AMINO ACID SEQUENCE OF HUMAN THROMBIN A CHAIN

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SUMMARY

The A chain isolated from biologically active human thrombin is thirteen residues shorter from the amino terminus than the A chain isolated from bovine thrombin. Automated Edman degradation of the reduced, alkylated A chain permitted the direct identification of 34 out of the 36 residues and established all tryptic overlaps. The NH_2-terminal residue is threonine. This residue is homologous to threonine-14 of the A chain of bovine thrombin. Human thrombin A chain has one half cystine linking it to the larger B chain. Methionine, histidine, valine and tryptophan are not present. There are nine acid residues (Glu, Asp) but none of their respective amides.

INTRODUCTION

The generation of biologically active thrombin (E.C. 3.4.4.13) from prothrombin proceeds through a series of activation steps which have been well defined for bovine prothrombin (1-5). The activation of commercially available human prothrombin complex has been examined (6). An altered human zymogen has been isolated (7) which has properties similar to bovine prethrombin (1,5) or intermediate 1 (3,4). The final step in the development of biologically active bovine thrombin is the hydrolysis of an arginine-isoleucine peptide bond by autoprothrombin C (Factor Xa) (3,5) and this peptide linkage has been established as the bond joining the COOH-terminus of the A chain to the NH_2-terminus of the B chain (8).

Human thrombin has been prepared on a large scale (9) and its properties are similar to bovine thrombin. Lanchantin et al. (10,11) have reported on the properties of human prothrombin and its conversion to thrombin in 25% sodium citrate. They have also given evidence (12) that two forms of human thrombin have been isolated. Multiple forms of human thrombin have also been reported elsewhere (13).

These forms of human thrombin apparently are similar in nature to the various forms of bovine thrombin already known (5,14-16). In the bovine species the first enzyme to be derived from prothrombin had only esterase activity. This was called prethrombin-E (5). By cleavage of a single Arg-Ile bond, the classical thrombin activity arises, and then by autolysis and loss of the B1 chain, only esterase activity remains. The latter is called thrombin-E (5). It may be that these three enzymes can also be obtained from human prothrombin and that there is really only one form of classical thrombin.

The purpose of this communication is to report on the amino acid sequence of the A chain of human thrombin. It contains 36 amino acid residues as compared with 49 for the corresponding chain from bovine thrombin (17).

MATERIALS AND METHODS

Human prothrombin complex (Cutter Laboratories, Berkeley, California), whose properties were studied extensively (6), was dissolved in distilled water and dialyzed in the cold (4° C) overnight. The final product, 15,000 units/ml, was converted to thrombin using purified coagulation factors in a five-component activating mixture (18). The thrombin solution was chromatographed on IRC-50 (19) and the purified thrombin was dialyzed and freeze-dried at pH 8.2.

Human thrombin (300 mg) was dissolved in 8 M urea and reduced and alkylated (20) using tritium labeled iodoacetic acid and the excess salts were removed by filtration over Sephadex G-10 in 50% acetic acid. The acetic acid was removed by rotary evaporation and the sample was then freeze-dried. The S-CM-thrombin was dissolved in 50% acetic acid and passed over a G-100 column (2.5 x 91 cm; flow rate 7 ml/hr) equilibrated in 50% acetic acid (14). The last peak eluted was the S-CM-A chain and it was refiltered on a G-50 column equilibrated in 0.1 M NH_4HCO_3 pH 7.8.

