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On the Structure of Histidine and Its Role in Enzyme Active Sites

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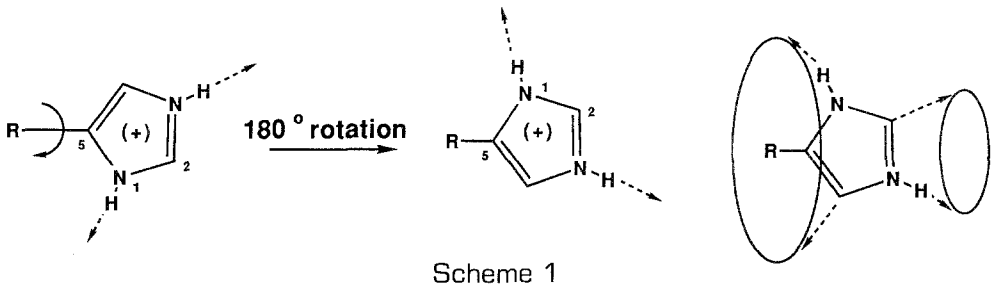
A structural refinement is proposed for the mechanistic details of the action of the serine proteases. The proposal involves ring flipping of the imidazole function of the histidine side chain as a vehicle for proton transfer. The geometric feasibility of this motion is established by molecular graphics analysis of the crystal structure of α-chymotrypsin. It is suggested that the shape of histidine is as important as its pK_a for its function at the active sites of enzymes.

Histidine is found at the active sites of a number of enzymes. Its function has generally been interpreted in terms of the pK_a of the imidazolium ion ($pK_a \simeq 7$), a feature that permits significant concentrations of both acidic and basic forms near neutrality. Our intent here is to bring attention to another attribute to the structure; its ability to transfer protons in a variety of directions.

The consequence of attaching the imidazolium function at C5 (rather than, say, at C2) is that rotation about the single bond permits the acidity of the NH bonds (or the lone pairs of the conjugate base) to be expressed in several directions. One possibility is depicted in Scheme 1, where 180° rotation results in proton translocations to opposite edges of the structure. The limits are defined by the two circles shown. To the extent that NH bonds of such heterocycles have moderately directional hydrogen-bonding characteristics [1], rotation is the most likely way to redirect acid–base character on this surface.

The use of rotation as a vehicle for proton transfer has a special significance for the action of the serine proteases. The catalytic triad is shown in a two-dimensional fashion in Scheme 2. The initial addition product conforms to the generally accepted sequence of events for this type of enzyme. The tetrahedral intermediate may be formed through the "charge relay mechanism," a notion supported by proton inventory studies [2] and recent mutagenesis experiments [3], although some controversy exists on this point [4]. Before breakdown of the tetrahedral intermediate to the acyl enzyme derivative can occur, the departing nitrogen must become protonated. This can be accomplished by rotation around the single bond indicated. The hydrogen bond involving the more basic syn lone pair [5] of the asparate is lost during the rotation but can be recovered as the system becomes poised for the breakdown of the intermediate. Subsequent deacylation of the enzyme by water acting as the nucleophile can be accomplished by a reversal of the sequence.



