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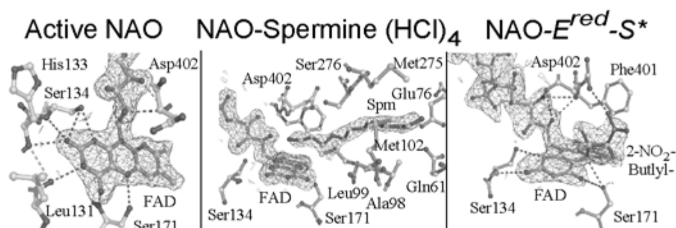
Nitroalkane Oxidase Trapped During Turnover Provides Mechanistic Insights Into The FAD-Dependent Enzyme. A. Nagpal[a], M.P. Valley[b], P.F. Fitzpatrick[b], A.M. Orville[a], School of Chemistry & Biochemistry, Georgia Inst. of Technology[a], Atlanta, GA 30332, Dept. of Biochemistry & Biophysics, Texas A&M Univ.[b], College Station, TX 77843.

Nitroalkane oxidase (NAO) from *Fusarium oxysporum* converts nitroalkanes to the aldehyde or ketone and nitrite, and O_2 to H_2O_2. We have solved two crystal structures of NAO, but observe three enzyme states. During turnover of nitroethane in the presence of nitroethane anion, a covalent N5-FAD adduct is trapped (E^{red}-S^*). The α_4 structure of E^{red}-S^* was solved by MAD phasing of 52 Se-Met sites to 2.2 Å resolution in space group $P2_12_12_1$. Oxidized NAO crystallizes in space group $P3_221$ ($a = b = 104$Å, $c = 488$Å) with 6 subunits per asymmetric unit. The 2.07 Å resolution structure reveals three oxidized active sites and three sites that contain spermine $(HCl)_4$, a weak inhibitor. The α_4 structures in oxidized NAO and E^{red}-S^* differ by a 26° rotation of the subunits. Comparisons with acyl-CoA dehydrogenases, structural homologs of NAO, suggest structural bases for different substrate preference and reaction mechanisms utilized by the homologs.



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