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Short hydrogen bonds in the catalytic mechanism of serine proteases

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Abstract: The survey of crystallographic data from the Protein Data Bank for 37 structures of trypsin and other serine proteases at a resolution of 0.78–1.28 Å revealed the presence of hydrogen bonds in the active site of the enzymes, which are formed between the catalytic histidine and aspartate residues and are on average 2.7 Å long. This is the typical bond length for normal hydrogen bonds. The geometric properties of the hydrogen bonds in the active site indicate that the H atom is not centered between the heteroatoms of the catalytic histidine and aspartate residues in the active site. Taken together, these findings exclude the possibility that short "low-barrier" hydrogen bonds are formed in the ground state structure of the active sites examined in this work. Some time ago, it was suggested by Cleland that the "low-barrier hydrogen bond" hypothesis is operative in the catalytic mechanism of serine proteases, and requires the presence of short hydrogen bonds around 2.4 Å long in the active site, with the H atom centered between the catalytic heteroatoms. The conclusions drawn from this work do not exclude the validity of the "low-barrier hydrogen bond" hypothesis at all, but they merely do not support it in this particular case, with this particular class of enzymes.

Keywords: trypsin; serine proteases; low-barrier hydrogen bonds.

INTRODUCTION

Usually observed hydrogen bonds represent relatively weak interactions (around 10 kJ mol^{-1}). Approximate parameters for the O–H···O hydrogen bond in water are 2.8, 1.0 and 1.8 Å for the O···O ("heteroatom distance"), O–H and H···O interatomic distances, respectively.[1] However, the existence of short hydrogen bonds, or Speakman–Hadzi bonds, has been known for a long time.[2,3] In short hydrogen bonds, the overall bond becomes stronger as the heteroatom dis-

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tance becomes shorter and at a distance of 2.4 Å, the hydrogen becomes centered between the heteroatoms; this is largely a covalent bond.[4]

In 1992, Cleland and Frey[1,5–9] proposed that such short, or low-barrier hydrogen bonds (LBHB), when located between the substrate and the acid-base catalyst in the active sites of enzymes, can play an important role in several cases of enzyme catalysis. The theory of LBHB has emerged in recent years and implies short (< 2.5 Å) and very strong (40–80 kJ mol^{-1}) hydrogen bonds.[5,10–12] Such hydrogen bonds are formed if the donor and acceptor atoms are close to one another and display similar pK_a values. In LBHBs, the proton is shared by the donor and acceptor in a single, low-potential energy well, which implies that the barrier for proton transfer is eliminated.[13]

The LBHB catalytic hypothesis contains the following premises:[12]

(a) In hydrogen bonds located between the substrate and the acid-base catalyst in the active site, a large increase in bond strength can occur as the enzyme–substrate complex is converted into a reactive intermediate or a transition state.[12]

(b) Strong hydrogen bonds between bases of similar proton affinities could account for much of the required differential stabilization of enzyme-bound intermediates and transition states compared to the initial enzyme–substrate complex.[12]

(c) If the hydrogen bond strength is significantly less when the proton affinities of the donor and acceptor are dissimilar (such as in the enzyme–substrate complex), the energetic cost of forming the transition state should be substantially reduced.[8,12]

(d) Thus, the strength of the hydrogen bond depends on: its length, linearity, the nature of the microenvironment and the degree to which the pK_a values of the conjugate acids and the heavy atoms sharing the proton are matched.[8]

When a mechanism involves the formation of an unstable intermediate, the transition state for forming it will closely resemble the intermediate and the LBHB will also be found in the intermediate or a closely mimicking enzyme–inhibitor complex. It was proposed that such mimics of metastable intermediates at enzymatic sites may allow the direct observation of LBHBs by crystallographic methods.[8,12,14,15]

Following this lead, the crystallographic structures of the active sites of serine proteases, in free enzymes, in preparations complexed with products of the reaction and in enzyme complexes with ligands were systematically investigated in order to observe short hydrogen bonds. Unlike other classes of enzymes, a large number of structures of serine proteases have been solved at high resolution and these proteases are exceptionally suitable for the above-mentioned task.

Protein Data Bank

The RCSB-PDB Protein Data Bank was systematically examined for three-dimensional structures of serine proteases. The data were downloaded from the

website http://www.rcsb.org and are presented in Tables I and II; they summarize the free enzymes, enzyme–product complexes, and enzyme–ligand complexes

TABLE I. Summary of crystal structures of trypsin (entries 1–12) and other serine proteases (entries 13–18), at a resolution from 0.78–1.00 Å

Entry	PDB access code	Source of protease	Resolution Å	*R* value	pH[a]	Ligands bound in the active site[b]	Source
1	1PQ7	*Fusarium oxysporum* trypsin	0.80	0.109	5.0	Arginine	16
2	1FN8	*Fusarium oxysporum* trypsin	0.81	0.108	6.0	Gly–Ala–Arg	17
3	1FY4						
4	1FY5	*Fusarium oxysporum* trypsin	0.81	0.124	6.0	Gly–Ala–Lys	17
5	1GDN	*Fusarium oxysporum* trypsin	0.81	0.108	6.0	Gly–Ala–Lys	17
6	1XVO	*Fusarium oxysporum* trypsin	0.84	0.108	5.0	–	18
7	1PQ5	*Fusarium oxysporum* trypsin	0.85	0.098	5.0	Arginine	16
8	1GDQ	*Fusarium oxysporum* trypsin	0.93	0.099	6.0	Arginine	17
9	1HJ9	Trysin from bovine pancreas after structural damage	0.95	0.117	8.0	–	19
10	2AYW	Trypsin from bovine pancreas	0.97	0.138	6.0	ONO, MES	20
11	1HJ8	Trypsin from atlantic salmon	1.00	0.118	5.8	–	19
12	1PQ8	*Fusarium oxysporum* trypsin	1.00	0.128	4.0	Gly–Gly–Arg; Lys	16
13	2H5C	*α*-Lytic protease from *Lysobacter enzymogenes*	0.82	0.081	4.3	–	21
14	1SSX	*α*-Lytic protease from *Lysobacter enzymogenes*	0.83	0.087	8.0	–	22
15	2H5D	*α*-Lytic protease from *Lysobacter enzymogenes* complexed with MeOSuc–Ala–Ala–Pro–Val boronic acid	0.90	0.080	8.0	MSU–Ala–Ala–Pro–B2V	21
16	1EA7	*Bacillus sphaericus* sphericase	0.97	0.097	7.5	Sulfur atom	23
17	1GCI	*Bacillus lentus* subtilisin	0.78	0.099	5.9	–	24
18	1GVK	Porcine elastase acyl enzyme	0.94	0.122	5.0	Acetyl group	25

[a]pH of crystallization buffer; [b]most preparations contained SO_4^{2-} and Ca^{2+} and some contained bound benzamidine or glycerol. Abbreviations: ONO – 2-[2-({[4-(diaminomethyl)phenyl]amino}carbonyl)-6-methoxypyridin--3-yl]-5-{[(1-formyl-2,2-dimethylpropyl)amino]carbonyl)benzoic acid, MES – 2-(*N*-morpholino)-ethanesulfonic acid, MSU–Ala–Ala–Pro–B2V – Succinic acid monomethyl ester–Ala–Ala–Pro–Valine boronic acid

