Crystal Structures of Nitroalkane Oxidase: High Resolution Data Collection Strategy for Crystals with a Very Long Unit Cell Edge

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Nitroalkane oxidase (NAO) catalyzes the oxidation of neutral nitroalkanes to the corresponding aldehydes or ketones with the production of H_2O_2 and nitrite. Oxidized NAO readily crystallizes in a trigonal space group, diffracts to beyond 1.6 Å, but with a c unit-cell edge of 488 Å. These characteristics push data collection facilities to the limit. Using a combination of 2θ detector offsets, kappa geometry, and multiple data sets, a complete data set to 1.6 Å resolution has been achieved. To determine the structure we used crystals of a Se-Met enriched form of NAO, but trapped in a stable reaction intermediate complex (E^{red}-S). Although the orthorhombic unit cell of E^{red}-S* was smaller, it nevertheless required analysis of 52 Se sites with MAD phasing methods. The 2.2 Å structure of E^{red}-S* was then used to solve the structure of NAO in the large unit-cell crystal form.*

One of the research interests of the Orville lab is the structure/function analysis of enzymes that catalyze nitrite elimination from natural and "xenobiotic" nitrochemicals. Nitroaliphatic compounds are often produced by plants to inhibit the central metabolic enzymes of the TCA cycle in potential pathogens. Nitroalkane oxidase (NAO) is an FAD-dependent enzyme in fungus, which is induced by nitroalkanes and enables the microbe to obtain all its nitrogen from these types of compounds. Oxidized NAO crystallizes easily, which encouraged us to vigorously pursue the structure. However, the diffraction pattern observed with our in-house Cu Kα x-rays was very streaky, even at the maximum crystal-to-film distance (**Figure 1**). In contrast, our first diffraction images obtained at X26C clearly indicated a different story. The NAO crystals diffracted to beyond 1.6 Å resolution in space group $P3_221$, but with unit cell dimensions of $a = b$ =104 Å, c = 488 Å.

As of January 2005 only 158 x-ray structures (out of 29,040) deposited in the PDB contain a *c* axis unit cell edge greater than 450 Å. Moreover, only 16 of these structures were to at least 2.5 Å resolution. Thus, NAO would set the highest resolution record for a large unit cell, provided that high-resolution diffraction data could be collected, the phases could be solved, and the structure refined. We visited several beamlines at NSLS and APS to achieve these goals.

The observed x-ray diffraction pattern has a reciprocal relationship with the unit cell dimensions of the crystal in real space. Therefore, the resulting x-ray reflections for NAO crystals are very close together and appear as streaks with Cu Kα x-rays. In order to resolve closely spaced reflections, the crystal-to-detector distance must be sufficiently large to allow the reflections to diverge from each other before they reach the detector. However, this also significantly decreases the maximum Bragg angle of reflection, so consequently the high-resolution data are not observed.

To collect the high-resolution data of NAO, several data sets were merged.

Beamline X26C

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They were obtained with different detector vertical offsets (approximately equivalent to 2θ) and optimized kappa geometry to align the long cell axis approximately parallel to the crystal rotation axis. This strategy was first utilized at X26C to collect three data sets. The first two data sets used a vertical offset of 75 mm and a kappa of either 0° or 40°. The third data set was standard, employing no vertical or kappa offset. Merging these data sets yielded a 90% complete dataset to 2.5 Å resolution with I/σ(I) of 8.3 in the highest resolution shell. We subsequently collected data at APS beamline 14-BMC (operated by BioCARS) using the strategy established at X26C and a 150 mm maximum vertical offset. The merged data is 93.5% complete to 2.07 Å resolution, including 80% completeness in the 2.07 Å resolution shell, but still only 60% complete to 1.6 Å resolution. Further high-resolution studies are ongoing at several synchrotron beamlines.

To solve the structure, we took advantage of the fact that during NAO turnover of nitroethane a N5-(2-nitrobutyl)-1,5-dihydro-FAD adduct (**Figure 2**) can be trapped at low temperature (E^{red}-S*). This form of the enzyme crystallizes in a smaller, orthorhombic space group and contains one holoenzyme (α_4) per asymmetric unit. A Se-Met enriched form of E^{red}-S* was used for MAD data analysis (52 selenium sites) and solved to 2.2 Å resolution.

The larger unit cell for oxidized NAO contains 1½ holoenzymes (six subunits) per asymmetric unit. The structure was solved by molecular replacement using E^{red}-S* as a search model. The structure of oxidized NAO has been refined to 2.07 Å resolution. We are currently analyzing our structures to characterize a subtle rearrangement of subunits associated with the conversion of oxidized NAO to E^{red}-S*. The approximately 26° rotation for the subunits is unique to NAO, since analogous conformational changes are not observed in the acyl-CoA dehydrogenases, which are structural but not functional homologs of NAO.

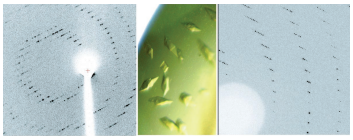

Figure 1. The x-ray diffraction patterns obtained from crystals of oxidized NAO. The diffraction pattern on the left was obtained with Cu Kα x-rays (1.54 Å), whereas the pattern on the right was collected with 1 Å synchrotron radiation. Synchrotron facilities provide a more intense, smaller, and focused x-ray beam. This enables longer crystal-to-film distances and the ability to resolve very closely spaced reflections from large unit cells.

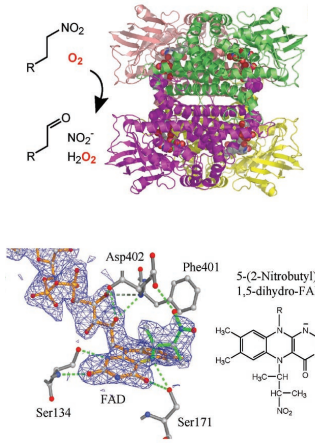

Figure 2. The structures of NAO and E^{red}-S* are homotetramers (top). The 2.2 Å resolution electron density for the N5-FAD adduct within the E^{red}-S* trapped intermediate (bottom) clearly indicates the presence of the 2-nitrobutyl moiety.