Supporting Information

X-ray studies of Recombinant Rat Kidney Long-Chain Hydroxy Acid Oxidase

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Analysis of Space Group and Subunit Arrangement

In the recorded X-ray diffraction data of LCHAO, the *l*-odd reflections, with observed mean structure factor amplitude $\langle F_o \rangle = 19.1$, were considerably weaker than the *l*-even reflections, for which $\langle F_o \rangle = 70.5$. This weakness when *l*-odd indicates that there is a high degree of pseudosymmetry that gives rise to the appearance of a smaller unit cell with half the volume of the asymmetric unit. In the course of the structure analysis it was found that by discarding all *l*-odd reflections a similar packing of the molecules to that of the large cell was obtained, but with only one tetramer in the asymmetric unit.

In the original large cell, there was some ambiguity in the space group assignment, $P2_12_12_1$ vs $P2_12_12$ since both give the same packing arrangement. The observation of extinctions of the $00l$ reflections when *l* was odd is indicative of $P2_12_12_1$, but because of the general weakness of the *l*-odd reflections these extinctions are not definitive. The space group assignment of $P2_12_12_1$ seems more reasonable than $P2_12_12$. $P2_12_12_1$ leads to the presence of a full octamer of subunits in the asymmetric unit; this might occur during crystallization through

formation of an octamer in solution just prior to its incorporation into the crystal lattice. $P2_12_12$ leads to the presence of two independent tetramers in the asymmetric unit with each octamer then being generated by a crystallographic 2-fold symmetry operation. Octamer formation might then occur during crystal growth by tetramers binding to previously bound tetramers. Ultracentrifugation studies have shown the tetramer to be the limiting form at low concentrations, and at higher concentrations it equilibrates with its dimeric and tetrameric forms [Phillips, 1976 #15].

The correct space group of the large cell was established by comparing the refinement behavior of the l -odd and l -even reflections in each space group. In $P2_12_12_1$, R for the l -even and l -odd reflections were 0.28 and 0.54, respectively, while for $P2_12_12$ these values were 0.25 and 0.67 (the model used having been refined in space group $P2_12_12$). It was also observed that the calculated structure factor amplitude $\langle F_c \rangle = 70.5$ for l -even but $\langle F_c \rangle = 14.5$ for the l -odd reflections. By rescaling the l -odd reflections the R in $P2_12_12_1$ fell to 0.46, a value consistent with l -even reflections having the same mean structure factor amplitude, while the R in $P2_12_12$ dropped only to 0.64. Thus, the l -odd reflections proved to be very sensitive to the correct assignment of space group symmetry.