The alkylated A chain was digested with trypsin for 6 hours at 37° C at an enzyme substrate ratio of 1:50 (w/w). The tryptic fragments were purified by preparative electrophoresis or preparative thin-layer chromatography. The amino

TABLE I

AMINO ACID SEQUENCES OF HUMAN AND BOVINE THROMBIN A CHAINS

HUMAN: Sequence Absent

BOVINE:* THR-SER-GLU-ASN-HIS-PHE-GLU-PRO-PHE-PHE-ASN-GLU-LYS-

1 ↓

HUMAN: THR-PHE-GLY-SER-GLY-GLU-ALA-ASP-CYS-GLY-LEU-ARG-

BOVINE: THR-PHE-GLY-ALA-GLY-GLU-ALA-ASP-CYS-GLY-LEU-ARG-

2 ↓ 3 ↓ 4 ↓

HUMAN: PRO-LEU-PHE-GLU-LYS-LYS-SER-LEU-GLU-ASP-LYS-THR-

BOVINE: PRO-LEU-PHE-GLU-LYS-LYS-GLN-VAL-GLN-ASP-GLU-THR-

5 ↓ 6

HUMAN: GLU-LYS-GLU-LEU-LEU-GLU-SER-TYR-ILE-ASP-GLY-ARG-

BOVINE: GLN-LYS-GLU-LEU-PHE-GLU-SER-TYR-ILE-GLU-GLY-ARG-

*Bovine sequence from Magnusson (17). Arrows indicate tryptic cleavage points for human chain only. Numbers refer to order of tryptic fragments used for amino acid analyses. Underlined residues are points of mutation. Only one of these involves more than one base change; namely, residue number 32, GLN to SER.

acid compositions of reduced, alkylated A chain, as well as the purified tryptic fragments, were determined using a Beckman 121M analyzer (21).

The S-CM-A chain was degraded with the use of a Beckman 890C sequencer using a modified DMAA peptide program. PTH derivatives were identified using gas chromatography (22) and a color-coded thin-layer chromatography technique (23).

RESULTS AND DISCUSSION

The amino acid sequence of the A chain of human thrombin is shown in Table I. The sequence of the bovine chain (17) is also presented for comparative purposes. It is most striking to note that there are thirteen residues missing from the NH_2-terminus of the human chain. In addition, there are eight residues which differ from the bovine. All but one of these differences represents a single

TABLE II

AMINO ACID COMPOSITION OF HUMAN THROMBIN A CHAIN AND ITS TRYPTIC FRAGMENTS

	S-CM-A Chain	TRY-1	TRY-2	TRY-3	TRY-4	TRY-5	TRY-6
Aspartic	2.91 (3)	1.13(1)			0.92(1)		0.97(1)
Threonine*	1.90 (2)	0.89(1)				0.90(1)	
Serine*	2.64 (3)	0.76(1)			0.72(1)		0.88(1)
Glutamic**	5.42 (6)	1.00(1)	1.00(1)		1.00(1)	1.00(1)	2.00(2)
Proline	1.09+(1)		0.87(1)				
Glycine	3.55 (4)	3.16(3)					1.35(1)
Alanine	1.07+(1)	0.85(1)					
CM-Cysteine	0.89+(1)	0.92(1)					
Valine							
Methionine							
Isoleucine	1.08+(1)						0.76(1)
Leucine	4.23 (5)	1.16(1)	0.91(1)		0.73(1)		1.77(2)
Tyrosine	0.93 (1)						0.88(1)
Phenylalanine	1.68 (2)	1.06(1)	1.12(1)				
Lysine	3.47 (4)		0.86(1)	1.00(1)	0.76(1)	0.82(1)	
Histidine							
Arginine	2.11 (2)	1.09(1)					0.94(1)
TOTALS	36	12	5	1	5	3	10

+ Average amino acid residue value used as integer.

* Threonine and serine values extrapolated from 72 hours hydrolysis.

** Glutamic acid used as integer for the tryptic fragments.

Numbers in parentheses represent nearest integer.

base change for the mutation to have occurred. One of the changes (bovine Glx-36 to human Lys-23) results in the formation of an additional tryptic fragment in the human chain.

The amino acid compositions of the reduced, alkylated human A chain and the tryptic fragments are given in Table II. The amino acid differences between the human and bovine chains (residues 14-49 only) are minor. There is an increase

(by 1) in basic residues in the human chain but a decrease (by 2) in the acidic residues. The human chain does not contain glutamine or asparagine so the net charge of the bovine and human chains are similar.

