

Characterization of AOC3 D386N mutant

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Vascular adhesion protein-1 (VAP-1, EC 1.4.3.6) also called an amine oxidase copper containing three (AOC3) is involved in the rolling and transmigration of leukocytes into the site of inflammation. The semicarbazide-sensitive amine oxidase (SSAO) activity of AOC3 is required for its role in adhesion and trafficking. AOC3 catalyses the oxidative deamination of primary and aromatic amines into the corresponding aldehydes. The reaction requires molecular oxygen and copper, and releases hydrogen peroxide and ammonia as byproducts. The reaction happens at the intrinsic topaquinoxinone cofactor made spontaneously at Y471. The transient covalent bond between the substrate and the cofactor of the substrate Schiff's base during the enzymatic reaction is postulated to be crucial in leukocyte transmigration. We have designed and characterized a general base mutant which is assumed to prevent the release of the substrate from the enzyme-substrate complex. Ideally, the mutant will be used in the substrate trapping of the unknown enzymatic substrate and the leukocyte counterpart of AOC3.

The general base of the human AOC3 is proposed to be D386. It abstracts the proton from the substrate Schiff's base complex enabling the hydrolysis of the aldehyde product. I have changed the general base to asparagine (D386N). In *Escherichia coli* a corresponding mutation (D383N) did not interfere the cofactor formation, but abolished the enzymatic activity. The binding properties of the mutant were not studied. The mutation was introduced by polymerase chain reaction and sequenced. For the protein production the construct was transfected into a mammalian cell line. The mutant protein was recognized by a monoclonal antibody against AOC3, as expected. This made it possible to sort the positive cells for a stable protein production cell line in Chinese Hamster Ovary cells. When measured from the cell lysates, the D386N mutation stopped the production of hydrogen peroxide of AOC3. The activity was at the same level than for inactive Y471F mutant and for the plain cell control. By using 1-10 micrograms of purified mutant protein we were not yet able to demonstrate significant increase in known ¹⁴C labeled pseudosubstrate, benzylamine, when compared to wt.