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

By I. S. SHAW, W. D. STEIN and C. H. WYNN.
(Department of Biological Chemistry, University of Manchester, M13 9PL)

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*

By G. R. BARKER and P. HODGES. (Department of Biological Chemistry, University of Manchester, M13 9PL)

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

places, either during isolation or on fractionation, to give fragments of different molecular weight. The merits of these two possible explanations will be discussed.

Although the transforming activity is associated largely with different chromatographic fractions of DNA in the two prototrophic strains used, the ratio of transforming frequencies for the three markers studied are the same.

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The Cell Wall of a Chlorhexidine-Resistant *Pseudomonas*

By A. DAVIES and W. ROBERTS. (*Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire*)

A strain of *Pseudomonas* capable of growth in media containing high concentrations of chlorhexidine had a lower capacity than normal strains for absorbing the bactericide from the growth medium (Bentley, Davies, Field & Roberts, 1968). This might be a reflection of an unusual wall structure. Bacteria were grown in Roux bottles at 30° in nutrient broth with 0.5% (w/v) glucose (150 ml./bottle) with and without chlorhexidine digluconate (100 µg./ml.) and were also grown on nutrient-agar plates from which they were scraped and suspended in 0.15 M-NaCl. Cells grown without chlorhexidine were centrifuged and washed with cold 0.15 M-NaCl; those grown with chlorhexidine were washed once with 0.1% (w/v) acetic acid and once in 0.1 M-phosphate buffer, pH 7.0, both at 2°. Cells were disrupted by shaking them with no. 12 Ballotini beads in a Mickle disintegrator for 15 min. at 2°. Cell walls were isolated by centrifugation and resuspension five times in M-NaCl and five times in water, and then fractionated by a method based on that of Clarke, Gray & Reaveley (1967). Comparative analytical values for the walls of the chlorhexidine-resistant bacterium and for *Pseudomonas aeruginosa* N.C.T.C. 1999 (in parentheses) were: % of N 13.2, 11.1 (9.6, 8.9); % of P 0.62, 0.54 (1.8, 1.8); % of lipopolysaccharide (aqueous phase after phenol extraction of the wall) 5.8, 11.2 (22.7, 27.2); % of P in the lipopolysaccharide 1.76, 1.0 (4.9, 4.4). Although the values varied with different growth conditions (in each pair of values the first value is for liquid culture, the second for culture on agar)

it was clear that the resistant *Pseudomonas* contained more nitrogen and considerably less phosphorus than *Ps. aeruginosa*. The greatest difference (eightfold) was in the amount of lipopolysaccharide phosphorus. The electrophoretic properties of these organisms differed: with *Ps. aeruginosa* 80% of the surface charge was due to a monobasic acid of pK about 4.5 and 20% to an acid of lower pK, whereas with the resistant organism the proportions were 94 and 6% respectively. Chlorhexidine readily precipitates nucleic acids from solutions and has far less effect on proteins (Hugo & Longworth, 1966; A. Davies, unpublished work); the resistant *Pseudomonas* may have acquired its resistance to chlorhexidine at least in part by a decrease in surface phosphate groups, thus decreasing the cell's ability to absorb the bactericide.

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Intracellular Accumulation of N-Acetylhexosamines in *Micrococcus lysodeikticus*

By A. J. GARRETT. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

Deprivation of bivalent cations increases the intracellular concentration of a mucopeptide precursor in *Bacillus subtilis* W23 (Garrett, 1968). Mg²⁺ or Mn²⁺ are specific activators of all the mucopeptide-synthesizing systems isolated so far, and as the particulate enzymes from *Micrococcus lysodeikticus* have the highest requirement for Mg²⁺ (Anderson, Meadow, Haskin & Strominger, 1966), the effects of bivalent-cation starvation on cell suspensions of this organism were studied.

M. lysodeikticus 2665 cells from exponentially growing cultures were resuspended (1.5–2.0 mg. dry wt./ml.) in 0.05 M-phosphate buffer, pH 7.0, containing DL-alanine, DL-glutamic acid, L-lysine, glycine (each 0.02%), glucose (1.0%) and chloramphenicol (0.005%). Incubation in this medium alone does not stimulate accumulation of N-acetylhexosamines, but EDTA (0.001 M) caused a 10–15-fold increase in 1 hr. Chromatography of the trichloroacetic acid-soluble material on Sephadex G-25 and on paper showed the presence of three nucleotide-N-acetylhexosamines. Two have been identified as the mucopeptide precursors, UDP-N-acetylmuramyl-Ala-Glu-Lys-Ala-Ala (44% of the hexosamine pool) and UDP-N-acetylglucosamine (32%). The third (24% of the pool) is a UDP derivative of an acidic hexosamine, with a uridine: