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DNA cleavage function of seryl-histidine dipeptide and its application

Review Article

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Summary. The double-stranded DNA or circular plasmid DNA can be cleaved by the Ser-His dipeptide by hydrolysis of the DNA-phosphodiester bond. The proper sequential order of the amino acids serine and histidine is apparently crucial in its unique cleavage activity as compared to the other di- or tri-peptides containing one of these amino acids. An inverted sequence of this dipeptide to a His-Ser linkage renders the peptide ineffective in the cleavage of DNA. In addition to the DNA cleavage function, Ser-His is also capable of cleaving other molecules, e.g., proteins, esters and RNAs. The cooperative actions of the hydroxyl group and the basic groups in the serine and histidine or related amino acids can be found in contemporary enzymes, such as DNase, serine proteases, lipases, esterases, chymotrypsin, trypsin, and elastase, etc. The Ser-His and related oligopeptides might have played important roles in the evolution of enzyme functions.

Keywords: Seryl-histidine dipeptide – DNA cleavage function – Evolution of enzymes

Introduction

During the past two decades, artificial nucleic acid cleavage agents have received considerable attention due to their very important applications in biochemistry and molecular biology (Åström and Strömberg, 2004; Boseggia et al., 2004; Yang et al., 2004; Gnaccarini et al., 2006). One of the driving forces behind studies of artificial nucleic acid cleavage agents is the desire to understand the catalytic mechanism of natural nucleases (Nomura and Sugiura, 2004). Another driving force originates from the fact that the site and sequence specific cleavage agents can be applied to the gene therapy (Cowan, 2001) as well as to general molecular biology. The ordinary cleavage agents without recognition systems also have potentially extensive applications in footprinting as cleaving agents, in hybridizations as probes for studying nucleic acids with high grade structures (Fox and Waring, 2001).

The amino acids serine (Ser) and histidine (His) function together in the active sites of various naturally occurring enzymes as direct participants in enzymatic reactions. They can be found in the active sites of serine proteases (Steitz and Shulman, 1982), lipases (Brady et al., 1990; Schrag et al., 1991; Contreras et al., 1996), esterases (Sussman et al., 1991), chymotrypsin, trypsin, and elastase (Carter and Wells, 1988; Sigman and Chen, 1990; Corey et al., 1995). Ser-His and Asp/Glu catalytic trial was also observed in the hydrolysis property of β-amyloid (Brzyska et al., 2001). The side chain hydroxyl of serine often serves as a nucleophile, while histidine possesses an imidazole side chain that can serve as a proton donating or accepting group in enzymes, and some serine protease mimics were designed and synthesized based these concepts (Madder et al., 2002; Catry and Madder, 2007).

The roles of Ser (or related amino acid residues) and His in peptide bond and ester bond cleavage reactions of enzymes are well documented, and the shared chemical characteristics in the cleavage of the peptide bond and the phosphodiester bond were further verified by the experimental result that the dipeptide seryl-histidine (Ser-His) itself can cleave proteins as well as DNA.

DNA cleavage activity of seryl-histidine

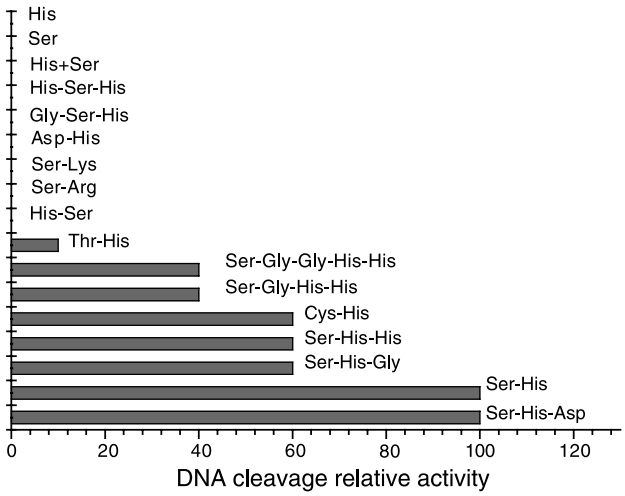
In the studies of the interaction between *N*-phosphoamino acids with DNA, it was found that the aged solution of *N*-phosphorus serine in a saturated histidine buffer exhibited DNA cleavage activity, not the fresh one. Finally, it was clarified that the seryl-histidine dipeptide which formed in the solution was responsible for the DNA cleavage (Ma, 1996).

Linear bacteriophage λ-DNA and circular plasmid DNA pBR322 were both gradually degraded into smears of progressively smaller fragments of heterogeneous sizes, after 72 h of incubation with seryl-histidine (Li et al., 2000). This DNA cleavage activity has no sequence specificity, which was demonstrated in a cleavage experiment using a radio-labelled oligonucleotide substrate. The nucleolytic activity in samples incubated with Ser-His could be detected over a wide pH range (from 5 to 9), with a pH value near the pKa of imidazole (pH 6) being optimal for cleavage at 37 °C. The rate of cleavage was temperature-dependent; incubation at 50 °C resulted in faster DNA cleavage than at 37 °C.

However, the DNA cleavage activity of Ser-His is much lower than DNase. For example: the DNA cleavage activity of 1.21 μg/μL Ser-His is equal to about 1/1000 of 7×10^{-3} unit/μL RQ1 DNase at 37 °C, that means, the specific activity of Ser-His is 5.0×10^{-3} unit/mg (Wan, 2000a).

Evaluation of related oligopeptides and the functional groups in DNA cleavage

To understand the role of Ser and His in DNA cleavage, different amino acid residues were used in replacement of Ser or His in the Ser-His dipeptide, or were added internally or to the *N*- or *C*-terminus of Ser-His. The cleavage activities are lost when Ser is replaced with any other amino acid except cysteine (Cys) or threonine (Thr). The histidine residue cannot be replaced by any of the amino acids tested, including lysine or arginine with positively charged side chains (Scheme 1). His-Ser, a dipeptide identical to Ser-His in chemical composition but in reverse sequence, is also inactive.The cleavage activities of Ser-His are reduced or lost when an amino acid is added to its *N*-terminus but are retained when one or more amino acids are added to the *C*-terminus. It was also found that amino acids could be added between Ser and His without abolishing the cleavage activity.



Scheme 1. DNA cleavage activities of Ser-His and related oligopeptides

Table 1. DNA cleavage activities of Ser-His and structure related compounds

Compounds	DNA cleavage activities	Optimum pH
Ser-His	+++++	6.0
Boc-Ser-His	−	
Ser-His-OMe	++	6.0
Ser-Ala	+	5.0
Ser	−	
Ser-OMe	+	5.0
Thr-OMe	+	5.0
Ethanolamine	+	5.0
Diethanolamine	+	5.0
Triethanolamine	+	5.0
Isopropanolamine	+	5.0

Table 2. The major structure features in docked complexes

Peptide	DNA cleavage activity	Dist. (Å)[a]	Binding points[b]
Ser-His	+++++	3.7	α-NH_3–$(PO_4)_1$ (2H-bonds) OH–$(PO_4)_1$ C(O)NH–$(PO_4)_2$ Imidazol–$(PO_4)_2$
Cys-His	+++	3.6	α-NH_3–$(PO_4)_1$ SH–$(PO_4)_1$ C(O)NH–$(PO_4)_2$ Imidazol–$(PO_4)_2$
Thr-His	±	3.1	α-NH_3–$(PO_4)_1$ OH–$(PO_4)_2$
Asp-His	–	–	α-NH_3–$(PO_4)_1$
Ser-Arg	–	4.9	α-NH_3–$(PO_4)_1$ NH=$C(NH_2)_2$–$(PO_4)_2$
His-Ser	–	5.4	α-NH_3–$(PO_4)_1$ (2H-bonds) C(O)NH–$(PO_4)_2$

[a] Distance between nuleophile O(S) atom of the peptide side chain and P atom of the trinucleotide

[b] $(PO_4)_1$ and $(PO_4)_2$ stand for the two neighbor phosphates

These experimental results implicated the hydroxyl (or mercapto) functional groups of the *N*-terminal amino acid residues and the imidazole functional group of histidine as the requisite groups for cleavage.

Some compounds which share different degrees of similarity in structure with Ser-His were also tested for DNA cleavage (Wan, 2000a; Wan et al., 2000b). Their cleavage activities relative to Ser-His are summarized in Table 1.

The amino group is vital to cleavage activity, and the common structural feature of the above compounds with DNA cleavage activity in Table 2 is that there are hydroxyl and amino groups in the vicinal carbons. Histidine residues are known to play a prominent role in the catalytic mechanism of many protein enzymes, including ribonucleases and other phosphoesterases, and some artificial cleavage agents that contained imidazole groups have been developed to catalyze the cleavage of DNA and RNA substrates (Breslow, 1991; Lipscomb and Strater, 1996; Santoro et al., 2000; Beloglazova et al., 2004; Reddy et al., 2004; Zhao et al., 2005). It was found that the imidazole is not the crucial group for the cleavage activity, but is very important for enhancing the activity. Consequently, Ser-His is the most efficient DNA cleaving agent among all the above compounds. The *C*-terminal carboxyl group also is not essential for the cleavage activity, but the esterification of carboxyl group drastically reduced the cleavage activity. The difference in activity may be accounted for the free carboxyl group of Ser-His, which could improve the solubility of Ser-His in water.

