

Essay

Not peer-reviewed version

The Role of the Oxygen Hole in Hydrolases

[Bent Havsteen](#) *

Posted Date: 22 August 2025

doi: 10.20944/preprints202508.1643.v1

Keywords: electron transfer; serine proteases; thermodynamics; enzyme activation; enzyme kinetics



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Essay

The Role of the Oxygen Hole in Hydrolases

Bent H. Havsteen

Department of Biochemistry, University of Kiel, Olshausenstrasse 40, D-24098 Kiel, Germany.

benthavs@gmail.com

Abstract

The oxygen hole is ubiquitous in hydrolase and lowers the activation energy of the transition state. Its geometry invites the formation of a low energy tetrahedron facilitating electronic movements. In effect it functions as a switch.

Keywords: electron transfer; serine proteases; thermodynamics; enzyme activation; enzyme kinetics

A large number of proteinases and esterases contain an oxygen hole, which appears to play a key role in the lowering of the activation energy of the transition state [1]. The structure of the apparently conserved oxygen hole includes two hydrogen bonds flanking a conserved gly residue near the conserved ser residue in the active site. The other conserved residues of the oxygen hole are contributed by the substrate. The oxygen hole invites the formation of a low energy tetrahedron, the carbon of which links ser195 to the carboxyl carbon atom of the substrate. The role of the oxygen hole appears to be to form a particularly dense structure of tetrahedral geometry, which possesses a minimal surface area to volume ratio, near that of a sphere. The advantage of such a structure to enzymatic catalysis is the minimisation of the distances of electronic movements, thus lowering the energies required for electronic transitions. The dense tetrahedron appears to contain 4 electrons. It appears to be more strongly bound to the active site than the initial binding site is. Such a construction would facilitate the evolution of efficient mechanisms to aid the survival under the prevailing environmental conditions. Evidence of the importance of an unpaired electron in the oxygen hole might be gained by the inclusion in the enzyme assay of a free radical catching compound e.g., cys, glutathion or ascorbate [2]. The unpaired electron of the O-hole may lower the activation energy by raising the energy of the ground state. The unpaired electron originates from the N atom of the substrate amide group and may run along the his57-asp102 charge relay to perturb the electric charge state of the environment. This could release a conformational change promoting the product release.

Inclusion compounds [3], which surround the primary catalyst, e.g., yttrium in methanogenic bacteria, may operate by a similar effect, because they confine the electrons to the vicinity of the reaction center.

A large number of proteases and hydrolases with serine in the active site appear to act in a similar way, which is characterized by the formation of an oxygen hole that contains the bond destined to be cleaved. Since the evolution has fine tuned and generalized this mechanism, an attempt to discern the individual steps in the sequence and their characteristic kinetic and thermodynamic properties must be appropriate and timely.

Step 1. The initial binding of the substrate to the active site: $E + S \rightleftharpoons ES$

k_{12} and k_{21} are known from temperature jump experiments with specific inhibitors [4] to be: 5×10^6 L.s/M and 10^3 /s, resp.

Step 2. Compression of the hydrophobic site: $ES \rightleftharpoons ES'$, $k_{23} = 10^2$ - 10^3 s⁻¹, $k_{32} = 10^2$ - 10^3 s⁻¹.

This step is accompanied by the formation of the difference spectrum found by Wootton and Hess at 290 nm [5].

Step 3. Twisting of the sensitive bond to permit the formation of the internal tetrahedron mentioned above [6]:

$ES' \rightleftharpoons ES''$, $k_{34} \rightleftharpoons k_{43}$, $k_{34} = k_{43} = 10^2$ - 10^3 s⁻¹.

Step 4. Orbital pairing: $ES^{\cdot\cdot} \rightleftharpoons E^{\cdot\cdot}$

An unpaired electron on the serine hydroxyl group of the active site forms a pair of opposite spin direction with an unpaired electron of the oxygen atom in the bond to be split.

Step 5. Product release: $ES^{\cdot\cdot} \rightleftharpoons E + P$.

The proposed pairing of two singular electrons is the following: One electron from the oxygen atom of the serine hydroxyl group and the other from the oxygen of the substrate group, which is to be broken, form a pair of electrons with opposite spin. That might also, in enthalpy and mechanism, resemble the formation of a covalent bond. The single bond covalent radius of H is 0.28 Å. The bond energies of H-H, C-C and O-O are 436, 356 and 146 kJ/mol, resp.

The proposed oxygen-oxygen electronic interaction could lower the energy of the transition state by an amount comparable to the one observed at the oxygen hole.

The positive p-orbits of the singular electrons of the serine sidechain oxygen and the oxygen atom of the target linkage, resp., form a bond of the π -bond type, thus creating an acyl bond and releasing the first product, the one with a terminal amino group. Subsequently, the oxygen atom of the attacking water molecule cleaves the serine bound substrate fragment by offering a more stable product, the carboxyl terminated fragment.

This argument leads to the conclusion that a transient π -bond formed by two p-orbitals, one from each of the two oxygen atoms mentioned. That π -bond stabilizes the acyl-enzyme stage of the catalytic process.

Conflicts of Interests: The author declares no conflicts of interest.

References

1. Robertus, J. D., Kraut, J., Alden, R. A. and Birktoft, J. J. (1972) Subtilisin; a stereochemical mechanism involving transition-state stabilization. *Biochemistry* **11**, 4293–4303 <https://doi.org/10.1021/bi00773a016>
2. Till, U. and Thielmann, K. (1989) Pathobiochemie Edition Medizin VCH., Weinheim
3. Saenger, W. (1980) Cyclodextrin Inclusion Compounds in Research and Industry. *Angew. Chem. Int. Ed. Engl.* **19**, 344–362 <https://doi.org/10.1002/anie.198003441>
4. Havsteen, B. H. (1967) The kinetics of the two-step interaction of chymotrypsin with proflavin. *J. Biol. Chem.* **242**, 769–771 [https://doi.org/10.1016/S0021-9258\(18\)96271-X](https://doi.org/10.1016/S0021-9258(18)96271-X)
5. Wooton, J. F. and Hess, G. P. (1960) Evidence for the involvement of a tryptophan residue in the reaction of diisopropylphosphorofluoridate with alpha-chymotrypsin. *Nature* **188**, 726–728 <https://doi.org/10.1038/188726a0>
6. Havsteen, B. H. (1969) 5—Perturbation and Flow Techniques. In *Physical Principles and Techniques of Protein Chemistry* (Leach, S. J., ed.), pp 245–289, Academic Press <https://doi.org/10.1016/B978-0-12-440101-3.50011-4>

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.