TWO STAGES IN THE POLYMERIZATION OF FIBRIN

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The type of gel and duration of polymerization of fibrin are both determined by the terminal values of the ionic strength (μ) and pH, irrespective of when the process takes place. The main reaction of fibrin polymerization is slow in its course and is independent of μ and pH. These parameters affect the clotting time and determine the degree of polymerization at which the products formed are capable of gelatinization, the final process. If the values of μ and pH are low, the "early" polymerization products can react in this way.

Specific inhibitors of fibrin polymerization – fibrinogen and the macromolecular product of its enzymic hydrolysis – are known to inhibit directly only the early stage of the process [3].

In this investigation changes in ionic strength (μ) and pH in the course of polymerization of fibrin until its completion by gelatinization (clotting) were studied.

EXPERIMENTAL METHOD

Fibrin monomer [2, 4] was used in the experiments. The methods were described previously [1]. The precise experimental conditions are given in the captions to the tables and figure. The values of μ or pH were changed to allow modification of the type of gel formed. High values of μ or pH are favorable for the formation of thin gels, low values favor the formation of coarse gels.

TABLE 1. Effect of Increase in Ionic Strength (μ) during Self-Assembly of Fibrin on Gel Formation 0.05M Tris-HCl buffer, pH 7.65 + NaCl; μ: initial 0.16, final 0.30 (addition of a further 0.1 ml 1.65 M NaCl in buffer to 0.9-ml final volume of mixture); fibrin concentration 0.36 mg/ml. With μ = 0.16 clotting time equals 185 sec, E_{600nm} of gel 0.115].

Time during which mixture kept at μ = 0.16 before increase in μ (in sec)	Clotting time (in sec)	Interval between increase in μ and clotting (in sec)		Diffusion of light by gel E_{600} nm
		expected	found	
0	520		520	0,016
40	520	480 (520—40)	480	0,013
80	487	440 (520—80)	407	0,016
120	510	400 (520—120)	390	0,015
140	511	380 (520—140)	371	0,019
150	505	370 (520—150)	355	0,034

EXPERIMENTAL RESULTS AND DISCUSSION

The results of increasing the value of μ from 0.16 to 0.3 are given in Table 1 for one of the experiments. This change in μ gives the same increase in the duration of the clotting time and the same decrease in turbidity of gel whether carried out shortly before the end of the process or right at its beginning. It was hitherto taken for granted that the lengthened clotting time of fibrin monomer caused by an increase in μ was based on slower polymerization [5]. It was therefore expected that only slight delay of clotting would occur in experiments in which polymerization took place almost to the end under favorable conditions of a lower μ (salt was added after a long delay) and that clotting would be greatly retarded in the experiments in which a high value of μ acted as an unfavorable factor throughout the process. In fact, the reaction ended at the same time. This result shows that μ has virtually no effect on the velocity of the basic polymerization process. A different explanation must be sought for the well-

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TABLE 2. Effect of Decrease in Ionic Strength (μ) in Self-Assembly of Fibrin on Diffusion of Light and Syneresis of Gels (0.05 M Tris-HCl buffer, pH 7.65 + NaCl; μ: initial 0.32, final 0.16. Fibrin concentration before dilution 0.8 mg/ml, buffer dilution 0.33 mg/ml. Clotting time at μ = 0.32 and fibrin concentration 0.8 mg/ml was 410 sec, in fibrin concentration 0.33 mg/ml it was 690 sec, and at μ = 0.16 and in fibrin concentration 0.33 mg ml it was 210 sec. Initial weight of all gels 2.45 g)

μ	Time of keeping reaction mixture at μ = 0.32 before dilution (in sec)	Diffusion of light by gels $E_{600\,nm}$	Syneresis	
			weight of gels after separation from walls of tube (in mg)	degree of syneresis (loss of weight of gel in %)
0,32	without dilution	0,025	could not be determined	—
0,16	0	0,125	430	82,5
0,16	30	0,130	442	82,0
0,16	90	0,125	445	81,9
0,16	150	0,132	450	81,7
0,16	200*	0,130	440	82,0