Based on the evaluation of each function group of seryl-histidine in DNA cleavage reaction, another compound seryl-histamine amide, a mimic of Ser-His without carboxyl group, was synthesized and then testing shown it had the ability to cleave DNA (Sun et al., 2003; Zhu et al., 2004; Liu et al., 2005).

Molecular modeling on DNA cleavage activity of seryl-histidine and related dipeptides

A series of three-dimensional models of dipeptide. . .5′-TpTpdC-3′ complexes have been constructed using the molecular docking program FlexiDock encoded in Sybyl (Sun et al., 2004; Zhong et al., 2004). The major structural features in the complexes are summarized in Table 2.

A contactable O(S)–P distance is a necessary condition for the DNA cleavage. In the cases of Ser-His and Cys-His, the distances between the nuleophile O or S atom of peptide and the P atom of trinucleotide in their complexes (O–P: 3.7 Å and S–P: 3.6 Å, respectively) are both within a contactable range for the O(S) atom conducting nucleophilic attack to the phosphoric ester (Table 4). This may lead to a formation of a pentacoordinate phosphotriester transition state followed by cleavage of the ester bond, and therefore result in the DNA cleavage.

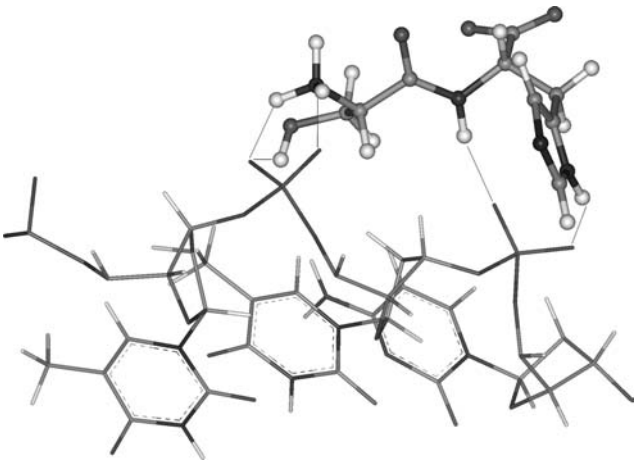
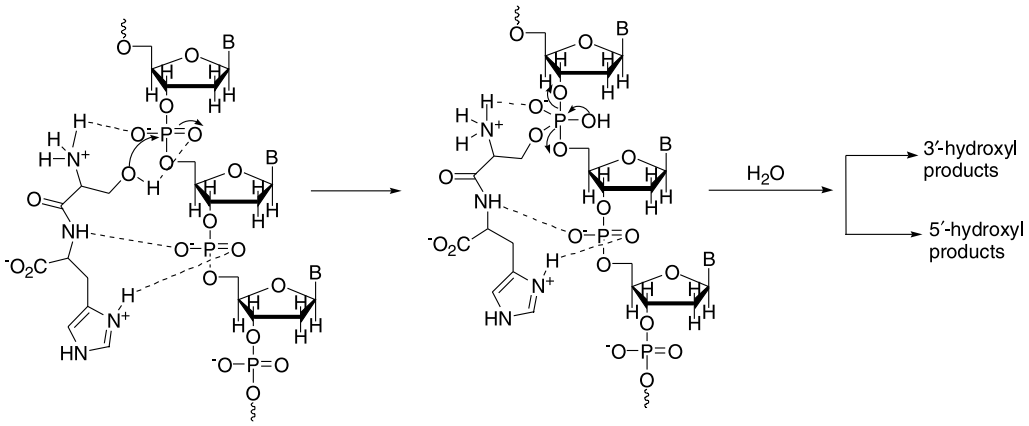


Fig. 1. The docked structure of Ser-His binding with 5′-TpTpdC-3′. Five pairs of H-bonds are marked by pink lines (for an interpretation of the reference to colour in this figure, the reader is referred to the online version of this paper under www.springerlink.com)

Another key structure feature is that the dipeptide must bind to two neighbor phosphates in the DNA backbone simultaneously. For Ser-His–5′-TpTpdC-3′, the Ser moiety binds to one phosphate through H-bonds and electrostatic interaction, while the His moiety binds to the other phosphate. The α-amino group of serine forms two H-bonds with the two oxygen atoms of the phosphate, and the hydroxyl group of serine forms one H-bond with one oxygen atom of the phosphate (Fig. 1). There are two other H-bonds between the amide or imidazole group of His moiety and another phosphate, which provide more recognition sites for constructing the complex. This may play an essential role in keeping the binding geometry and facilitate nucleophilic attack by bringing the DNA closer.

