EVOLUTIONARY CONSTANCY OF PRIMARY STRUCTURE IN AN α(A)-CHAIN FRAGMENT OF FIBRINOGEN

T.SÖDERQVIST and B.BLOMBÄCK

Dept. of Blood Coagulation Research, Karolinska Institut, Stockholm

Received 21 January 1970
Revised version received 11 March 1970

1. Introduction

The α(A)-chain of fibrinogen from several animal species seems to contain, in its *N*-terminal portion, the structural features responsible for the narrow substrate specificity of thrombin when acting on fibrinogen during blood clotting. During this process, fibrinopeptide A is split off from the *N*-terminal end of the α(A)-chain. The rate of surviving mutations in fibrinopeptide A has been estimated as 90 PAMs in 100 m.y. [1]. The most pronounced evolutionary changes are restricted to the *N*-terminal region of the peptide. The *C*-terminal region, on the other hand, which is close to the thrombin vulnerable arginyl-glycine bond, has been fairly constant during mammalian evolution [2]. This paper gives evidence for further sequence isologies in the α(A)-chain of different species.

2. Methods

Fibrinogen from sheep, ox, rabbit and horse plasma was prepared [3] and treated with CNBr in formic acid [4]. The fragments were separated on Sephadex G-100. The K_{av}-value of fraction 2 from all species corresponds to that of the *N*-terminal "disulfide knot" of human fibrinogen [4]. Thrombic digestion (30 NIH units/ml) of fraction 2 [4] released fibrinopeptides A and B. In all four species a second anodic yellow spot was found as well. *N*-Terminal α(A)-, β(B)- and γ-chains of the disulfide knot were prepared by reduction with dithiothreitol [5] (4 moles/mole disulfide [6]), alkylated with iodoacetic acid-^{3}H (4 μCi/μmole; 8 moles/mole DTT), and subsequently separated on Sephadex G-100. Fractions yielding fibrinopeptide A and the anodic yellow spot on digestion with thrombin were refiltered on Sephadex G-50. This procedure yields an essentially pure α(A)-chain fragment [4] as judged by electrophoresis. The fragment was digested with trypsin and the tryptic peptides separated by two-dimensional thin-layer electrophoresis (pH 5.5 and 6.4) - chromatography [7]. The plates were stained with the ninhydrin reagent and reagents for arginine, tyrosine, tryptophan and histidine. The ninhydrin positive spots were in addition eluted and assayed for radioactivity. Human α(A)-chain served as reference in our studies. Fingerprint analysis was therefore also performed on samples to which tryptic digest of the human α(A)-chain fragment had been added. Peptides overlapping with the human peptides and showing the same color reaction were considered to have identical or very similar sequence (isologous [8]).

3. Results

The fingerprint patterns of human, sheep, ox, rabbit and horse α(A)-chain fragments are shown in fig. 1. In all species except the rabbit there are present seven main tryptic peptides. In the rabbit α(A)-chain there seems to be an additional trypsin-susceptible bond since eight peptides are discernible. Three spots, TRY-2, TRY-6 and TRY-7 have the same location and color reactions in all species. TRY-2 corresponds to the tripeptide Gly-Pro-Arg (residue 17–19) in the human α(A)-chain (fig. 2). TRY-6 corresponds to the carboxy methyl cysteinylpeptide (residue 45–50) and TRY-7 to homoserine (residue 51) in the human se-

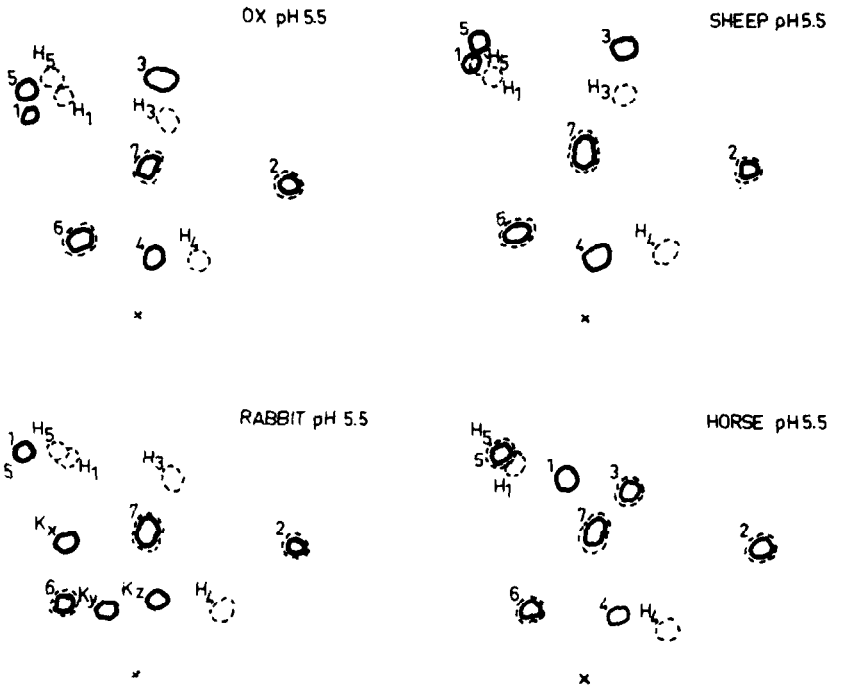

Fig. 1. "Fingerprint" patterns of ox, sheep, rabbit and horse α(A)-chain fragments, 40–70 μg of animal fragments mixed with equal amounts of human α(A)-chain fragment and digested with trypsin (enzyme: substrate 1 : 50) for 4 hr. Tryptic peptides were separated by electrophoresis on thin-layer plates coated with 0.5 mm cellulose (300 V and 15 mA in 1 hr 15 min; pH 5.5 or 6.4) and by ascending chromatography, *n*-BuOH: pyridine: HAc: H_2O (15 : 10 : 3 : 12 v/v), for 3½ hr. Species-specific spots in full circles, numbers refer to respective tryptic peptides. K_X, K_Y, K_Z: unidentified rabbit peptides. Human spots are in broken circles.

