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Some comparative aspects of the fibrinogen–fibrin conversion

Species-specific differences have been demonstrated to exist among the N-terminal amino acids of mammalian fibrinogens[1–3]. The variability appears to be confined to the end-groups which disappear from the parent protein following thrombin action during clotting. The new N-terminal residues of the resulting fibrins are those of glycine, suggesting that the conversion of all mammalian fibrinogens is a function of the cleavage of X–glycine bonds. The known preferential specificity of several mammalian thrombins (human, bovine, and rabbit) for arginyl esters[4], coupled with the positive identification of arginine in the C-terminal position of human[5] and bovine[6] fibrinopeptides, further suggests that the clotting of fibrinogen is generally initiated by the hydrolytic cleavage of an arginyl–glycine peptide bond. Another consistency among the mammalian fibrinogens has been the detection of a pair of N-terminal tyrosines in both the fibrinogen and fibrin of 8 mammalian species examined. These relationships are summarized in Table I.

TABLE I

PREVIOUSLY REPORTED N-TERMINAL RESIDUES OF MAMMALIAN FIBRINOGENS AND FIBRINS

	Ox	*Human*	*Pig*[2]	*Sheep and goat*[2]	*Horse and dog*[2]	*Rabbit*[2]
Fibrinogen	Glu[98] NAT-Ala[11]	Ala[1]	Ala	Ala Gly	Thr	Val Ala
	Tyr[88]	Tyr[1]	Tyr	Tyr	Tyr	Tyr
Fibrin**	Gly[98]	Gly[1]	Gly	Gly	Gly	Gly
	Tyr[88]	Tyr[1]	Tyr	Tyr	Tyr	Tyr
Clot liquor	Glu[98] NAT-Ala[11]	Ala[1,5]	Ala	Ala Gly	—***	Val Ala

* NAT, *N*-acetyl threonine.

** Human fibrin obtained by clotting human fibrinogen with human thrombin; all other fibrinogens clotted with bovine thrombin.

*** Clot liquors of horse and dog not examined for end groups.

Lamprey-eel (*Petromyzon marinus*) fibrinogen is similar in its physico-chemical properties to mammalian fibrinogens and can be clotted by mammalian thrombins[7]. At least one negatively charged fibrinopeptide is cleaved from the molecule during its conversion to fibrin. The present study was undertaken in order to gain further details concerning the conversion of fibrinogen derived from this primitive vertebrate.

Purification of the lamprey-eel fibrinogen was carried out as described previously[7]. The preparations, containing 85–92 % clottable proteins, were divided into two portions, one of which was converted into fibrin. In three of these experiments clotting was effected using lamprey-eel thrombin; in a fourth, human thrombin was used.

The fibrinogens and fibrins were treated with Sanger's reagent (1-fluoro-2,4-dinitrobenzene) as described earlier[8]. After acid hydrolyses of 8 and 24 h, the ether-soluble α-DNP-amino acids were quantitatively determined following separation on two-dimensional paper chromatograms using the solvent systems of toluene–pyridine–chloroethanol–ammonium hydroxide and 1.5 M phosphate buffer[9]. DNP-

aspartic acid was distinguished from DNP-glutamic acid in a one-dimensional run with *tert.*-amyl alcohol–phthalate[9]. Suitable corrections were applied for hydrolytic losses[10]. For estimating the moles of DNP-amino acids per 340000 unit of protein, it was assumed that the actual protein contents were equal to 80 % of the dry weights of the DNP-proteins.

In all cases, the positions of the major ether-soluble DNP-amino acids derived from lamprey-eel fibrinogen corresponded to those of aspartic acid and serine. Similarly, lamprey fibrin, produced by the action of either lamprey or human thrombin, gave mainly N-terminal glycines and serines. The quantitative values for DNP-aspartic acid and DNP-glycine were quite consistent, but the DNP-serine contents of the preparations varied considerably. Occasionally, one or two anomalous spots appeared on the chromatograms of the 8-h hydrolysates, but since these disappeared after 24 h hydrolysis, it was assumed that they represented incompletely hydrolyzed DNP-peptides. The estimated moles of DNP-amino acids per 340000 unit of protein are reported in Table II. In nearest whole numbers, these data describe a fibrinogen unit containing 2 N-terminal aspartic acids and 1 or more serines. The fibrin apparently has 4 N-terminal glycines and 1 or more serines. The disappearance of the N-terminal aspartic acid is typical of the release of the fibrinopeptide material during clotting and of its DNP- derivative being removed from the system during the washing of the DNP-fibrin[8].

