Technical Papers

Species Specificity of Fibrinogen as Revealed by End-Group Studies[1]

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The studies on bovine fibrinogen carried out during the past few years have established the principles involved in the clotting phenomenon (*1–6*). As has been known for some time, the actual gelation phase of clotting is preceded by the enzymic action of thrombin on fibrinogen. The altered fibrinogen is ready to aggregate into a clot whenever the ionic strength and the pH of the medium are suitable (*7*). The change that fibrinogen undergoes prior to aggregation has been characterized by a change in the N-terminal residues of the protein from glutamic acid to glycine (*2, 4*). Since after the action of thrombin α-amino groups of glycine are left on fibrin and since the enzyme was found to be specific for arginyl esters (*8*), it is possible to predict that bovine thrombin splits some arginyl-glycine bonds of fibrinogen. During the enzymic phase of the clotting reaction nonprotein nitrogen (ca. 3%) is liberated from fibrinogen in the form of fibrino-peptide (*3, 5*). Fibrino-peptide is an acidic unit of the fibrinogen molecule and represents highly charged centers at physiological pH (*6*). The removal of these centers by thrombin would eliminate some repelling charges from fibrinogen, and the discharged sites on the protein could serve as contact points for neighboring molecules. Once the fibrin particles are aligned in an oriented fashion, secondary forces would strengthen their association (*1*).

It is believed that the clotting of fibrinogen reveals a story that has a significance beyond the borders of blood coagulation itself, and it may be an indication of how certain principles are being utilized in building biological fibers. The mechanism represented by the fibrinogen-fibrin transformation may be a common pattern in the biogenesis of a number of proteins. The enzymically altered protein displays quite different properties from that of the primary one, although it differs very little from it in molecular and chemical constitution. In the case of fibrinogen, the altered protein acquires the property of spontaneous fiber formation through the loss of localized charges. The concept of such relatively minor change during clotting is in agreement with all experimental data so far.

The above picture of the clotting reaction was derived from experiments using bovine material. It was deemed of considerable importance to investigate whether species-specific differences could be demonstrated by studying human fibrinogen and its clotting by human thrombin. The present note reports on the N-terminal residues of human fibrinogen and on the changing of these groups in association with human thrombin. The end-group assay was carried out by Sanger's technique (*9, 10*) similar to the work performed on bovine material (*4*). Human fibrinogen (ca. 85% clottable) was prepared by ammonium sulfate fractionation (*11*) from the alcoholic Fraction I of plasma. Fibrin was separated after clotting with thrombin[2] by dissolving the clot in urea and precipitating fibrin by dilution with saline (*4*). Table 1 shows the comparative findings on bovine and human substrates.

TABLE 1

N-TERMINAL RESIDUES
(moles/g of protein)

	Bovine	Human
Fibrinogen	Tyrosine (1 mole/ca. 220,000 g) Glutamic acid (1 mole/300,000–500,000 g)	Tyrosine (1 mole/ca. 220,000 g) Alanine (1 mole/ca. 300,000 g)
Fibrin	Tyrosine (1 mole/ca. 220,000 g) Glycine (1 mole/ca. 100,000 g)	Tyrosine (1 mole/ca. 220,000 g) Glycine (1 mole/ca. 120,000 g)

Beside the end groups mentioned in Table 1, other amino acids were also found with free α-amino groups, but their quantities (1 mole/1,000,000 g of protein) suggested that they were derived from impurities in the fibrinogen preparation, and not from the clotting protein itself. It transpires from these investigations that species-specific differences between bovine and human fibrinogens are revealed by the assay of the N-terminal amino acid residues. The determinations of these groups seem to provide a fairly sensitive sign of species specificity in the case of fibrinogen, similarly to that found for certain hemoglobins (*12*). It is interesting to note that in fibrinogen the peptide chain (terminating in glutamic acid in bovine, and in alanine in human material) undergoing most obvious alteration during clotting carries a sign of species specificity.

On the whole, one may conclude that while there are certain species-specific differences at the molecular level in fibrinogen, the basic principles involved in the clotting reaction seem to be the same for both bovine

[1] This work was started at the Department of Biomolecular Structure of the University of Leeds, England. Thanks are due to the Regional Blood Transfusion Service of Leeds for a supply of human plasma.