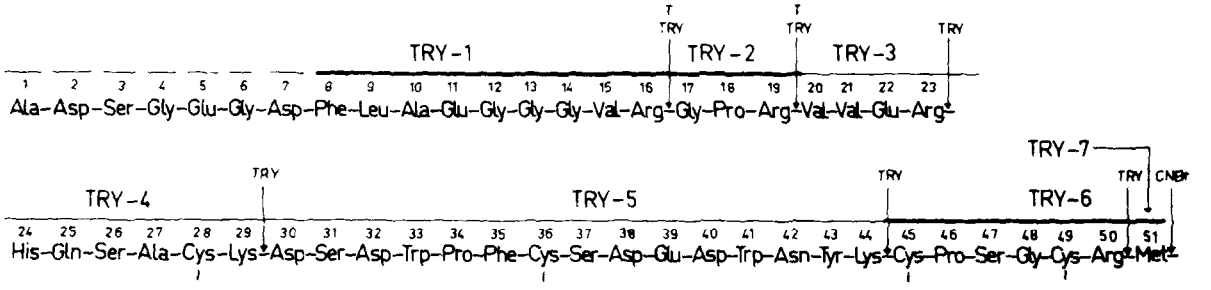

Fig. 2. Tentative sequence homology of tryptic peptides from α(A)-chain fragments [9]. The arrows indicate cleavage sites with thrombin (T) and trypsin (TRY). Bold-face lines indicate regions with low substitution rate and broken lines regions with high substitution rate.

quence. Fibrinopeptides A are in all species acidic at pH 5.5 and have about the same R_f-values. Peptide 2 gave in all species a bright yellow color with ninhydrin. Peptide 6 gave a greyish-yellow color and contained as judged from the radioactivity of the spot two Cys/2 residues when compared to peptides 5, 4 and K_x. In digests of horse α(A)-chain, the Sakaguchi positive peptide 3 overlapped human TRY-3 (residues 20–23). In ox and sheep but not in rabbit, a peptide of only a slightly different position was observed. This peptide is therefore most likely isologous to human TRY-3.

All digests furthermore contained a peptide that gave positive color reactions for tyrosine and/or tryptophan. It was in this respect similar to human TRY-5 and had roughly the same electrophoretic mobility and R_f-value. As judged by radioactivity, it seemed in all species to contain one Cys/2 residue. Peptide 5 is therefore probably isologous to human TRY-5.

In ox, sheep and horse, peptide 4 (containing one Cys/2) is probably isologous to human TRY-4 though it does not give a positive His-reaction. It is premature to assign isologies to the peptides K_x, K_y and K_z of rabbit. These are most likely derived from a segment, corresponding to the sequence Val_{20}-Lys_{29} in human α(A)-chain.

4. Discussion

The fingerprint patterns from the five species investigated give a tentative picture of the evolution of the *N*-terminal region of the α(A)-chain in fibrinogen. At least two slowly changing regions are found: The sequence from Phe_8 to Arg_{19} and the sequence from Cys_{45} to Met_{51}. It is of particular interest that Phe_8 and the Arg_{16}-Gly_{17}-Pro_{18}-Arg_{19} sequence around the thrombin-susceptible Arg_{16}-Gly_{17}-bond has not changed since the earliest common divergence of the lagomorph, primate, perissodactyl and artiodactyl stocks. This stability may account for the narrow specificity of thrombin, which out of a large number of available arginyl bonds splits at any appreciable rate only two in the α(A)-chain and one in the β(B)-chain. The constancy of the peptide segment Cys_{45}-Met_{51} suggests that the covalent structure as maintained by the disulfide bridges, likewise has been of importance for the function of fibrinogen throughout mammalian evolution. Our preliminary results suggest that the conformation around the split-point during evolution may have been balanced by compensatory substitutions. For example only rabbit exhibits a profound change in the constant region of fibrinopeptide A [2], and has also a change in the fingerprint pattern of the tryptic peptides (TRY-X, TRY-Y and TRY-Z) on the other side of the bond split.

Acknowledgements

This work was supported by a grant from the U.S. National Institutes of Health, S-RO1-HE07379-06.

References

[1] M.O.Dayhoff, Atlas of Protein Sequence and Structure (National Biomedical Research Foundation, Silver Spring, Md. 1969) Vol. 4.
[2] B.Blombäck and M.Blombäck, Primary structure of animal proteins as a guide in taxonomic studies, in: Chemotaxonomy and Serotaxonomy, ed. J.G.Hawkes (Academic Press, London, 1968) p. 3.
[3] B.Blombäck and M.Blombäck, Arkiv Kemi 10 (1956) 415.
[4] B.Blombäck, M.Blombäck, A.Henschen, B.Hessel, S.Iwanaga and K.R.Woods, Nature 218 (1968) 130.
[5] W.W.Cleland, Biochemistry 3 (1964) 480.
[6] W.L.Zahler and W.W.Cleland, J. Biol. Chem. 243 (1968) 716.
[7] M.Blombäck, B.Blombäck, E.F.Mammen and A.S.Prasad, Nature 218 (1968) 134.
[8] M.Florkin, A Molecular Approach to Phylogeny (Elsevier, Amsterdam, 1966) p. 6.
[9] B.Blombäck, Brit. J. Haematol. 17 (1969) 145.