TABLE II

MAJOR N-TERMINAL AMINO ACIDS OF LAMPREY-EEL FIBRINOGEN AND FIBRIN

Estimated moles per 340000 unit protein. The calculations presume that the actual protein content is equal to 80 % of the dry DNP-protein. The numbers in parentheses indicate what percentage of the DNP-amino acids has been presumed to remain after hydrolysis. Tr = traces.

*Preparation**		*Glycine* 8 h (36 %)	*Aspartic acid* 8 h (82 %)	*Aspartic acid* 24 h (58 %)	*Serine* 8 h (86 %)	*Serine* 24 h (70 %)
I	Fibrinogen	Tr	1.6	2.0	0.5	0.8
	Fibrin	2.9	Tr	Tr	0.5	0.8
II	Fibrinogen	Tr	1.7	2.1	0.6	0.9
	Fibrin	3.5	Tr	Tr	1.4	1.7
III	Fibrinogen**	0.5	1.7	2.2	3.4	4.0
	Fibrin**	3.5	Tr	0	1.9	2.4
IV	Fibrinogen	0.1	1.4	1.3	0 6	—
	Fibrin	1.8	0.8	0.4	0.6	—

* Preparations I, II, and III were clotted with lamprey-eel thrombin. Preparation IV was clotted with human thrombin.

** These preparations were treated with 5 mM urea and then exhaustively dialyzed against distilled water before the treatment with FDNB[8] was undertaken.

These data are compatible with the assumption that an X-glycine bond is cleaved in the clotting of lamprey-eel fibrinogen. That this is an arginyl–glycine bond may be inferred from the known preferential specificities of the two thrombins used[7]. It is likely that the splitting of an Arg–Gly link is a characteristic event in fibrinogen conversion for all vertebrates, signifying a considerable restraint in the evolution of this region of the fibrinogen molecule.

The N-terminal serine appears to be a part of that peptide chain which in mammals ends in tyrosine. In light of the extreme variability of the fibrinopeptide N-terminals (Table I), the conserved mammalian tyrosine end group—common to both fibrinogen and fibrin—may have a specific function which results in its being selected for during evolution. It will be interesting to find where among the vertebrates the N-terminal tyrosine makes its first appearance.

We wish to thank Dr. W. LOVE for providing the lamprey-eel plasma. This work was aided at Amherst College by a grant from the Rockefeller Foundation and at Northwestern University by grant H-2212 of the National Heart Institute, U.S. National Institutes of Health.

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Received August 16th, 1962

Biochim. Biophys. Acta, 69 (1963) 161–163

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Steroids. CCXXIII*.
Color reagent for steroids in thin-layer chromatography**

Colorimetric reactions have proved valuable for quantitative and qualitative analysis. In the case of steroids which do not exhibit ultraviolet absorption, colorimetric reactions are indispensable for their location in chromatograms. Such reactions are also used as supporting evidence for structural groupings in steroids, although in general colorimetric reactions are not specific enough to be used as primary evidence.

The general, non-specific reagent described here uses vanillin in H_2SO_4–ethanol. Vanillin was used by FREREJAQUÉ[2] to detect digitalis glucosides on paper with formation of blue colors. MCALEER AND KOZLOWSKI[3,4] used vanillin in H_3PO_4 to detect sapogenins and 17-hydroxy-20-ketopregnanes on paper. The former produced various colors while the latter gave orange spots on a background of yellow. The latest reported use of vanillin for the detection of steroids employed this reagent in $HClO_4$ to detect pregnanetriols in paper chromatography[5].

The advent of thin-layer chromatography[6] has presented a medium in which

* See ref. 1 for steroids CCXXII.
** Contribution No. 283.