TABLE II. Summary of crystal structures of trypsin at resolution 1.02–1.28 Å

Entry	PDB access code	Source of trypsin	Resolution Å	*R* value	pH[a]	Ligands bound in the active site[b]	Source
1	1S0Q	Bovine pancreas	1.02	0.112	8.0	c	26
2	1S0R	Bovine pancreas	1.02	0.112	8.0	c	26
3	1GDU	*Fusarium oxysporum*	1.07	0.104	6.0	Gly–Ala–Arg	17
4	1XVM	*Fusarium oxysporum*	1.10	0.144	5.0	Gly–Ala–Arg	18
5	2AH4	Guanidinobenzoyl--trypsin	1.13	0.12	5.0	GBS	27
6	2AGI	Leupeptin–trypsin covalent complex	1.14	0.117	5.0	Ace–Leu–Leu––Arg	27
7	2AGE	Succinyl-AAPR-trypsin acyl-enzyme	1.15	0.12	6.0	Suc–Ala–Ala––Pro–Arg	27
8	1UTN	Bovine pancreas	1.15	0.113	8.0	TRS, benzylamine	28
9	1UTO	Bovine pancreas	1.15	0.139	8.0	2-Phenylethyl-amine	28
10	1UTQ	Bovine pancreas	1.15	0.138	8.0	c	28
11	1Y59	Bovine pancreas trypsin mutant complexed with inhibitor	1.20	0.123	7.0	TL1	29
12	2BLV	Bovine pancreatic trypsin before a high dose X-ray "burn"	1.20	0.107	–	c	30
13	2BLW	Bovine pancreatic trypsin after a high dose X-ray "burn"	1.20	0.108	–	c	30
14	2F91	Crayfish trypsin complexed with peptide inhibitor SGTI	1.20	0.139	4.6	Peptide inhibitor SGTI-32AA	31
15	1J8A	Bovine pancreas	1.21	1.21	7.4	TRS	32
16	1PPZ	Trypsin complexed with an inhibitor MIS	1.23	0.14	5.0	MIS	16
17	1PQA	Bovine pancreas trypsin complexed with PMSF	1.23	0.141	5.0	SEB	16
18	2A31	Bovine pancreas trypsin complexed with borate	1.25	0.14	8.0	BO4, PG3	33
19	2AGG	Succinyl-AAPK-trypsin acyl-enzyme (bovine pancreas)	1.28	0.125	6.0	Suc–Ala–Ala––Pro–Lys	27

[a]pH of crystallization buffer; [b]most preparations contained SO_4^{2-} and Ca^{2+} and some contained bound glycerol; [c]no ligand was bound in the active site. Abbreviations: GBS – 4-guanidinobenzoic acid, Ace–Leu–Leu–Arg – – *N*-acetyl–Leu–Leu–Arg, Suc–Ala–Ala–Pro–Arg – succinyl–Ala–Ala–Pro–Arg, TRS – 2-amino-2-hydroxymethyl-propane-1,3-diol, TL1 – 2,5-bis-*O*-{3-[amino(imino)methyl]phenyl}-1,4:3,6-dianhydro-D-glucitol [2,5-*O*,*O*--bis-(3',3"-amidinophenyl)-1,4:3,6-dianhydro-D-sorbitol], MIS – monoisopropylphosphorylserine, PMSF – phenylmethylsulfonyl fluoride, SEB – ortobenzylsulfonyl-serine, BO4 – borate ion, PG3 – guanidine-3-propanol, Suc–Ala–Ala–Pro–Lys – succinyl–Ala–Ala–Pro–Lys

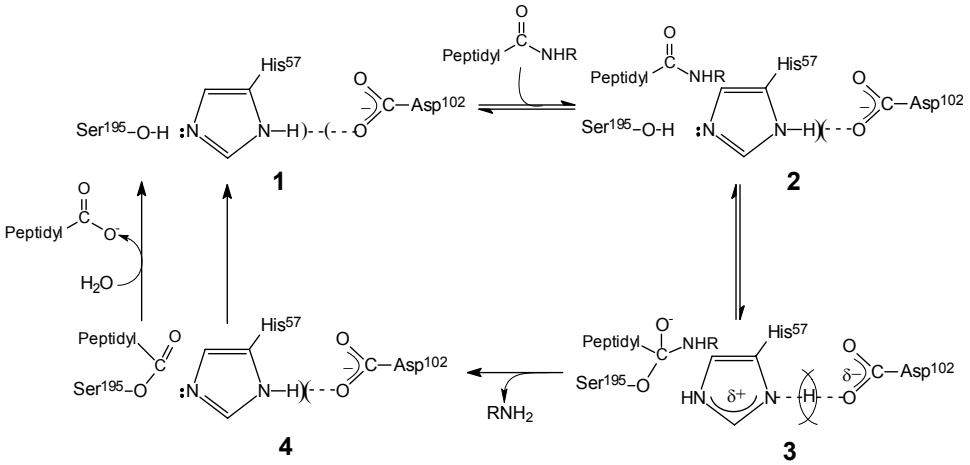
that were examined in this work. It is important to emphasize that the data were surveyed up to 1 January, 2007 and, therefore, Tables I and II present the complete crystallographic information on the active sites of serine proteases up to that date. The distances between atoms and the angles between the bonds were estimated with the aid of two computer programs; first, the Deep View/ /Swiss PDB Viewer program, v. 3.7, downloaded from the ExPASy Proteomics website (http://www.expasy.org/spdbv), and second, the RasWin Molecular Graphics, Windows Version 2.6 program, downloaded from the website of the University of Massachusetts Amherst (http://www.umass.edu/microbio/rasmol/getras.htm).

