Ser-His catalyses the formation of peptides and PNAs

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ABSTRACT

The dipeptide seryl-histidine (Ser-His) catalyses the condensation of esters of amino acids, peptide fragments, and peptide nucleic acid (PNA) building blocks, bringing to the formation of peptide bonds. Di-, tri- or tetra-peptides can be formed with yields that vary from 0.5% to 60% depending on the nature of the substrate and on the conditions. Other simpler peptides as Gly-Gly, or Gly-Gly-Gly are also effective, although less efficiently. We discuss the results from the viewpoint of primitive chemistry and the origin of long macromolecules by stepwise fragment condensations.
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1. Introduction

One of the fundamental and largely unsolved questions in the origin of life is the biogenesis of long and specific co-oligopeptide sequences, the gate to enzymes and/or transport proteins. The N-carboxy anhydride (NCA) amino acid condensation brings to 10–20 residues long homo- and co-oligopeptides [1–4], but the achievement of longer sequences remains elusive. A possible effective prebiotic route to long polypeptides resides in the condensation of peptide fragments, catalyzed by very simple peptides. In addition to several reports focusing on the catalytic activity of simple peptides [5–10], it was recently shown that seryl-histidine (Ser-His) acts as protease and phosphoesterase [11]. On the basis of microscopic reversibility principle, a protease – e.g., α-chymotrypsin, or Ser-His – can also catalyze the reverse reaction, i.e. the formation of peptide bonds, which is well documented in the literature [12,13] (a similar use of transpeptidases has also been similarly reported [14]).

The availability of the Ser-His might be arguable in an origin of life context. Whereas serine is considered a prebiotic amino acid [15], histidine is not. There are reports, however, on the prebiotic synthesis of histidine [16], so that the formation of Ser-His might be seen as plausible.

We describe how Ser-His and other simple peptides can effectively catalyze the synthesis of peptide bonds with a yield comparable to that of α-chymotrypsin, although in a more sluggish time-scale. We argue here that the repeated fragment condensation may in principle represent a simple gate for the biogenesis of polypeptides. We have also extended the use of Ser-His to the synthesis of peptide nucleic acid (PNA) oligomers. PNAs are nucleic acid analogues, having a polypeptide backbone and purine/pirimidine bases as side chains, being capable of Watson–Crick base pairing with natural nucleic acids [17,18].

2. Materials and methods

2.1. Peptide synthesis

The ethyl or methyl esters of amino acids and peptides (50 mM) were added as DMF solution to the nucleophilic amino acid derivatives (e.g. H-Leu-NH$_2$ or similar; 50 mM), dissolved in aqueous buffer (generally, 100 mM sodium borate pH 10), so that the final DMF content in the aqueous solution was 6% (v/v). After addition of the catalyst (e.g. Ser-His, or α-chymotrypsin, etc.), and proper incubation time, the reaction was stopped by adding 10% v/v HCl

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1 N. The precipitate was collected by centrifugation (13 000 rpm, 15 min), dissolved in a measured amount of DMF and analysed by HPLC. The supernatant was also analysed by HPLC. The coupling product and the unreacted acyl donor are generally recovered in the precipitate. In contrary, the hydrolized substrate is found in the supernatant. Yields were calculated from HPLC peak areas; each compound was identified by mass spectrometry.

2.2. Synthesis and oligomerization of PNA building blocks

PNA thymine monomer was synthesized according to published procedure, and condensation was carried out as essentially described in the above.

Details on materials and methods are given in the Supplementary material.

3. Results and discussion

As shown in Chart S1 (in the Supplementary material), our approach is based on the Ser-His catalyzed aminolysis of ethyl esters. In particular we show first the formation of peptide bonds by reacting the ethyl ester of *N*-acetyl-phenylalanine (Ac-Phe-OEt), acting as acyl donor, with leucinamide (H-Leu-NH$_2$) [13]. The formation of the product (Ac-Phe-Leu-NH$_2$ is followed by reverse phase HPLC and mass spectrometry. This product, being sparingly soluble in water, forms quickly (within 1 minute), and in high yields (>60%), when the catalyst is α-chymotrypsin. The substrate Ac-Phe-OEt is also hydrolyzed to give about 35% Ac-Phe-OH (water soluble). In the following, we will refer to these two competitive reactions as coupling and hydrolysis.

