Cyanide Binding and Inhibition of a Purified Arylsulphatase of *Alcaligenes metalcaligenes*

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The arylsulphatase of *Alcaligenes metalcaligenes* shows, in the presence of inhibitors such as sodium cyanide and hydrazine hydrochloride, the characteristics of 'anticompetitive' inhibition (Dodgson, Spencer & Williams, 1956): a plot of $1/v$ against $1/s$, for various inhibitor concentrations, yields parallel lines instead of lines that intersect on either of the axes. A possible explanation is that the inhibitor combines with the enzyme only in the presence of substrate.

The enzyme was prepared to stage B as described by Dodgson, Melville, Spencer & Williams (1954) and was further purified by gel filtration on Sephadex G-50.

Enzyme incubated with cyanide and then dialysed against excess of buffer showed little decrease in activity towards *p*-nitrophenyl sulphate compared with a control that had not contained cyanide, but the activity of enzyme that had been incubated with cyanide in the presence of *p*-nitrophenyl sulphate was greatly decreased. This decrease increased with inhibitor concentration. These results suggest that the inhibition is irreversible, and similar tests with [^{14}C]cyanide showed that, after dialysis, significantly greater amounts of cyanide remained bound to the enzyme when it was incubated in the presence of substrate despite the high background binding when substrate was omitted. This was confirmed by passing the solutions through Sephadex G-75 after incubation, to ensure complete removal of unbound cyanide, when the difference between cyanide bound in the presence and absence of substrate was more significant. This background binding was probably due to the impurity of the enzyme preparation.

The initial rate of hydrolysis of *p*-nitrophenyl sulphate, both in the presence and in the absence of inhibitor, was substantially the same, but, in the presence of cyanide, it then fell off until inhibition was almost, or completely, total depending upon cyanide concentration. If the enzyme was pre-incubated with cyanide, on addition of substrate the initial rate of hydrolysis was somewhat less than in the above cases.

Further purification of the enzyme has been attempted by a combination of DEAE-cellulose and isoelectric-focusing techniques to eliminate the high background binding of cyanide in the absence of substrate.

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The Fractionation of Transforming Deoxyribonucleic Acid from *Bacillus subtilis*

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Ayad, Barker & Weigold (1968) reported the fractionation of transforming DNA on columns of methylated albumin-kieselguhr by stepwise elution by increasing concentrations of sodium chloride. Using this technique they were able to obtain partial separation of the DNA corresponding to the histidine B and tryptophan genes of *Bacillus subtilis* from that corresponding to the arginine gene.

The present study set out to discover whether a similar separation of the DNA corresponding to the adenine, histidine A and lysine genes could be obtained, by using *B. subtilis* strain 312, auxotrophic for adenine, histidine and lysine, as recipient, and two prototrophic strains, PR 312 and 234, as sources of donor DNA.

In agreement with the results of Ayad, Barker & Weigold (1968) both DNA types on fractionation gave three main u.v.-absorbing peaks measured at 260 m μ . Of these, peak 1 consisted of low-molecular-weight material with a sedimentation coefficient of less than 5s, with no transforming activity. Peaks 2 and 3 had sedimentation coefficients of 22s and 26s respectively, and both showed transforming activity. No separation of the markers had been achieved, but it was observed that, in the fractionation of strain PR 312 DNA, at least 95% of the transforming activity appeared in peak 2, whereas in the fractionation of strain 234 DNA at least 87% of the transforming activity appeared in peak 3.

The higher molecular weight of peak 3 is in accord with the results of Mandell & Hershey (1960) and Ayad, Barker & Weigold (1968). Melting-temperature and buoyant-density measurements indicated that peaks 2 and 3 are native and that the later fraction is richer in adenine and thymine, in agreement with Ayad, Barker & Weigold (1968) and Sueoka & Cheng (1962).

The results reported could be explained by assuming that the transforming activity for the adenine, histidine A and lysine markers are associated with regions of different overall base composition in the two strains studied. An alternative interpretation is that the DNA molecules from the two strains preferentially break in different