THE PROTEOLYTIC ACTION OF THE SNAKE VENOM ENZYMES ARVIN AND REPTILASE ON N-TERMINAL CHAIN-FRAGMENTS OF HUMAN FIBRINOGEN

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1. Introduction

Mammalian fibrinogens are built up by three peptide chains, α(A), β(B) and γ [1]. The N-terminal ends of these chains are linked together in a firm "disulfide knot" (DSK) by means of disulfide bridges [2]. When thrombin acts on fibrinogen small fibrinopeptides are split off from the N-terminal ends of the α(A) and β(B) chains. The result of this proteolysis is fibrin. The peptides, which have a molecular weight of less than 2000, are fibrinopeptide A and its analogues AP and AY from the α(A) chain and fibrinopeptide B from the β(B) chain [3]. Fibrinopeptide AY has the same amino acid sequence as fibrinopeptide A minus the N-terminal alanine; AP is fibrinopeptide A with a phosphoserine residue replacing serine at position 3 from the N-terminal end. "A" refers to A+AP+AY. Polymerization starts when fibrinopeptide "A" is released. In its limited proteolytic action, thrombin rapidly hydrolyses the arginyl-glycine bonds binding the fibrinopeptides to the rest of the molecule. However, some other arginyl or lysyl bonds can also be split. In this category is the arginyl-valyl bond, occurring 3 residues from the arginyl-glyceryl bond split when the A-peptide is released [2, 4]. The tripeptide in question, Gly–Pro–Arg, (see fig. 1), has been isolated from thrombic digests of DSK and α"A"-chain-fragments of DSK [2, 4], as well as from plasmic digests of sulfitolyzed α(A) chain-fragment [5].

It has been shown that the thrombin-like enzyme, Reptilase, from the venom of *Bothrops atrox* has a more limited proteolytic action than thrombin. Only fibrinopeptides "A" were reported to be released from native fibrinogen [6, 7]. The same pattern of proteolysis has been reported for highly purified Arvin, another snake venom enzyme from *Agkistrodon rhodostoma* [8].

In our studies on the purified disulfide knot we found it of interest to compare the action of these two thrombin-like enzymes on this substrate. It was found that other bonds, in addition to the ones mentioned above, could be split by the enzymes.

Abbreviations:

DSK: Disulfide knot
A : Human fibrinopeptide A
AP : Human fibrinopeptide AP
AY : Human fibrinopeptide AY
B : Human fibrinopeptide B
"A": Human fibrinopeptide A+AP+AY
α("A")-chain fragment: Designates the N-terminal part of the α("A")-chain fragment in the disulfide knot.

2. Materials and methods

DSK was purified from cyanogen bromide (CNBr) treated human fibrinogen by gel filtration and counter current distribution [9]. Thrombin (EC 3.4.4.13) was prepared as earlier described [10]. The final preparation had an activity of 368 NIH units per mg. Reptilase R was obtained from Pentapharm AG, Basel. The final preparation had an activity equivalent to 95 NIH (thrombin) units/mg. Arvin was obtained in solution from Twyford Laboratories, London. The activity was found to be equivalent to 670 NIH (thrombin) units/ml. Digestions with the enzymes were performed at pH 6.5 and 8.5 for 2–4 hr at 37° [4]. The enzyme concentration was from 6–30 NIH units and the substrate concentration 5–10 mg per ml of final mixture. The digests were freeze-dried.

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Low-voltage electrophoresis and fingerprint analysis on cellulose plates were performed as described earlier [4]. Gel filtration, chromatography on a Technicon Automatic Peptide Analyzer and amino acid analyses were performed as described [5, 11].

3. Results

Fig. 1 shows that all three enzymes released fibrinopeptides A and Ap from DSK and the α("A")-chain-fragment of DSK. Thrombin and Reptilase in addition released the Gly–Pro–Arg peptide which gives a yellow colour with ninhydrin. Arvin however, did not release this peptide. Instead another yellow peptide appeared. This peptide was isolated by preparative electrophoresis and by gel filtration and chromatography [5, 11]. Amino acid analysis gave the following residues: Glu 1.05, Pro 0.95, Gly 1.00, Val 1.77, Arg 1.98. The results suggested that a peptide containing the residues No. 17 to 23 of the α"A"-chain-fragment had been released by Arvin. Amino acid sequence with the manual PITC method of Edman [12] gave the expected sequence: Gly–Pro–Arg–Val–Val–Glu–Arg.

These results indicate that Arvin and Reptilase have different specifities.

The electrophoresis and fingerprint analysis also suggested that at least small amounts of B-peptide are indeed released by the venom enzymes when purified DSK and β(B)-chain-fragment prepared from DSK are used as substrate. From Arvin and Reptilase digests of DSK not only B-peptide but also small amounts of a longer fragment was isolated by gel filtration and chromatography. This fragment, by amino acid analysis, was shown to be identical with the first 42 residues of the β(B)-chain-fragment of the DSK [13]. Also other fragments are released, at least by Arvin, but not yet identified.

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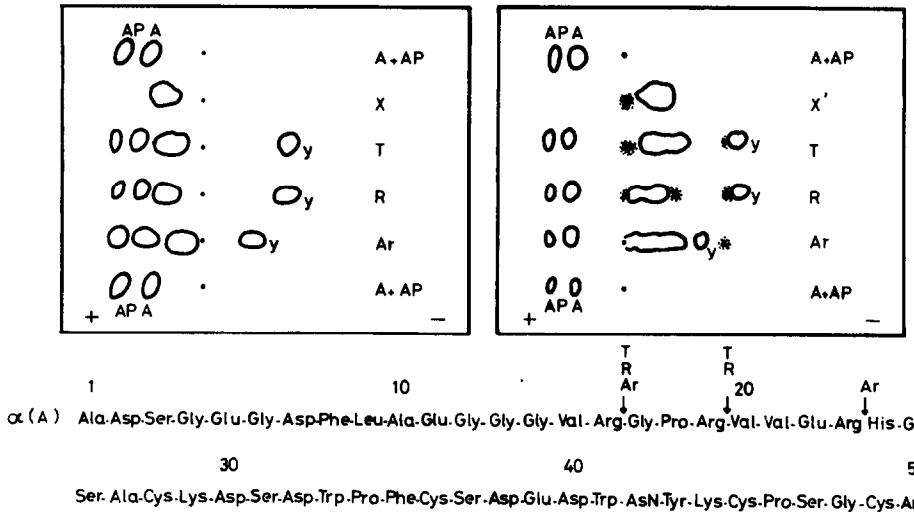

Fig. 1. Electrophoresis at pH 5.5 of thrombin, Reptilase and Arvin digests of α"A"-chain-fragment and DSK. Top left: substrate: α"A"-chain-fragment. Top right: substrate: DSK. X = undigested α"A"-chain-fragment; X^1 = undigested DSK. T = thrombin; R = Reptilase; Ar = Arvin. A = A-peptide [3]; AP = AP-peptide [3]. These were used as references. Spots were developed with ninhydrin spray. y = yellow spot. Below: Sequence of α"A"-chain-fragment of DSK from human fibrinogen [11].

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