When Ser-His (4 mM) is used instead of α-chymotrypsin (40 μM), we observe the same reactive pattern. After one-day incubation at room temperature, Ser-His brought to the formation of the desired coupling product in 10% yield, which is only six times lower that when α-chymotrypsin is used. Control experiments, without catalyst, or in the presence of imidazole, His, Ser, His plus Ser, and the isomer His-Ser, do not give any reaction in the allotted

time-scale (data not shown). Only in particular conditions (1 month, 25 °C) we measured a certain amount of uncatalyzed coupling, which corresponds to about 20% of the Ser-His catalyzed coupling. It is remarkable that His-Ser is not a catalyst for ester aminolysis; on the other hand, Li et al. [11] have already reported the lack of His-Ser hydrolytic activity when compared to Ser-His. The terminal serine amino group and/or the different stereo-electronic arrangement around imidazole ring may possibly explain this observation.

Fig. 1a shows the pH dependence of products for the reaction between Ac-Phe-OEt and H-Leu-NH$_2$. The formation of Ac-Phe-Leu-NH$_2$ is significant only at pH > 7, and reaches a plateau after pH 9. On the contrary, the hydrolysis of the substrate constantly increases in basic conditions. If Ser-His concentration is increased from 4 to 16 mM, the yield of coupling product increases by a factor 2.4 (Fig. 1b). Above 16 mM, the yield decreases and hydrolysis becomes favored. Fig. 1c illustrates the time course of Ser-His catalysed reaction between Ac-Phe-OEt and H-Leu-NH$_2$. The amount of coupling product constantly increases in time, reaching an asymptotic value of about 60% in 30 days. Interestingly, the amount of hydrolyzed substrate does not exceed 40%, and actually decreases after 7 days. The fact that Ser-His catalyzed coupling, although slowly, reaches high conversion yields is particularly important in a primitive fragment condensation scenario (see below). Finally, lower temperature favored the synthesis of the peptide (Fig. 1d), probably due to lower solubility of the product. By contrast, substrate hydrolysis is abundant at high temperature. Longer peptides can be also obtained, as shown in Table 1 (entries 1–6); di-, tri-, and tetra-peptides are formed in yields that depend on the nature of the substrates (0.5–31.5%).

In some cases (entries 4 and 5) the low solubility of acyl donor substrates did not allow the reaction to proceed effectively; but the solubility of the product can also affect the coupling yield (e.g., as in entry 6). When the ethyl ester and the nucleophilic amino groups are present in the same molecule, the substrate can oligomerize, as in the case of thymine PNA building blocks. In fact, Ser-His readily oligomerizes the peptide precursor 1 (Fig. S1) to
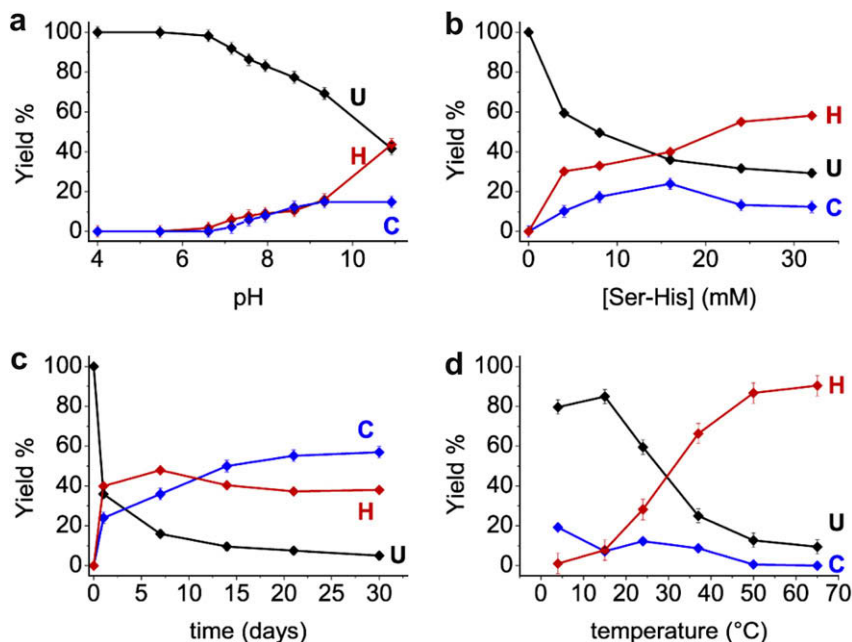