RESULTS

Mechanism of action of serine proteases

Serine proteases represent a large family of enzymes which catalyze the hydrolysis of peptide bonds in proteins and peptides or the hydrolysis of ester bonds. They include trypsin, chymotrypsin, and other serine proteases.[34]

Recently, Cleland[8] and Frey[35] proposed that the catalytic cycles of trypsin, chymotrypsin and other serine proteases proceed according to the mechanism given in Scheme 1.



Scheme 1. Catalytic cycle of chymotrypsin, drawn according to Frey[35] and Cleland.[8]

According to Cleland,[8] upon the binding of a specific substrate, the active site of chymotrypsin undergoes a compression which brings His-57 and Asp-102 close together. The required energy is provided by the binding energy derived from specific enzyme-substrate contacts. Since the difference in pK_a between the neutral His-57 and Asp-102 is more than 10 units, the hydrogen bond remains weak and cannot relieve the strain of compression. Protonation of Nε2 permits the formation of an LBHB between His-57 and Asp-102 because the pK_a are now

much more closely matched. The short hydrogen bond relieves the strain of compression (Scheme 1).

Accuracy of X-ray crystallographic data

The crystal structure of over 600 serine proteases and their complexes with substrate analogs and inhibitors have been reported and deposited in the Protein Data Bank.[36] However, only a small portion of these data was obtained at an atomic resolution (< 1 Å) or at a near atomic resolution (1.0–1.3 Å). When the crystallographic resolution becomes lower than 1.3 Å, the positioning of the hydrogen bond and the hydrogen atom becomes uncertain, because the error in estimation increases rapidly with decreasing resolution (Fig. 1). For this reason, from over 600 serine proteases reported in the literature, the crystal structure of two groups of proteases, the first group with a resolution less then 1 Å (Table I) and the second with a resolution of 1–1.3 Å (Table II), were examined in the present study.

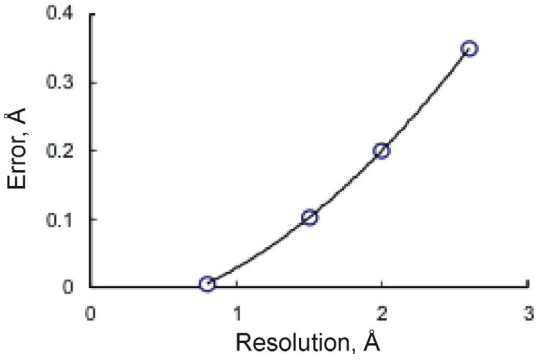


Fig. 1. Relationship between resolution and error.

Structure of serine proteases at a resolution below 1 Å

The enzyme structures analyzed in Table I were crystallized in most cases at pH 4.0–6.0, indicating that the catalytic aspartate was dissociated in most cases, while the catalytic histidine was mostly protonated. In Table I, the *R* values are in most cases below 0.12, indicating a high quality of crystallographic resolution. In the catalytic triad Ser···His···Asp (Scheme 1), the distance between the heteroatoms in Ser and His, for all structures listed in Table I, was always 2.80–3.10 Å (data not shown), indicating a normal hydrogen bonding distance between the hydroxyl oxygen of serine and imidazole nitrogen of histidine.[34]

On the other hand, the distance between the heteroatoms in histidine and aspartate in the catalytic triad was shorter, from 2.64–2.77 Å (Table III). The difference between the heteroatoms in Ser and His was always shorter than that between the heteroatoms in His and Asp. The average difference was 0.2 Å, which is significant because at this resolution the error was less than 0.02 Å.