[2] We acknowledge the gift of alcoholic Fraction I of human plasma from Dr. R. Murray, and a sample of human thrombin from Dr. W. H. Seegers.

and human material. The disappearance of the glutamic acid N-terminal residues from bovine fibrinogen during clotting foreshadowed the cleavage of fibrinopeptide from the protein. Similarly, the disappearance of the alanine N-terminal residues from human fibrinogen upon clotting with homologous thrombin suggests the existence of human fibrino-peptide. Moreover, the postulated human fibrino-peptide would have to differ from the demonstrated bovine one, at least in its N-terminal residues.

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Manuscript received August 24, 1953.

The Growth and Shrinkage of Aerosols

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When a nonvolatile aerosol, e.g., one composed of minute droplets of dioctyl phthalate (DOP) suspended in air, is introduced into a flask whose bottom and side walls are wetted with a master solution containing DOP and a miscible volatile solvent such as toluene, the aerosol droplets grow with extreme rapidity by absorbing the ambient vapor of the volatile solvent. Equilibrium is reached in a few seconds or less and persists for a considerable period of time as judged by the constant positions of the red bands in the Higher Order Tyndall Spectra produced when a beam of white light is directed through the aerosol.

The extent of growth is determined by the fixed partial pressure of toluene from the master solution; for the smaller droplets there is the additional effect of curvature of the droplet upon the vapor pressure of the volatile component (Kelvin effect) to be taken into account. When correction is made for the curvature effect, La Mer and Gruen (*1*) have shown that this procedure furnishes a very rapid, simple, and exceedingly accurate method of determining particle radii that are too small to measure without growth.

The present investigation involves the reverse process; namely, the shrinkage of aerosols. Monodisperse aerosols of DOP of the order of 0.10 μ in radius were first produced in a La Mer-Sinclair homogeneous generator (*2–5*) and grown over master solutions of DOP and toluene. By observations of the Higher Order Tyndall Spectra, the particle radius was then obtained. The grown aerosol was then passed into a second vessel containing a master solution with a lower proportion of the volatile component toluene. From the angles of the Higher Order Tyndall Spectra, it was observed that the aerosol diminished in size, and attained equilibrium with the second master solution almost immediately. Shrinkage experiments were carried out using master solutions of various compositions, and similar phenomena were observed in all cases.

Some typical results are given below. The radius of the grown aerosol for each of the two master solutions (r_g) is followed by the radius of the initial aerosol (r_i) calculated as shown in the La Mer-Gruen paper, allowing for the Kelvin effect and assuming that equilibrium has been set up between the aerosol and the master solution. The compositions of the master solutions are indicated by numbers, $K = 2$, 3, 4; these K values signify that a 2-, 3- or 4-fold increase in the radius of the aerosol droplets would be expected if the Kelvin effect was ignored; i.e., the solution for which $K = 2$ has the composition $[(2)^3 - 1]$ parts by volume of toluene to 1 part of DOP. In practice of course, because of the Kelvin correction, the grown aerosol does not attain the size suggested by the K value.

Experiment		r_g (microns) exptl.	r_i (microns) calcd.
(a)	$K = 3$	0.520	0.194
	$K = 2$	0.365	0.190
(b)	$K = 4$	0.590	0.187
	$K = 3$	0.510	0.192
(c)	$K = 4$	0.605	0.191
	$K = 2$	0.355	0.186

The agreement within the limits of experimental error between the values of r_i for the two master solutions in each set of results indicates then that the growth and shrinkage of the aerosol under these experimental conditions is an equilibrium process.

Attempts have also been made to ascertain whether or not the presence of a surface film on the aerosol had any effect in reducing the rate of shrinkage when it was placed over the second master solution. If the surface of the droplet became contaminated, the contamination should remain on the periphery if the contaminant lowers the surface tension. With decreasing size, the skin of the contaminant would shrink and might impede the rate of evaporation of the volatile component by forming a complete surface. It was hoped that experiments of this type might furnish an explanation for the frequently reported failure of smogs to evaporate when the relative humidity became lower than the equilibrium value. Since it is difficult to find a suitable surface contaminant that is not soluble in toluene, growth and shrinkage experiments were made with pure sulfuric acid aerosols over master solutions of H_2SO_4-H_2O. Oxidized hydrocarbon oils could be