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Bromination of Indoles by Vanadium Bromoperoxidase: Products, Selectivity, Mechanism, and Enzyme-Substrate Complex.

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Abstract

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The reactivity of a series of simple substituted indoles in the bromination catalyzed by vanadium bromoperoxidase (V-BrPO) was investigated, and the results were interpreted on the basis of the binding of the substrate to, or near to the active site of the enzyme.

In the presence of bromide and hydrogen peroxide, V-BrPO catalyzed the bromination and the oxidation of simple substituted indoles. The products formed were in agreement with initial attack of the C2-C3 double bond by an oxidized bromine species, and no reaction on the benzene ring was observed. The rearrangement of the bromonium intermediate and the formation of the final products depended of the nature of the substituent. In general, 2-substituted indoles yielded the 3-bromo product whereas the 3-substituted indoles gave the 3-oxo derivative. When indole was used as a substrate, 3-bromoindole, oxindole and indigo were the main products formed. The mechanism of indigo formation was investigated in further details and it was found to occur *via* oxidative coupling of 3-bromoindole and indoxyl and did not require molecular oxygen.

Kinetic studies showed that V-BrPO reacted with indoles preferentially over many others substrates, including monochlorodimedone, phenol red, and

oxidation of a second equivalent of hydrogen peoxide. The reactivity of V-BrPO differed greatly with the reactivity observed for HOBr and strongly suggested binding of indoles to V-BrPO.

The fluorescence of 2-phenylindole was quenched by V-BrPO with a quenching constant (in 100 mM tris buffer at pH 8.3 and 21 ^{o}C) of 1.1 x 10^{5} M^{-1}. The quenching was not consistent with dynamic interactions and was therefore interpretated as being due to static interactions (binding) between 2-phenylindole and V-BrPO. Labeling of V-BrPO with photoreactive 5-azidoindoles resulted in highly specific insertion of the probes in the enzyme and confirmed that indoles bind to V-BrPO.