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Short Communications

On the Thrombin-Fibrinogen Reaction in Different Species

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The fibrinogen molecule is built up by three pairs of peptide chains [1,2] two of which are proteolytically attacked by thrombin. During this cleavage two peptides named A and B are released from the N-terminal end of parent molecule.[3-6] The amino acid sequences of these peptides vary considerable between different species. However, certain regions of the fibrinopeptides have changed less than others in the course of mammalian evolution. This is particularly true with respect to the C-terminal region of the A-peptide.[7-9] Peptide A is released at a higher initial rate than peptide B in several mammalian species (man, ox, horse, and rabbit).[5,6,10,11] The release of this peptide seems to initiate fibrin formation.[6]

It is well known that the interaction between thrombin and fibrinogen shows species specificity.[12-17] It is possible that differences in the structure of fibrinopeptide A might reflect this species specificity in the rate of reaction between different fibrinogens and thrombins. It has recently been found [18] that the "fibrinopeptide A" in lamprey eel fibrinogen was not split off by mammalian thrombin. On the other hand lamprey eel thrombin could easily produce this cleavage. The amino acid sequence of the lamprey eel peptide A [18] was strikingly different from mammalian fibrinopeptides.

Data on the thrombin-fibrinogen reaction rate correlated to structural features in fibrino-peptides might give information on possible specific requirements for thrombin action in these structures. Such data are contained in this report.

Fibrinogen, (fraction I-4) was prepared as described earlier.[19] The fibrinogens were dissolved in 0.275 M NaCl, containing 0.025 M Tris (tris(hydroxymethyl)aminomethane), pH 7.2 and dialyzed against that buffer. The protein concentrations varied between 10 and 16 mg/ml and the coagulability between 91 and 97 %. The fibrinogen solutions were diluted to a protein concentration of 8 mg/ml with Tris-NaCl buffer and then with an equal volume of distilled water.

Bovine thrombin (about 200 NIH * units/mg) was prepared as described elsewhere.[20,1] From the other species thrombin was prepared from fraction II + III (Cohn's method 6 [21]) by adsorption on $BaSO_4$ followed by elution with 0.18 M sodium citrate. The eluate was dialyzed against Tris buffer, pH 7.2, ionic strength 0.1. The prothrombin was transformed to thrombin by addition of calcium ions, homologous brain thromboplastin [22] (man, dog, rabbit) or a crude phospholipid fraction ** (horse) and the supernatant after adsorption of fraction II + III on $BaSO_4$ (factor V source), all reagents at optimal concentration. The thrombins were further purified by fractionation [1] with methanol. The specific activity of the different thrombins varied between 6 and 46 NIH units/mg. The different thrombins were assayed on bovine fibrinogen against bovine thrombin as standard and the activity expressed in NIH-units. The thrombin was dissolved and diluted to a concentration of about 1 NIH-unit/ml in a solvent of the following composition: 0.015 M calcium chloride, 0.019 M sodium chloride, 0.016 M trisodium citrate, and 0.056 M imidazole, pH 7.2. The thrombin solutions were kept on ice in siliconized test tubes during the experiment. The clotting

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** "Inositin lipid" from Associated concentrates, Inc., N. Y.

Table 1. Relative thrombin activity when different thrombins and fibrinogens are cross-reacted.
(NIH units obtained in the reaction between bovine fibrinogen and bovine thrombin was arbitrarily chosen as 100)

		FIBRINOGEN				
		Ox	Man	Horse	Dog	Rabbit
THROMBIN	Ox	100	114	61	141	142
	Man	106	119	64	164	166
	Horse	109	100	71	130	134
	Dog	118	127	80	196	217
	Rabbit	110	124	60	127	132

procedure was as follows: 0.2 ml of thrombin solution was added to 0.2 ml of fibrinogen solution, at 37°, and the clotting times determined. They varied between 8 and 25 sec. The clotting times of any particular combination was expressed as thrombin units by interpolation on the bovine thrombin-fibrinogen standard curve. Dilution curves of the different thrombins were parallel with the bovine standard curve.

As can be seen from Table 1 there was no greater difference in activity of the different thrombins when reacted with ox, human, and horse fibrinogen. Human and particularly dog thrombin showed a somewhat higher activity when reacted with dog and rabbit fibrinogen.

Fibrinogens from ox and man showed roughly the same reactivity towards all thrombins whereas dog and rabbit fibrinogens showed a higher reactivity. Horse fibrinogen reacted slower with all thrombins. The latter findings are essentially in keeping with those reported by Stormorken.[15] However, in the present study no definite "species specificity" of thrombin could be observed.