The normally allowed van der Waals distance for interatomic contacts between oxygen and nitrogen is 2.7 Å, indicating the presence of short hydrogen bonds only in several crystal structures in Table II. Since proton imaging was feasible at this resolution, it was possible to determine in most cases the extent of linearity of the H bond; in most cases the angle was 160–170°, indicating that the H bonds were nearly linear (Table III).

TABLE III. Putative hydrogen bonds in the catalytic triad of trypsin and other serine proteases at a resolution 0.78–1.00 Å, from Table I

Entry	PDB access code	Putative hydrogen bonds between the catalytic aspartate and histidine	Distance Å	Hydrogen bond, Å		Angle deg.
				OD···H	H–ND1	
1	1PQ7	ASP99A (OD2)···HIS 56A (ND1)	2.77	1.92	0.86	166.1
2	1FN8	ASP102A (OD2)···HIS 57A (ND1)	2.75	1.92	0.86	164.0
3	1FY4	ASP102A (OD2)···HIS 57A (ND1)	2.75	1.91	0.86	162.8
4	1FY5	ASP102A (OD2)···HIS 57A (ND1)	2.75	1.92	0.86	163.1
5	1GDN	ASP102A (OD2)···HIS 57A (ND1)	2.76	1.92	0.86	163.1
6	1XVO	ASP99A (OD2)···HIS 56A (ND1)	2.75	1.91	0.86	165.6
7	1PQ5	ASP99A (OD2)···HIS 56A (ND1)	2.75	1.91	0.86	165.6
8	1GDQ	ASP102A (OD2)···HIS 57A (ND1)	2.75	1.92	0.86	166.1
9	1HJ9	ASP102A (OD2)···HIS 57A (ND1)	2.73	a	a	a
10	1AYW	ASP102A (OD2)···HIS 57A (ND1)	2.70	a	a	a
11	1HJ8	ASP102A (OD2)···HIS 57A (ND1)	2.69	a	a	a
12	1PQ8	ASP99A (OD2)···HIS 56A (ND1)	2.74	1.90	0.86	166.7
13	2H5C	ASP102A (OD1)···HIS 57A (ND1)	2.76	1.93	0.85	163.9
14	1SSX	ASP102A (OD1)···HIS 57A (ND1)	2.77	1.95	0.83	170.4
15	2H5D	ASP102A (OD1)···HIS 57A (ND1)	2.73	1.77	1.01	158.6
16	1EA7	ASP34A (OD1)···HIS 71A (ND1)	2.65	1.79	0.86	174.9
17	1GCI	ASP32 (OD2)···HIS 64 (ND1)	2.64	1.57	1.12	157.5
18	1GVK	ASP102B (OD2)···HIS 57B (ND1)	2.67	a	a	a

[a]No corresponding data were found in the Protein Data Bank

Structure of serine proteases at a resolution 1.0–1.3 Å

The structures of trypsin crystallized at pH 5.0–8.0 and solved at 1.02–1.28 Å resolution are shown in Tables II and IV. At this resolution, proton imaging was possible only in few cases. The *R* values were in most cases below 0.14, indicating a satisfactory quality of the crystallographic resolution. In the catalytic triad Ser···His···Asp (Scheme 1), the distance between the heteroatoms in Ser and His for the structures listed in Table II was always 2.8–3.1 Å (data not shown), indicating again a normal hydrogen bond distance between the hydroxyl oxygen of serine and the imidazole nitrogen of histidine.

On the other hand, the distance between the heteroatoms in histidine and aspartate in the catalytic triad was shorter, from 2.57–2.77 Å; the exceptions are entries 9, 12, 16, and 19 in Table IV, where the difference was in the opposite direction (data not shown). We have no ready explanation for these exceptions, except that the lower resolution may afford, in some cases, some degree of uncertainty. The difference between the heteroatoms in Ser and His, and in His and

Asp is this time less significant, because at this resolution the error is up to 0.06 Å. At this resolution, proton imaging is possible only in a few cases and, therefore, the linearity of the H bond can be determined in only a few cases (Table V).