Religation of DNA cleavage fragments and the deduction of cleavage mechanism

It was also found that fragments with 3′-hydroxyls and 5′-phosphates were among the DNA cleavage products generated by Ser-His, since the fragments could be re-ligated by DNA ligase The generation of these terminal groups is consistent with hydrolysis of phosphodiesters by Ser-His, and would not be characteristic of cleavage of the DNA by a free-radical mechanism (Li et al., 2000). Combining all the evidence, the DNA cleavage mechanism is postulated as shown in Scheme 2.



Scheme 2. Proposed mechanism for DNA cleavage by Ser-His. *Dash lines* represent H-bonds between Ser-His and oligonucleotide

Application of seryl-histidine as a DNA nicking agent

Nick translation is a commonly used method for labeling DNA to make DNA hybridization probes (Kuhn et al., 2003). In this approach, the use of DNase I to generate nicks in double-stranded DNA presents an inherent drawback, because the enzyme's high rate of reaction causes significant fragmentation and shortening of the hybridization probes. Like DNase, Ser-His randomly nicks DNA, but the dipeptide has a much lower rate of reaction that enables more complete labeling of the DNA probes with less fragmentation (Li et al., 2002). DNA probes labeled through nick translation using Ser-His as the DNA nicking agent were consistently larger in size and exhibited significantly higher specific activities, and enhanced hybridization signals in Southern blot analyses compared to control DNA probes that were made using DNase I as the nicking agent. Furthermore, the degree of nicking and consequently the quality of the probes could be easily controlled by adjusting the temperature and time of the Ser-His nicking reaction.

The possible roles of seryl-histidine in the early evolution of enzymes

Protein self-splicing provides another example of the Ser/His catalytic dyad. This peptide bond cleaving process invariably uses Ser or Cys at the *N*-terminus and His (plus an asparagine (Asn) at the *C*-terminus of an internal protein sequence (intein) to enable cleavage at the splice junctions and the rejoining of the external protein sequences (exteins). More interestingly, the spliced intein always has Ser or Cys at its *N*-terminus and His-Asn at its *C*-terminus, and functions as a homing endonuclease to cleave chromosomal DNA (Kane et al., 1990; Perler et al., 1994; Liu, 2000; Xu, 2005). It seems reasonable to speculate that these Ser and His residues may function not only in the intein splicing reaction, but in the subsequent DNA cleavage as well.

The dipeptide Ser-His is the shortest peptide ever reported to have multiple cleavage activities. Results of preliminary experiments indicate that in addition to DNA cleavage, Ser-His is also capable of cleaving protein, ester, and RNA (Li et al., 1997, 2000). Because of its ability to interact with multiple classes of biological molecules over such wide ranges of physical and chemical conditions, Ser-His and related oligopeptides may have played important roles, either independently or as cofactors to RNA, in the hypothetical 'RNA world' (Joyce and Orgel, 1993; Roth and Breaker, 1998; Joyce, 2007) from which the modern 'protein world' emerged. The ability of Ser-His to retain its multiple cleavage activities when amino acids are added internally or to its *C*-terminus demonstrates the extraordinary evolutionary capacity of the dipeptide Ser-His. Since the number of catalytically active combinations available for the evolution of polypeptide enzymes is limited by the relatively small number of functional groups provided by the naturally occurring amino acids, it is not surprising that the successful combination of Ser and His is repeatedly selected in the evolution of diverse groups of enzymes.

Conclusion

The cooperative actions of the hydroxyl group and the basic groups in serine and histidine or related amino acids can be found in contemporary enzymes, such as serine

proteases, lipases, esterases, chymotrypsin, trypsin, and elastase, etc. Ser-His and related oligopeptides with DNA and protein cleavage activity may have played important roles in the evolution of enzyme functions. We hope our results may shed light on the molecular recognition of the biological system as a whole, especially on the understanding of the general interaction mechanism between DNA and proteins.

Furthermore, Ser-His can substitute for DNase I as a DNA nicking agent to produce superior DNA probes and hybridization. This application can be extended easily to making non-radioactive nick translation probes. When associated with appropriate targeting molecules, Ser-His or related oligopeptides may be able to produce site-specific nucleic acid and/or protein cleavage.

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