Scheme 1

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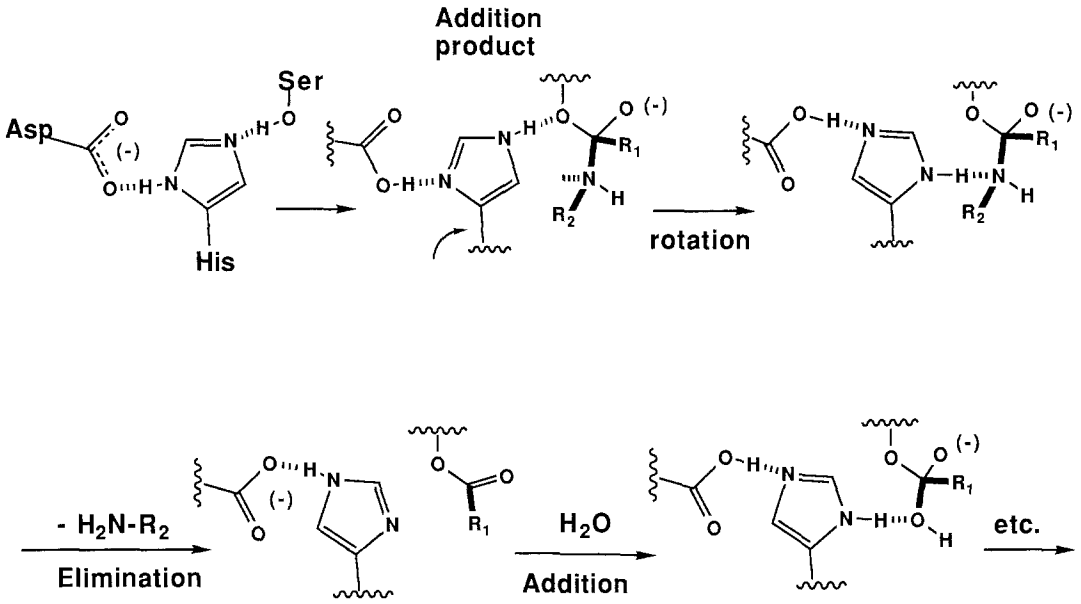
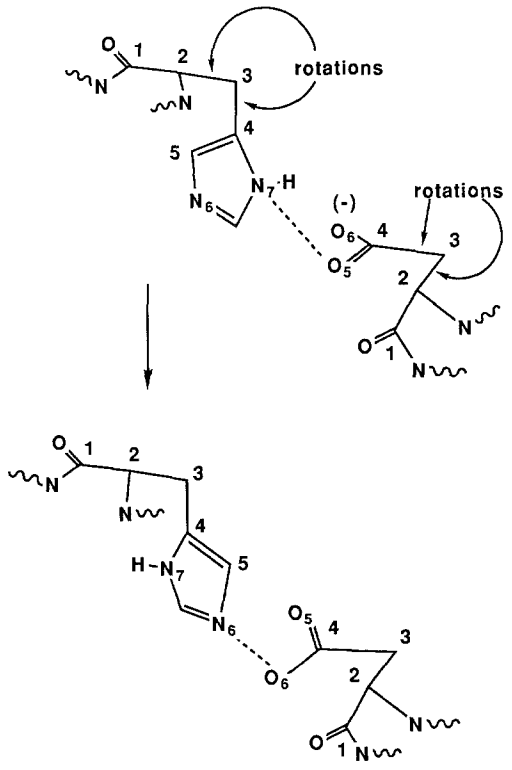


Scheme 2

The geometric feasibility of this process in three dimensions was tested for α-chymotrypsin by examining the most recently refined crystal structure [6]. The dihedral angles [7] of the bonds involved are shown in Table 1 for both the ground-state structure and the proposed intermediate (after ring flipping), in which the proton is ready for transfer to the departing nitrogen. In addition to the rotation of the imidazole, the carboxyl function of the aspartate also needs to undergo a moderate motion to insure reasonable stereoelectronics for the new hydrogen bond. These heavy-atom motions, summarized in Scheme 3, can be accomplished within the active site without steric problems involving the substrate, other side chains of the enzyme, or its peptide backbone. For an ideal hydrogen bond between N6 and O5 some dislocation of the peptide chain must occur. In general, there must be some flexibility at the active site of most enzymes: they must bind the ground states, intermediates, products, the transition states that connect them. Deformation of both substrate and enzyme is likely to occur along the reaction pathway.

Table 1. Structural Parameters

	Ground state		After rotation
Dihedral angles (°)			
His			
1234	−51		−78
2345	85		−75
Asp			
1234	64		44
2345	166		−108
Distances (Å)			
N_7–O_5	2.61	N_6–O_6	3.04
N_7–O_6	3.31	N_6–O_5	2.57



Scheme 3

In summary, we propose that these structural details and stereoelectronics of histidine may be as significant as its pK_a in this and other enzymes where it performs at the active site [8]. It may be possible to

engineer the ring flipping feature into model systems [9] and we are working toward this goal.

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NOTE ADDED IN PROOF: In accord with the suggestion above, there is evidence that the protease and esterase activities of chymotrypsin involve different N atoms of the imidazole ring. (West, J. B.; Scholten, J.; Stolowich, N. J.; Hogg, J. L.; Scott, A. I.; Wong, C.-H. *J. Am. Chem. Soc.*, **1988,** *110,* 3709).

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