TABLE IV. Crystal structures of trypsin at a resolution 1.02–1.28 Å

Entry	PDB access code	Putative hydrogen bonds between the catalytic aspartate and histidine residue	Distance Å
1	1S0Q	ASP743A (OD2)···HIS 699A (ND1)	2.76
2	1S0R	ASP84A (OD2)···HIS 40A (ND1)	2.75
3	1GDU	ASP102A (OD2)···HIS 57A (ND1)	2.72
4	1XVM	ASP99A (OD2)···HIS 56A (ND1)	2.71
5	2AH4	ASP102X (OD2)···HIS 57X (ND1)	2.78
6	2AGI	ASP102X (OD2)···HIS 57X (ND1)	2.75
7	2AGE	ASP102X (OD2)···HIS 57X (ND1)	2.75
8	1UTN	ASP102A (OD2)···HIS 57A (ND1)	2.73
9	1UTO	ASP102A (OD2)···HIS 57A (ND1)	2.76
10	1UTQ	ASP102A (OD2)···HIS 57A (ND1)	2.76
11	1Y59	ASP102T (OD2)···HIS 57T (ND1)	2.74
12	2BLV	ASP102A (OD2)···HIS 57A (ND1)	2.73
13	2BLW	ASP102A (OD2)···HIS 57A (ND1)	2.73
14	2F91	ASP102A (OD2)···HIS 57A (ND1)	2.74
15	1J8A	ASP102A (OD2)···HIS 57A (ND1)	2.72
16	1PPZ	ASP99A (OD2)···HIS 56A (ND1)	2.97
17	1PQA	ASP99A (OD2)···HIS 56A (ND1)	2.57
18	2A31	ASP102A (OD2)···HIS 57A (ND1)	2.78
19	2AGG	ASP102X (OD2)···HIS 57X (ND1)	2.81

TABLE V. Putative hydrogen bonds in the catalytic triad of trypsin at resolution 1.02–1.28 Å

Entry	PDB access code	List of putative hydrogen bonds in the catalytic triad	Distance Å	Hydrogen bond, Å		Angle deg.
				OD···H	H–ND1	
3	1GDU	ASP102A (OD2)···HIS 57A (ND1)	2.72	1.89	0.86	160.4
4	1XVM	ASP99A (OD2)···HIS 56A (ND1)	2.71	1.89	0.86	160.7
16	1PPZ	ASP99A (OD2)···HIS 56A (ND1)	2.97	1.73	1.43	140.0
17	1PQA	ASP99A (OD2)···HIS 56A (ND1)	2.57	1.82	0.86	144.4
18	2A31	ASP102A (OD2)···HIS 57A (ND1)	2.78	1.85	1.00	153.2

CONCLUSIONS

Binding of ligands. Most of the analyzed enzyme structures given in Table I (obtained at a resolution < 1 Å) were crystallized in the presence of salts and contain salt ions, sometimes bound in the active site, such as SO_4^{2-}, Na^+, Ca^{2+} or citrate, or ligands, such as glycerol, benzamidine or aniline. Detailed information concerning these ligands is not included in Table I in order to save space. Furthermore, several preparations contained arginine, lysine, or arginine peptides bound in the active site, which mimic the products of the reaction. In addition, two preparations had bound inhibitors ONO (entry 10) or valine boronic acid (entry 15). In one occasion (entry 18), the serine residue in the active site was acetylated.

The analyzed enzyme structures given in Table II (resolution 1.0–1.3 Å) contained similar ligands, including imidazole. Furthermore, some preparations contained inhibitors bound in the active site, benzylamine (entriy 8) and TL1 (entry 11). In addition, in several preparations the serine residue in the active site was acylated (entries 5, 6, 7, and 19).

Hydrogen bonds in the active site. In the catalytic triad Ser···His···Asp, there are two hydrogen bonds. In the preparations listed in Table I (resolution < 1 Å), the length of the hydrogen bonds between the heteroatoms in His···Asp were in each preparation shorter than the hydrogen bond between the heteroatoms in Ser···His, on average by 0.2±0.02 Å. In the preparations listed in Table II (resolution 1–1.3 Å), the length of the hydrogen bonds between the heteroatoms in His···Asp were in most cases shorter than the bond between the heteroatoms in Ser···His, on average by 0.2±0.06 Å; however, in a few cases, the situation was reversed, *i.e.*, the former hydrogen bonds were longer than the latter, on average by 0.1±0.06 Å (entries 9, 12, 16, and 19).