Fig. 1. Reaction between Ac-Phe-OEt and H-Leu-NH$_2$. The yields of the coupling product (Ac-Phe-Leu-NH$_2$, "C", blue), hydrolysis product (Ac-Phe-OH, "H", red), and of the unreacted substrate (Ac-Phe-OEt, "U", black) are shown. (a) Dependence on the pH; 4 mM Ser-His in Britton–Robinson buffer, 25 °C/ 3 days. (b) Dependence on the Ser-His concentration: Ser-His in sodium borate (100 mM, pH 10), 25 °C/1 day. (c) Reaction kinetics: 16 mM Ser-His in sodium borate buffer (100 mM, pH 10), 25 °C. (d) Dependence on the temperature: 4 mM Ser-His in sodium borate buffer (100 mM, pH 10), 6 days.

Table 1
Yields of peptides and PNAs, as catalysed by Ser-His and (Gly)$_n$.

Entry #	Catalyst	Conditions	Acyl donor	Free amine	Coupling product	Yield (%)
1	Ser-His	[a]	Ac-Phe-OEt	H-Leu-NH$_2$	Ac-Phe-Leu-NH$_2$	13.3
2	Ser-His	[a]	Ac-Phe-OEt	H-Phe-NH$_2$	Ac-Phe-Phe-NH$_2$	31.2
3	Ser-His	[a]	Ac-Phe-OEt	H-Leu-Phe-NH$_2$	Ac-Phe-Leu-Phe-NH$_2$	27.4
4	Ser-His	[a]	Z-Ala-Phe-OMe	H-Leu-NH$_2$	Z-Ala-Phe-Leu-NH$_2$	2.0
5	Ser-His	[a]	Z-Ala-Phe-OMe	H-Phe-NH$_2$	Z-Ala-Phe-Phe-NH$_2$	0.5
6	Ser-His	[a]	Z-Ala-Phe-OMe	H-Leu-Phe-NH$_2$	Z-Ala-Phe-Leu-Phe-NH$_2$	6.3
7	Ser-His	[b]	PNA monomer (1)	PNA monomer (1)	PNA dimer (2)	6.5[f]
8	Ser-His	[b]	PNA monomer (1)	PNA monomer (1)	PNA trimer (3)	3.6[f]
9	Ser-His	[b]	PNA monomer (1)	PNA monomer (1)	PNA tetramer (4)	9.1[f]
10	Ser-His	[c]	H-Phe-OEt	H-Phe-OEt	H-Phe-Phe-OEt	0.5–3
11	Ser-His	[d]	H-Phe-OEt + H-Trp-OEt	H-Phe-OEt + H-Trp-OEt	H-(Phe)(Trp)-OEt	0.1
12	Gly	[e]	Ac-Phe-OEt	H-Leu-NH$_2$	Ac-Phe-Leu-NH$_2$	11.9
13	Gly-Gly	[e]	Ac-Phe-OEt	H-Leu-NH$_2$	Ac-Phe-Leu-NH$_2$	13.2
14	Gly-Gly-Gly	[e]	Ac-Phe-OEt	H-Leu-NH$_2$	Ac-Phe-Leu-NH$_2$	14.2