As compared with bovine prothrombin, there is evidently a difference in the activation characteristics of human prothrombin. The final generation of bovine thrombin results in the removal of a 109 amino acid chain whose sequence is already known (24). Our preliminary results for the amino acid sequence of human O fragment indicate that it does not contain an extra thirteen amino acids at its COOH-terminus. The possibilities for these residues are (a) they are genetically missing in the biosynthesis of human prothrombin or (b) they are removed during the activation of human prothrombin by the five-component system or as a result of autolysis based on the predicted proteolytic specificity of thrombin (25). We favor the latter interpretation.

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REFERENCES

1. Marciniak, E., and Seegers, W.H. (1966) Nature 209, 621-622.
2. Aronson, D.L., and Menache, D. (1966) Biochemistry 5, 2635-2640.
3. Heldebrant, C.M., and Mann, K.G. (1973) J. Biol. Chem. 248, 3642-3652.
4. Owen, W.G., Esmon, C.T., and Jackson, C.M. (1974) J. Biol. Chem. 249, 594-605.
5. Seegers, W.H., Walz, D.A., Reuterby, J., and McCoy, L.E. (1974) Thrombosis Res. 4, 829-859.
6. Seegers, W.H., Sakuragawa, N., and McCoy, L.E. (1972) Thrombosis Res. 1, 33-46.
7. Tishkoff, G.H., Williams, L.C., and Brown, D.H. (1971) Thromb. Diath. Haemorrh. 24, 325-333.
8. McCoy, L.E., Walz, D.A., and Seegers, W.H. (1973) Thrombosis Res. 3, 357-361.
9. Fenton, J.W., Campbell, W.P., Harrington, J.C., and Miller, K.D. (1971) Biochim. Biophys. Acta 229, 26-32.
10. Lanchantin, G.F., Friedman, J.A., and Hart, D.W. (1965) J. Biol. Chem. 240, 3276-3282.
11. Lanchantin, G.F., Friedman, J.A., and Hart, D.W. (1967) J. Biol. Chem. 242, 2491-2501.
12. Lanchantin, G.F., Friedman, J.A., and Hart, D.W. (1973) J. Biol. Chem. 248, 5956-5966.

13. Gorman, J.J., and Castaldi, P.A. (1974) Thrombosis Res. 4, 653-673.
14. Seegers, W.H., Reuterby, J., Murano, G., McCoy, L.E., and Agrawal, B.B.L. (1971) Thromb. Diath. Haemorrh. Suppl. 47, 325-337.
15. Mann, K.G., and Batt, C.W. (1969) J. Biol. Chem. 244, 6555-6557.
16. Kingdon, H.S., Vogel, C.N., Uhteg, L.C., and Lundblad, R.L. (1974) Fed. Proc. 33, 1389 (abst. 938).
17. Magnusson, S. (1970) Thromb. Diath. Haemorrh. Suppl. 38, 97-104.
18. Baker, W.J., and Seegers, W.H. (1967) Thromb. Diath. Haemorrh. 17, 205-213.
19. Seegers, W.H., McCoy, L.E., Kipfer, R.K., and Murano, G. (1968) Arch. Biochem. 128, 194-201.
20. Hirs, C.H.W. (1967) Methods in Enzymology, Vol. XI, Hirs, C.H.W. (Ed.), Academic Press, New York.
21. Moore, S., and Stein, W.H. (1954) J. Biol. Chem. 211, 893-906.
22. Pisano, J.J., and Bronzert, T.J. (1969) J. Biol. Chem. 244, 5597-5607.
23. Walz, D.A., and Reuterby, J. (in preparation).
24. Reuterby, J., Walz, D.A., McCoy, L.E., and Seegers, W.H. (1974) Thrombosis Res. 4, 885-890.
25. Walz, D.A., Seegers, W.H., Reuterby, J., and McCoy, L.E. (1974) Thrombosis Res. 4, 713-717.