Crystallographic evidence for the mechanism in Scheme 1. In this communication, a total number of 37 enzyme structures of trypsin and other serine proteases, obtained at a resolution 0.78–1.28 Å, were examined (Tables I and II). It is interesting to note that no structure was reported in the literature for chymotrypsin with a resolution better than 1.4 Å.

The information obtained from the structures of active sites of serine proteases may be compared with the mechanism presented in Scheme 1. The normally allowed van der Waals distance for interatomic contacts between oxygen and nitrogen is 2.7 Å. There are only a few distances slightly shorter than 2.7 Å, four in Table III and one in Table IV. According to Scheme 1, short hydrogen bonds are formed between the catalytic aspartate and histidine and not between serine and histidine. Indeed, in accordance with this, the hydrogen bonds between aspartate and histidine are in most cases shorter than the hydrogen bonds between serine and histidine. The formation of an LBHB requires a hydrogen bond on a straight line between the heteroatoms, with the H atom nearly centered between them. Tables II and V indicate that the H atom is nearly collinear, the angle between the heteroatoms and the hydrogen is in most cases 160–170°. However, the H atom is nearly centered between the heteroatoms in only a few cases; this is not surprising, since the H atom is fully centered only in transition states which are unstable. In addition, the hydrogen atom is centered only when the pK_as of heteroatoms are matched, which occurs only in unstable transition state structures.

Thus, the survey performed in this work of crystallographic structures obtained at high resolution indicates that in serine proteases they do not comply with the LBHB hypothesis. Warshel and coworkers suggested earlier that LBHBs cannot be observed by crystallographic methods because the transition state structures are inherently unstable.[37–40] Also, recent quantum chemistry modeling of serine proteases do not support the LBHB concept.[41,42]

However, it must be emphasized that the findings reported in this work do not exclude the validity of the LBHB hypothesis at all, but merely show that the data do not comply with the hypothesis for this particular class of enzymes. Recently, a survey of critical hydrogen bond lengths in lactate and alcohol dehydrogenase was reported.[43] It was found that short hydrogen bonds were clustered, to a very high degree, exactly at the bond breaking position in complexes of alcohol dehydrogenase with its substrates or their analogs. This finding suggests indirectly that the LBHB hypothesis may be valid for alcohol dehydrogenases. A similar clustering of short hydrogen bonds was not found in the case of lactate dehydrogenases.

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И З В О Д

КРАТКЕ ВОДОНИЧНЕ ВЕЗЕ У КАТАЛИТИЧКОМ МЕХАНИЗМУ СЕРИНСКИХ ПРОТЕАЗА

ВЛАДИМИР ЛЕСКОВАЦ[1], СВЕТЛАНА ТРИВИЋ[2], ДРАГИЊА ПЕРИЧИН[3], МИРА ПОПОВИЋ[2] И ЈУЛИЈАН КАНДРАЧ[3]

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Преглед кристалографских података из " Protein Data Bank " за 37 структура трипсина и других серинских протеаза на разлагању од 0,78–1,28 Å, показују присуство водоничних веза у активном центру ензима, које се образују између каталитичког серина и аспартата, и у просеку су дужине 2,7 Å. То је типична дужина за нормалну водоничну везу. Геометријска својства водоничних веза у активном центру показују да H атом није центриран између хетероатома каталитичког хистидина и аспартата. Када се све сабере, ови налази показују да се "водоничне везе ниске баријере" не образују у основном стању структура активног центра које су истражене у овом раду. Хипотеза "водоничних веза ниске баријере", коју је недавно предложио Cleland, захтева присуство кратких водоничних веза од 2,4 Å у активном центру, са H атомом центрираним између хетероатома. Закључак овог рада никако не искључује валидност хипотезе "водоничних веза ниске баријере", али не подупире ову хипотезу у случају испитиване класе ензима.

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