[a] 50 mM reactants (each), 4 mM Ser-His, 24 h, 25 °C.
[b] 10 mM PNA monomer; 1.5 mM Ser-His, 35 h, 25 °C (Numbers 1, 2, 3, 4 refer to structures shown in Figure S1).
[c] 270 mM H-Phe-OEt; 22 mM Ser-His, 14 days, 4 °C.
[d] 50 mM reactants (both), 16 mM Ser-His, 7 days, 60 °C.
[e] 50 mM reactants (each), 4 mM Gly (or Gly-Gly; or Gly-Gly-Gly), 15 days, 4 °C.
[f] Calculated from the theoretical 100% yield of that product, as the only product in the mixture; chemical structures are shown in Fig. S1.

give the corresponding PNA dimer 2, trimer 3, and tetramer 4 in moderate yields (Table 1, entries 7–9). Also in this case, the driving force comes from the low solubility of products. Notice that the tetramer is formed in abnormal high yields, when compared with the lower oligomers. In this reaction, Ser-His is about 75 times less effective than α-chymotrypsin. Similarly, dimers of H-Phe-OEt and/or H-Trp-OEt can be obtained, although in lower yields (Table 1, entries 10–11).

From the mechanistic viewpoint, the Ser-His reaction represents an interesting case of organocatalysis. It may proceed through a Ser-*O*-ester intermediate, which in turn reacts with an amine to give the peptide bond. The lack of reactivity displayed by His-Ser, however, may also suggest the involvement of the Ser-His free amino group. Further studies are in progress in our laboratory in order to elucidate the reaction mechanism. Importantly, the effect of histidine on salt-induced peptide formation has been recently reported [19,20].

One advantage of Ser-His, and simple peptides in general, is that they can be active under conditions where large enzymes like α-chymotrypsin are inhibited or denatured. Thus, in the presence of 50 mM cupric ions, Ser-His is 1.5 times more effective than α-chymotrypsin; typical denaturing conditions (6 M urea) completely inhibit α-chymotrypsin, whereas Ser-His is still active (Fig. S2).

Interestingly, the simpler and certainly more prebiotic Gly, Gly-Gly and Gly-Gly-Gly, act similarly to Ser-His – see entries 12–14 of Table 1 (see also Fig. S3). It is noteworthy, however, that the coupling yield is about 13%, whereas Ser-His, in similar conditions, gave ca. 40% yield. The glycine-based peptide formation catalysis has been reported [9,10,21,22].

The present preliminary work shows the feasibility of using simple peptides for the condensation of peptide fragments. In principle, then, short peptides, which form spontaneously – for example by the NCA condensation – may combinatorially give origin to a library of longer peptides thanks to short-peptide catalysis. Of course, in order to proceed towards longer chains, successive, multiple condensation steps are needed.

The latter goal can be achieved by changing the experimental conditions at each step so that the insoluble products may become soluble, as in the dry-wet or laguna scenario proposed for the origins of life [23,24]. A synthetic analogue of this reaction scheme has been recently presented by us, in which the multiple condensation steps have been carried out by the Merrifield method. In this way, a de novo 44-residue folded protein has been synthesized [25].

In conclusion, short peptides such as Gly-Gly, Gly-Gly-Gly, and Ser-His, that could have been formed before the very biogenesis of specific macromolecules, can act as catalysts for the condensation of activated peptide fragments. The substrates used in this study (protected amino acids or PNAs ethyl esters) should be mainly considered as a model system, in order to provide a proof of principle; but it is conceivable that other activated amino acids (thioesters, for example, which have been extensively investigated in this respect) can react similarly, whereas free amino acids cannot.

Acknowledgments

Appendix A. Supplementary data

Materials and methods; HPLC, mass spectrometry and NMR characterizations of the products. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.11.052.

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