TRYPTIC CLEAVAGE OF HOMOARGINYL BONDS IN THE OXIDIZED GUANIDINATED DERIVATIVE OF A BOVINE TRYPSIN INHIBITOR (KUNITZ INHIBITOR)

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The recent report of tryptic cleavage of some peptide bonds involving homoarginine [1] prompts us to publish results observed during a study on the guanidinated derivative of the Kunitz trypsin inhibitor isolated from beef tissues. The molecule is comprised of a single polypeptide chain of 58 residues, 4 of which are lysines. Chemical modifications of individual lysines leading to derivatives which do not fit the specificity of trypsin can be studied because lysines alternate with arginines in the sequence and overlapping peptides containing a single lysyl residue can be isolated after tryptic hydrolysis [2, 3]. Guanidination has been employed to determine the respective roles of the α- and ϵ-amino groups of the inhibitor in the interaction with trypsin; it was observed that under the described conditions [4], 3.87 lysyl residues were converted into homoarginyl residues (yield of the guanidination: 97%) and that the α-amino group is apparently not modified. The guanidinated inhibitor is fully active [4, 5] but oxidation destroys the inhibiting capacity.

Performic acid oxidation, tryptic hydrolysis (molar ratio enzyme/substrate 1/250; pH 8.0; 60 min; 37°) and isolation of peptides by peptide mapping were carried out as previously described [6, 7]. Fig. 1 shows the tryptic peptide map of the oxidized guanidinated inhibitor. The peptides were analyzed [8] and identified from their composition (table 1), allowing to locate the bonds split by trypsin. The yields of cleavage at the 4 homoarginyl bonds were approximately determined assuming complete splitting of arginyl bonds, a condition observed in the trypsin digest of the oxidized inhibitor [6, 7], and using peptides T_3 and T_9 as references.

The four homoarginines are in positions 15, 26, 41 and 46. It is clear from table 2 that peptide bonds of Har-15* and Har-41 are weakly and not split, respectively. This result was expected for the bond Har-41–Arg-42 because the non modified bond Lys-41–Arg-42 is virtually intact after 60 min of tryptic hydrolysis [7]. In the case of the sequence Har-15–Ala-16–Arg-17, the proximity of an arginyl residue is presumably the cause of the poor cleavage of the

* Har: Homoarginyl residue

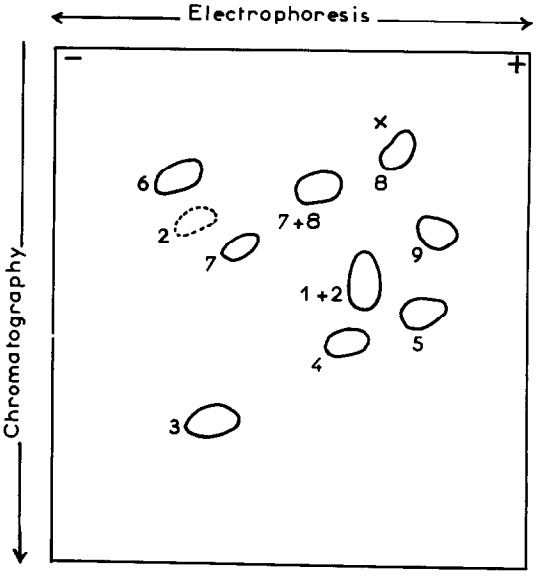

Fig. 1. Tryptic peptide map of the oxidized guanidinated derivative of the Kunitz trypsin inhibitor.

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Table 1
Amino acid composition of tryptic peptides from oxidized guanidinated inhibitor (number of residues per mole of peptide).

Amino acid	T_{1+2}	T_2*	T_3	T_4	T_5	T_6	T_{7+8}	T_7	T_8	T_9
Lys										
Arg	2.44	1.00	0.70		0.80	0.95	0.98		0.95	
Homo Arg	1.09			1.00		0.80	0.97	0.83		
$CySO_3H$	1.82				1.48		0.96		0.82	1.20
Asp	1.00			0.93			2.66	1.80	0.79	
Thr	0.97				0.97					1.02
Ser							0.73		0.50	
Glu	1.20				1.16		1.13		1.00	
Pro	2.97									
Gly	1.85				2.77					2.00
Ala	1.11	1.00		1.00	1.00	1.00	1.00		1.20	1.35
Val					0.87					
Met O_2							1.01		0.96	
Ile			2.00							
Leu	1.00				1.00					
Tyr**	0.29			1.13	0.38					
Phe	0.97			0.82	1.00		1.00	1.00		
Actual number of residues	17	2	3	5	13	3	11	4	7	5

* Amino acid composition estimated by paper chromatography.
** Tyrosine is largely destroyed when hydrolysis is performed on peptides eluted from paper.

Table 2
Tryptic hydrolysis at the 4 homoarginyl peptide bonds of oxidized trypsin inhibitor.

Peptide	Sequence	Approximate yield
T_{1+2}	1 15 17 Arg-Pro-Asp-Phe-$CySO_3H$--Leu-Glu--Pro-Pro-Tyr-Thr-Gly-Pro-$CySO_3H$-*Har*-Ala-Arg	85%
T_2	17 Ala-Arg	5–10%
T_{4+5}	not detected	—
T_4	26 Tyr-Phe-Tyr-Asn-Ala-*Har*	75%
T_5	39 Ala-Gly-Leu-$CySO_3H$-Glu-Thr-Phe-Val-Tyr-Gly-Gly-$CySO_3H$-Arg	80%
T_6	41 42 Ala-*Har*-Arg	100%
T_{7+8}	46 53 Asn-Asn-Phe-*Har*-Ser-Ala-Glu-Asp-$CySO_3H$-$MetO_2$-Arg	10–15%
T_7	46 Asn-Asn-Phe-*Har*	75%
T_8	53 Ser-Ala-Glu-Asp-$CySO_3H$-$MetO_2$-Arg	60%

homoarginyl bond. However the splitting is apparently complete when a lysyl residue occupies the place of the homoarginine [6, 7]. This result clearly confirms that a peptide bond involving a homoarginyl residue is split more slowly by trypsin than the similar lysyl bond.

For Har-46, the cleavage is rather high (about 70–80%) but not complete and an overlapping peptide can be isolated (table 2). The best splitting seems to be for Har-26 because the yields of peptides T_4 and T_5 are high and the overlapping peptide was not detected. It can be noted that the two nearest arginyl residues are far from Har-26 in the sequence (at positions 17 and 39); other factors such as the nature of neighbouring residues are probably involved.

The results obtained on the Kunitz trypsin inhibitor are in agreement with those of Seidl and Leiner [1] on the Bowman-Birk inhibitor and α-lactalbumin. Furthermore, we observe that the cleavage of homoarginyl bonds is often not complete under the usual conditions of trypsin hydrolysis. The cleavage can probably be improved by proper modifications of time or enzyme concentrations. It is of interest to mention that Baines et al. [9] have described the trypsin-catalysed hydrolysis of derivatives of homoarginine. The K_m (app) is considerably higher for the esters of α-N-toluene-p-sulfonyl-L-homoarginine used as substrates than for the corresponding arginine compounds, and on the other hand the deacylation rate of trypsin is lower with the first series than with the second. From the results observed for the four homoarginyl residues of the guanidinated inhibitor, it can be deduced that homoarginyl peptide bonds are substrates for trypsin but the cleavage, under the conditions used for peptide mapping, is very poor for the bonds Har–Arg and Har–X–Arg.

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