 at 50, 100 and 250 mM-NaCl, designated respectively peaks A, B and C. The purification achieved was 200-fold for peak B and 90-fold and 120-fold for peaks A and C. Enzyme B was eluted in two peaks of activity from Sephadex G-200, an included and excluded peak, and enzymes A and C were both totally excluded from the gel.

The K_m values for the three enzymes with *p*-nitrophenyl phosphate as substrate were 0.46, 0.37 and 0.44 mM and the pH optimum was pH 10.2 in each case. Enzyme A was the most resistant to urea inactivation, 55% of the activity remaining in 3.0 M-urea compared with 20% with enzymes B and C. The urea inhibition was non-competitive. On polyacrylamide-gel electrophoresis, enzyme B moved as a single band and had the highest mobility, enzyme A separated into three bands and enzyme C into two bands of activity. One component of enzyme A and of enzyme B was retarded after neuraminidase treatment. Extensively dialysed samples of the three enzymes were activated by Mg^{2+} , all to different degrees. EDTA inactivated enzymes A, B and C, the inactivation being a function of time and concentration; the activity was only partially restored by addition of Mg^{2+} .

The three enzymes varied in their heat stability but were all inhibited to the same extent (20%) by L-phenylalanine. The ratio of alkaline phosphatase activity to inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) activity was not constant through all the steps of purification (Moss *et al.* 1967); the ratios for enzymes A, B and C were 13, 20 and 12 respectively.

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The Human Pseudocholinesterase System

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Multiple forms of human pseudocholinesterase have been demonstrated on starch-gel (La Motta, McComb, Noll, Wetstone & Reinfrank, 1968) and polyacrylamide-gel (Juul, 1968) electrophoresis. It has been shown (Svensmark, 1961) that there are several molecules of *N*-acetylneuraminic acid (NANA) associated with each molecule of pseudocholinesterase, but that these play no part in the enzyme function (Augustinsson & Ekedahl, 1962).

A 460-times enriched preparation of the enzyme was obtained from pooled outdated plasma by ammonium sulphate precipitation and DEAE-cellulose fractionation at pH 4.2. Polyacrylamide-gel electrophoresis at pH 8.9 followed by enzyme detection with butyrylthiocholine iodide showed that some enzyme components of whole serum were lost during this purification process. The consistency of the distribution of the enzyme components of whole serum on polyacrylamide gels under various conditions of gel-pore size and buffering indicated that the heterogeneity may not be completely explained by assuming polymer forms for the enzyme (La Motta *et al.* 1968).

The enriched enzyme preparation, after treatment with neuraminidase (EC 3.2.1.18) for 7 days at 30° was quite homogeneous on polyacrylamide-gel electrophoresis and its mobility was less than that of the major component of the heterogeneous (6–10 components) control. Both the neuraminidase-treated preparation and its control yielded three fractions on elution from Sephadex G-75 with dilute ammonia solution at pH 8.2. The two major protein fractions were poorly resolved and eluted at about the void volume and the third minor fraction was eluted at about twice the void volume. Whereas enzyme activity was associated