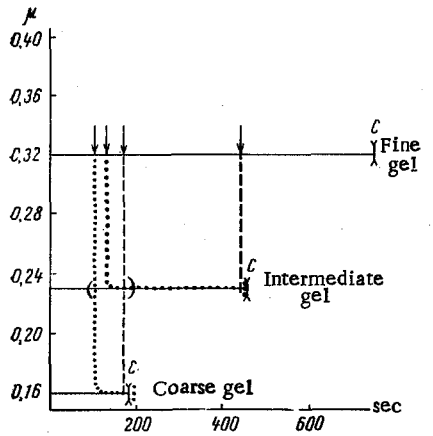

Fig. 1. Self-assembly of fibrin with constant and changing values of μ. Experimental conditions as in Table 2. Horizontal lines: course of polymerization. C) Time of clotting in sample with constant μ. Dots or crosses represent times of clotting after decrease in μ. Arrows indicate dilution of reaction mixture. Abscissa, time (in sec); ordinate, μ.

marked dependence of the clotting time on μ which has repeatedly been observed (more rapid clotting with a low value of μ). It seems probable that the polymerization of fibrin (which can be regarded essentially as self-assembly) consists of two processes: 1) a slow reaction which can be called primary polymerization and 2) rapid aggregation creating a gel. If this is true, the required explanation is as follows: a low value of μ enables the "earlier" polymerization products to take part in reaction, and 2) this leads to rapid clotting. A decrease in ionic strength leads to the formation of coarse gels instead of fine, because of strengthening of interactions between the opposite electrical charges of the polymerized fibrin [1]. Possibly these strong electrostatic interactions lead to the formation of coarse gels from the less complex "early" products of fibrin polymerization.

The hypothesis that with a low value of μ the gel is formed by "early" reaction products independent of μ can be tested. It can be concluded from this hypothesis in the course of polymerization at a high value of μ a potential state of preparedness for gelatinization must arise long before the end, and this may be manifested by a decrease in μ. If the "early" products of self-assembly, forming the gel, are associated through electrostatic interactions, the stage of gelatinization itself must take place extremely rapidly; and if the required degree of preparedness exists, dilution of the solution must give instantaneous clotting. This must occur if the time chosen for the transition to a low value of μ is chosen to correspond to the time of clotting of a mixture with unchanged μ, equal to that obtained in the experiment by dilution.

These hypotheses were confirmed by the experiments. This applies both to the clotting time and to the turbidity and syneresis of the resulting gels – indices by which the coarse gels are distinguished from fine (Fig. 1; Table 2).

It was important to determine whether changes in pH gave the same effects as changes in μ. Raising the pH from 6.8 to 8.8 with an ionic strength of 0.13-0.14 lengthened the clotting time of the fibrin monomer more than threefold and switched the self-assembly from coarse to fine structures; if this increase in pH was carried out during self-assembly the effect obtained was similar to that of increasing μ (Table 1). In the experiments in which the pH was lowered from 8.8 to 6.8, potential preparedness for clotting was found

and it appeared at an earlier stage of the process, just as in the experiments in which a change was made from high to low values of μ (Fig. 1). On the basis of these results the self-assembly (polymerization) of fibrin can thus be regarded as a combination of two processes: primary polymerization and terminal aggregation. In the experiments described above these processes could be observed separately. Of course, unlike primary polymerization, aggregation was highly dependent on both μ and pH, and the more favorable the medium for the formation of salt bridges the sooner it began. Primary aggregation is evidently based on interaction between nonionic groups of fibrin molecules and produces structures of the fine type, whereas the terminal stage takes place chiefly through electrostatic interaction and yields coarse structures [1]. Both structural modifications probably always take place in fibrin gels, but the quantitative ratio between them varies. At low values of μ and pH primary polymerization is sharply limited and the relative proportion of coarse structures increases, while at higher values it decreases. This view provides an explanation for the existence not only of typical coarse and fine gels, but also of various intermediate forms.

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