Comparison of Two Dioxygenases from *Pseudomonas putida*

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Catechol 2,3-dioxygenase and homoprotocatechuic 2,3-dioxygenase were purified from the same strain of *Pseudomonas putida*. Molecular weights and subunit sizes were similar, but amino acid compositions showed some marked differences.

Most of the dioxygenases investigated to date are those that catalyze fission of the benzene nucleus. Their substrates carry, as a minimum, two phenolic hydroxyl groups, which are located either on adjacent carbon atoms, as in catechols, or across the nucleus in the para position, as in gentisic or homogentisic acids. This communication is concerned with two *meta* fission dioxygenases that were purified by other workers from different organisms: catechol 2,3-dioxygenase (EC 1.13.11.2; CA dioxygenase) and 3,4-dihydroxyphenylacetate (homoprotocatechuic) 2,3-dioxygenase (EC 1.13.11.15; HP dioxygenase). These enzymes catalyze reactions of the same type (Fig. 1), but each fails to attack the other's substrate; moreover, later enzymes of the catabolic pathways exhibit similar narrow substrate specificities.

We considered that it would be of interest, particularly in connection with theories of the evolutionary origins of catabolic pathways, to compare the properties of these enzymes when they were isolated from the same source; namely, the strain of *Pseudomonas putida* designated *Pseudomonas* U (British NCIB 10015). When this organism grows with phenol (1) and 4-hydroxyphenylacetic acid (8) as sole carbon substrates, CA dioxygenase and HP dioxygenase, respectively, are induced. R. C. Bayly (personal communication) has informed us that some strains derived from the original culture of *Pseudomonas* U isolated in 1963 and maintained separately in various laboratories since that time now fail to utilize 4-hydroxyphenylacetic acid; he also reports that the relevant enzymes do not appear to be plasmid borne in our strain.

Cell extracts were prepared as previously described (1, 8) from *Pseudomonas* U grown in aerated mineral salts media containing either phenol (0.03%) or 4-hydroxyphenylacetate (0.05%) as carbon sources. For each enzyme, a quantity of cell extract containing 2.4 to 3.0 g of protein was treated with protamine sulfate to remove nucleic acids; this was followed by precipitation of enzyme with ammonium sulfate (55% saturation), gel filtration through Sephadex G-200, and column chromatography first on diethylaminoethyl-cellulose-Sephadex A-50 and then on hydroxylapatite (5). In the last two steps, enzymes were eluted, respectively, with linear concentration gradients of sodium chloride and potassium phosphate (pH 7.0). The resulting preparations of CA dioxygenase (specific activity, 112 U/mg) and HP dioxygenase (specific activity, 28 U/mg) each gave a single band in discontinuous gel electrophoresis with 7.5% polyacrylamide gels (4, 5). When molecular weights were determined by ultracentrifugation, single peaks were observed throughout sedimentation velocity experiments, and data from sedimentation equilibrium (5, 10) also indicated that both preparations were homogeneous. Molecular weights for the single subunits shown by electrophoresis in the presence of sodium dodecyl sulfate (5, 9) were measured. These physical properties (Table 1) were very similar, and indeed, on this basis, the proteins showed closer resemblances to each other than they did to enzymes that catalyzed the same reactions in other organisms. Thus, CA dioxygenase from *Pseudomonas arvilla* (6) and HP dioxygenase from *Pseudomonas ovalis* (7) both had molecular weights of 140,000, and the for-

![Fig. 1. Reactions catalyzed by CA dioxygenase (R=H) and HP dioxygenase (R=CH₃-COOH).](http://jb.asm.org/)
mer enzyme appeared to have three subunits (6). However, like the enzyme from *P. arvilla*, CA dioxygenase from *Pseudomonas U* was very unstable in the absence of 10% acetone, and this solvent was present throughout purification, HP dioxygenase was not significantly stabilized by organic-solvent additions.

The similarities in molecular characteristics of the two dioxygenases from *Pseudomonas U* might suggest a common evolutionary origin, but this is not supported when amino acid compositions are compared (Table 2). Hydrolyzes of samples were carried out in evacuated, sealed tubes in 6 N HCl at 110°C for 24, 48, and 72 h, and values for threonine and serine were obtained by extrapolation to zero time. Half-cystine was determined after performic acid oxidation. The total number of residues recorded for the subunits was 274 in each case. The frequency of occurrence for CA dioxygenase was leucine > aspartate > glutamate and for HP dioxygenase was alanine > glutamate > glycine. Other striking differences were noted for serine and proline.

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