The present results have been interpreted on the basis of fibrinopeptide A structure (Fig. 1). As mentioned above the release of the A-peptide seems to initiate the fibrin formation, whereas peptide B is released much slower. Furthermore, a considerable part of the C-terminal part of peptide A has been preserved during vertebrate evolution and seems therefore to have been subjected to natural selection. It is therefore possible that a unique amino acid sequence in the C-terminal part of the fibrinopeptide A is a part of the specific requirements for the action of mammalian thrombins. From what is known about preservation of amino acid residues in fibrinopeptides during mammalian evolution,[7,9,23] this unique sequence would be expected to be represented by the arginine at position 1, the Glu-Gly sequence at positions 6—5 and the phenylalanine at position 9 from the C-terminal end. The leucine or isoleucine at position 8 might also be included in this specific sequence. The present results are not contradictory to such an interpretation of the phylogenetic findings. Furthermore, they give experimental evidence that many of the mutations, which do have occurred in the C-terminal nonapeptide of fibrinopeptide A apparently have not to any greater extent effected the thrombin fibrinogen interaction. However, an occurrence of a histidine residue in position 7 as in horse

19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

Ox H-Glu-Asp-Gly-Ser-Asp-Pro-Pro-Ser-Gly-Asp-Phe-Leu-Thr-Glu-Gly-Gly-Gly-Val-Arg-OH

Man H-Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-Arg-OH

Horse H-Thr-Glu-Glu-Gly-Glu-Phe-Leu-His-Glu-Gly-Gly-Gly-Val-Arg-OH

Dog H-Thr-AsN-Ser-Lys-Glu-Gly-Glu-Phe-Ilu-Ala-Glu-Gly-Gly-Gly-Val-Arg-OH

Rabbit H-Val-Asp-Pro-Gly-Glu-Ser-Thr-Phe-Ilu-Asp-Glu-Gly-Ala-Thr-Gly-Arg-OH

Fig. 1. Amino acid sequence of fibrinopeptide A from ox, man, horse, dog, and rabbit (*cf.* Ref. 9).

1. In a previous report[8] the sequence for rabbit fibrinopeptide A was erroneous at positions 2, 3, 4, 7, and 8.
2. In human and dog fibrinogen some of the serine residues at position 14 are phosphorylated (*cf.* Ref. 7).
3. The bond cleaved by thrombin is an arginylglycine linkage.
4. AsN = asparaginyl.

fibrinogen might be less favourable for this reaction. It should also be pointed out that a change from isoleucine to leucine at position 8 (compare rabbit and dog with the other species) might also effect the reaction in question.

The present preliminary investigation does not give any information on other possible functional areas in the fibrinogen molecule beyond the arginyl-glycine bond split by thrombin.

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Structure of Thiuret Hydrochloride Hemihydrate

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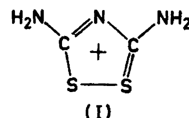
The unsaturated five-membered disulphide ring of the thiuret ion (I) possesses a sextet of π-electrons and is pseudoaromatic. However, the sulphur-sulphur



bond length 2.088 ± 0.012 Å, reported by Foss and Tjomsland [1] from a crystal structure analysis of thiuret hydroiodide, indicates that the ring conjugation does not extend over the sulphur-sulphur bond. Support for this idea derives from a structure investigation of 3,5-diamino-1,2-dithiolium iodide.[2] For this compound, which is isomorphous with and analogous to thiuret hydroiodide, a sulphur-sulphur bond length of 2.08 ± 0.02 Å has been found.[2] Molecular orbital calculations [3] show that for the sulphur-sulphur bond in the unsubstituted, 1,2-dithiolium ion a bond length of about 2.00 Å should be expected; this agrees with the found sulphur-sulphur bond in some phenyl-substituted 1,2-dithiolium salts.[4,5] A reason why the sulphur-sulphur bond is longer in thiuret hydroiodide and 3,5-diamino-1,2-dithiolium iodide than in the unsubstituted 1,2-dithiolium ion, may be the effect of the amino groups on the ring conjugation.[2] Attention should, however, also be paid to the environment of the disulphide group. There are in thiuret hydroiodide, and consequently also in the isomorphous 3,5-diamino-1,2-dithiolium iodide, close contacts between the iodide ion and the sulphur atoms. Those of the close contacts which occur in the approximately linear arrangement