calcoaceticus is an allosteric enzyme exhibiting negative co-operativity towards the co-

enzyme and ADP.

The physiological significance of negative co-operativity has been discussed by Conway & Koshland (1968). Negative co-operativity insulates an enzyme from the effects of large fluctuations in ligand concentrations. However, when an enzyme exhibits negative co-operativity towards a substrate or a coenzyme and also towards an effector, it is possible to obtain a situation where the rate also can be modulated depending on the relative magnitude of the homotropic and heterotropic constants and the relative concentrations of substrates, coenzymes and the different effectors. Although it is premature to extrapolate results obtained with an isolated enzyme to situations in vivo, the possibility that modulation of benzoylformate decarboxylase might be another element of control of mandelate degradation cannot be discounted. This possibility is further indicated by the observation that the benzoylformate decarboxylase of an unrelated organism, Aspergillus niger, showed an intermediary plateau region in its saturation with thiamin pyrophosphate and is inhibited by ATP (Jamaluddin, 1971).

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Purification and Properties of an Inducible β-Lactamase from Pseudomonas aeruginosa N.C.T.C. 8203

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Members of the β-lactamase family of enzymes (EC 3.5.2.6) catalyse the hydrolysis of the β-lactam bond of penicillins and cephalosporins. An inducible cell-bound β-lactamase of Pseudomonas aeruginosa N.C.T.C. 8203 was described by Jago et al. (1963), Sabath & Abraham (1964) and Sabath et al. (1965). They found that high concentrations of inducer were required for its production, that it hydrolysed cephalosporins at a much greater rate than penicillins but showed a higher affinity for the latter and that it was strongly inhibited by methicillin and cloxacillin. This β-lactamase has now been purified and some of its physical and chemical properties have been determined.

Ps. aeruginosa N.C.T.C. 8203 was grown in shake flasks in CCY medium (Gladstone & Fildes, 1940). Addition of 6-aminoopenicillanic acid as an inducer to a final concentration of 1 mg/ml (Garber & Friedman, 1970) to cultures in mid to late exponential phase (12×10⁹ bacteria/ml of growth medium) gives high enzyme production after 2h with minimum lysis of the organism. The amount of cell-bound β-lactamase formed corresponds to 1% of total cell protein. The enzyme is released from the cells by freezing and thawing. After removal of cellular material by centrifugation (16000g for 1h), the enzyme is purified in two chromatographic steps (Table 1), to give an electrophoretically homogeneous preparation. In this way 20–25 mg of pure β-lactamase can be isolated from 1 litre of culture.
Table 1. Purification of the β-lactamase from *Ps. aeruginosa N.C.T.C. 8203*

<table>
<thead>
<tr>
<th>Stage in purification</th>
<th>Total activity against cephalosporin C substrate* (enzyme units)</th>
<th>Yield (% of activity)</th>
<th>Total protein from 1 litre of culture (mg)</th>
<th>Specific activity (enzyme units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-thawed cells</td>
<td>950000</td>
<td>100</td>
<td>3000</td>
<td>315</td>
</tr>
<tr>
<td>Centrifugation and dialysis</td>
<td>913000</td>
<td>96</td>
<td>1290</td>
<td>710</td>
</tr>
<tr>
<td>Sephadex CM-50, pH5.9, salt-gradient elution</td>
<td>787000</td>
<td>83</td>
<td>65.5</td>
<td>12000</td>
</tr>
<tr>
<td>Sephadex DE-50, pH8.7, salt-gradient elution</td>
<td>662000</td>
<td>69.5</td>
<td>23.5</td>
<td>28000</td>
</tr>
</tbody>
</table>

* At pH8.0, 30°C.
Table 2. *Amino acid composition of β-lactamase from Ps. aeruginosa N.C.T.C. 8203*

The amino acid composition was determined after hydrolysis for 24, 48 and 72h at 105°C, and the values given are based on the assumption of a molecular weight of 42000.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>β-lactamase Residues/molecule</th>
<th>Amino acid</th>
<th>β-lactamase Residues/molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>33.9</td>
<td>Ile</td>
<td>14.3</td>
</tr>
<tr>
<td>Thr*</td>
<td>16.1</td>
<td>Leu</td>
<td>50.1</td>
</tr>
<tr>
<td>Ser*</td>
<td>16.3</td>
<td>Tyr</td>
<td>18.6</td>
</tr>
<tr>
<td>Glu</td>
<td>39.2</td>
<td>Phe</td>
<td>11.6</td>
</tr>
<tr>
<td>Pro</td>
<td>27.0</td>
<td>Lys</td>
<td>20.4</td>
</tr>
<tr>
<td>Gly</td>
<td>34.7</td>
<td>His</td>
<td>8.3</td>
</tr>
<tr>
<td>Ala</td>
<td>44.4</td>
<td>Arg</td>
<td>26.2</td>
</tr>
<tr>
<td>Val</td>
<td>20.4</td>
<td>Trp†</td>
<td>8.0</td>
</tr>
<tr>
<td>Met†</td>
<td>4.2</td>
<td>Cys†</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* Extrapolated to zero time.
† Cysteine was determined as cysteic acid and methionine as its sulphone after performic acid oxidation.
‡ Determined spectrophotometrically.

