Crystallographic analysis has been undertaken to determine what features permit this dual functionality while maintaining fairly strict substrate specificity. Three-dimensional structure of the native molecule as well as that of the enzyme-inhibitor complex prepared by "affinity alkylation" technique are of interest in this context.

The enzyme, a tetramer of about 100,000 daltons of identical subunits, was crystallized in the space group P43212 with 221222. The unit cell dimensions are a = 106.44 and c = 203.44 Å (Ghosh, D. and Duax, W. L., J. of Biol. Chem. 261, 1306-1308 (1986)). The complete asymmetric unit contains 32 identical subunits, two of each subunit being 3-fold related by proper rotation. 

The atomic model was prepared at 6 Å resolution, and the molecular model was refined at 3.0 Å resolution. The R values on the structure factor amplitudes for the native-to-Pt derivative and the native-to-Au derivative were 17% and 15%, respectively. The heavy atom positions were determined by the difference Patterson method and confirmed by direct methods using MULTAN to phase the "d" structure. Meaningful phase sets were selected based on high values of the absolute figure of merit and low values of the residual. 

The reflections for the three principal derivative sets were chosen by trial and error to remove weak links in the convergence map. Each derivative had two or three phase sets that were significantly better than the rest. For the Pt derivative, three of the four sites determined by the difference Patterson method were the first three peaks in the E map, and the fourth was the highest in a second E map, which was origin shifted from the first. For the Au derivative, two of the four sites located from the difference Patterson map were the highest peaks in the best E map and the other two were the first two peaks in the next best map. Thus each derivative has four major binding sites, one for each subunit of the tetramer in the asymmetric unit. 

These derivatives do not appear to have any major sites in common. It is not yet possible to determine if either derivative also has minor sites. Work is currently in progress towards the calculation of multiple isomorphous replacement phases. 

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