The molecular weight of the β-lactamase is 42000±1000, as determined by sodium dodecyl sulphate—polyacrylamide-gel electrophoresis and chromatography on Sephadex G-100. This value is higher than that for most β-lactamases, although an R-factor-mediated enzyme from *Escherichia coli* R-1818 has been reported to have a molecular weight of 44100 (Dale, 1971). The inducible enzyme also resembles the *E. coli* R-1818 β-lactamase in its very sharp pH—activity profile, with maximum activity at pH8.0. The enzyme is stable within wide limits of pH(4–10) at 4°C and 27°C, but is temperature-labile above 40°C. The amino acid composition has been determined (Table 2). One mol of cysteine is found/mol of enzyme. This has been shown by the use of Ellman’s reagent to be inaccessible in the native protein but to react readily in the protein denatured with 6M-guanidinium chloride. Other properties of the β-lactamase are: pI7.5; ε 6.95×10⁴ M⁻¹cm⁻¹ at 280nm; ε²₅₀ 16.5 at 280nm; catalytic-centre activity 1.96×10⁴ molecules/min (cephalosporin C substrate, pH8, 30°C).

A substrate profile for the inducible β-lactamase has been described previously (Sabath et al., 1965). Some penicillins not available at that time have now been tested as substrates and inhibitors of the enzyme. No binding is detected when the 6-substituent of benzylpenicillin (β-configuration) is inverted to epibenzylpenicillin (α-configuration). 6β-Aminopenicillanic acid (Lund & Tybring, 1972) is hydrolysed relatively slowly (25% of benzylpenicillin). Carbenicillin, an α-carboxybenzylpenicillin is a strong competitive inhibitor of the inducible β-lactamase (Kᵢ 4×10⁻⁷M). Inhibition of a range of β-lactamases of Gram-negative bacteria including *Ps. aeruginosa* by this antibiotic has been described previously (Bobrowski & Borowski, 1971).

Inactivation of some β-lactamases in the presence of certain substrates or substrate analogues has been reported (Crompton et al., 1962; Gouveiritch et al., 1962; Dyke, 1967; Zyk & Citri, 1967). The rate of hydrolysis of cephalosporin 87/312 (O’Callaghan et al., 1971) by the inducible β-lactamase falls off exponentially. Since there are two concomitant processes, substrate hydrolysis and enzyme inactivation, the phenomenon is best demonstrated at high substrate/ enzyme concentration ratios. At very high substrate concentrations, complete inactivation of the enzyme occurs almost instantaneously. To test whether the substrate-induced inactivation is reversible, the experiment was repeated on a larger scale by dialysing a concentrated solution of β-lactamase against successive fresh solutions of substrate. An enzyme preparation was obtained that had lost 95% of its activity, and no activity was recovered after chromatography on Sephadex G-25 to remove small molecules. Therefore under these conditions the inactivation appears irreversible.

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Phosphoglucone Isomerase in Teleostean Fish
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The glycolytic enzyme phosphoglucone isomerase (p-glucose 6-phosphate ketol-isomerase, EC 5.3.1.4), which catalyses the interconversion of glucose 6-phosphate and fructose 6-phosphate, has been studied in a number of mammalian species, and it is now evident that the mammalian enzyme is coded for by a single autosomal gene locus (e.g., see Carter et al., 1972). The mammalian enzyme is a dimer of molecular weight about 120000 and the specific activity of the purified enzyme is in the range 500–1000 units/mg at 30°C. Studies of amino acid compositions have revealed homologies between the enzymes from different species (Noltmann, 1972).

The same major isoenzymes have been found to occur in the tissues of individual mammals, and this isoenzyme has been shown to be under the control of one gene locus.

Here we describe some of the molecular properties of two genetically independent isoenzymes of phosphoglucone isomerase, recently described in some fish (Actinopterygians) (Avise & Kitto, 1973; Dando, 1973).

The two electrophoretically distinct isoenzymes of phosphoglucone isomerase (termed isoenzymes PGI-A and PGI-B) have been shown to exhibit tissue-dependent distribution, e.g. isoenzyme PGI-B is found predominantly in conger (Conger conger L.) white muscle whereas the conger liver shows predominantly isoenzyme PGI-A, which is also found in heart, spleen and erythrocytes. In most Actinopterygian species isoenzyme PGI-B is less positively charged on electrophoresis at pH 6.8, and therefore moves more slowly towards the cathode than does isoenzyme PGI-A. In some species, and in certain tissues, hybrid PGI-A-PGI-B isoenzymes are observed if the two phosphoglucone isomerase gene loci are expressed in the same tissue (Dando, 1973), but this is not true for the conger.

Molecular weights have been examined by using gel filtration through thin layers of Sephadex G-150 and we found that both liver and muscle enzymes had molecular weights in the range 130000–140000. Eight diverse Actinopterygian fish species all showed values in this range [Conger conger L., Gadus morhua L., Notopterus kiprat, Sprattus sprattus L., Scyllohrinus caniculus L., Branciostoma lancioluteum (Pallas), Lampetra sp. and Triturus sp.).]