Savvidou, G., Klein, M., Grey, A. A., Dorrington, K. J., & Carver, J. P. (1984) *Biochemistry 23*, 3736-3740.
Sheares, B. T., & Robbins, P. W. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1993-1997.
Sox, H. C., Jr., & Hood, L. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 975-982.
Spiegelberg, H. L., Abel, C. A., Fishkin, B. G., & Grey, H. M. (1970) *Biochemistry 9*, 4217-4223.
Stanley, P. (1984) *Annu. Rev. Genet. 18*, 525-552.
Staudt, L. M., & Gerhard, W. (1983) *J. Exp. Med. 157*, 687-704.
Swiedler, S. J., Freed, J. H., Tarentino, A. L., Plummer, T. H., Jr., & Hart, G. (1985) *J. Biol. Chem. 260*, 4046-4054.
Tai, T., Setsuko, I., Yamashita, K., Muramatsu, T., & Kobata, A. (1975) *Biochem. Biophys. Res. Commun. 65*, 968-974.
Takahashi, N., Ishii, I., Ishihara, H., Mori, M., Tejima, S., Jefferis, R., Endo, S., & Arata, Y. (1987) *Biochemistry 26*, 1137-1144.
Taniguchi, T., Mizuochi, T., Beale, M., Dwek, R. A., Rademacher, T. W., & Kobata, A. (1985) *Biochemistry 24*, 5551-5557.
van Beek, W. P., Smets, L. A., & Emmelot, P. (1975) *Nature (London) 253*, 457-460.
Vliegenthart, J. F. G., Dorland, L., & van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem. 41*, 209-374.
Warren, L., Blithe, D. L., & Cossu, G. (1982) *J. Cell. Physiol. 113*, 17-22.
Warren, L., Baker, S. R., Blithe, D. L., & Buck, C. A. (1983) in *Biomembranes* (Nowotny, A., Ed.) Vol. 11, pp 53-77, Plenum Press, New York.

Effect of Homo Poly(L-amino acids) on Fibrin Assembly: Role of Charge and Molecular Weight

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ABSTRACT: Positively charged molecules such as protamine, leukocyte cationic protein, and the carboxyl terminus of platelet factor 4 have been shown to increase fibrin fiber thickness. Synthetic homo poly(L-amino acids) were used to explore the role of charge and molecular weight of cationic molecules on fibrin assembly. The effects of poly(L-lysine) (PLL), poly(L-glutamic acid) (PLG), poly(L-aspartic acid) (PLA), poly(L-histidine) (PLH), and poly(L-arginine) (PLArg) on the assembly and structure of fibrin gels were studied by using light-scattering techniques. At a PLG (M_r 60 000) concentration of 80 μg/mL and a PLA (M_r 20 000) concentration of 64 μg/mL, neither of these negatively charged polymers produced a detectable change in either fibrin assembly kinetics or final structure. Positively charged PLArg (16 μg/mL) caused a 30% increase in fibrin fiber mass/length ratio without calcium. In contrast, PLH (16 μg/mL), also positively charged, had no effect in the absence of $CaCl_2$ but produced a 40% increase in fiber mass/length ratio with 5 mM $CaCl_2$. At concentrations as low as 1 μg/mL, positively charged PLL increased the initial fibrin assembly kinetics and led to larger fiber mass/length ratio. The impact on fibrin mass/length ratio was equivalent for three different molecular weight preparations of PLL (M_r 25 000, 90 000, and 240 000). The lack of a molecular weight effect on fiber thickness and the low polymer concentrations required to produce the perturbation argue against an excluded volume effect as the mechanism by which lateral fiber growth is augmented. Mechanisms by which poly(L-amino acids) may perturb fibrin assembly are discussed.

Fibrinogen normally circulates as a soluble asymmetric protein, M_r 340 000, until the amino-terminal peptide, fibrinopeptide A, is enzymatically removed by thrombin. The subsequent fibrin polymerization kinetics and resulting fibrin structure are exquisitely sensitive to and modified by the microenvironment in which fibrin assembly occurs (Jones & Gabriel, 1988). Small shifts in pH, ionic strength, or calcium concentration during the assembly process result in dramatic changes in the material properties of fibrin (Ferry, 1947; Shulman et al., 1953a,b; Shen et al., 1975; Latallo et al., 1962; Carr et al., 1986a,b). Agents with known positive charge, including protamine (Stewart et al., 1969), dextran (Carr & Gabriel, 1980), and hydroxyethyl starch (Carr, 1986), have been shown to modify the final clot structure. Recently, native plasma proteins such as immunoglobulins (Gabriel et al., 1983), histidine-rich protein (Leung, 1986), leukocyte cationic protein (Carr et al., 1986a,b), thrombospondin (Bale et al., 1986), platelet factor 4 (Carr et al., 1987), and actin (Janmey et al., 1985) have been reported to alter fibrin assembly kinetics and fibrin material properties. The roles of molecular charge and size of nonpolymerizing perturbing molecules in determining fibrin assembly kinetics and structure remain incompletely examined. This study reports the effects of charged synthetic homo poly(L-amino acids) of varying size and charge

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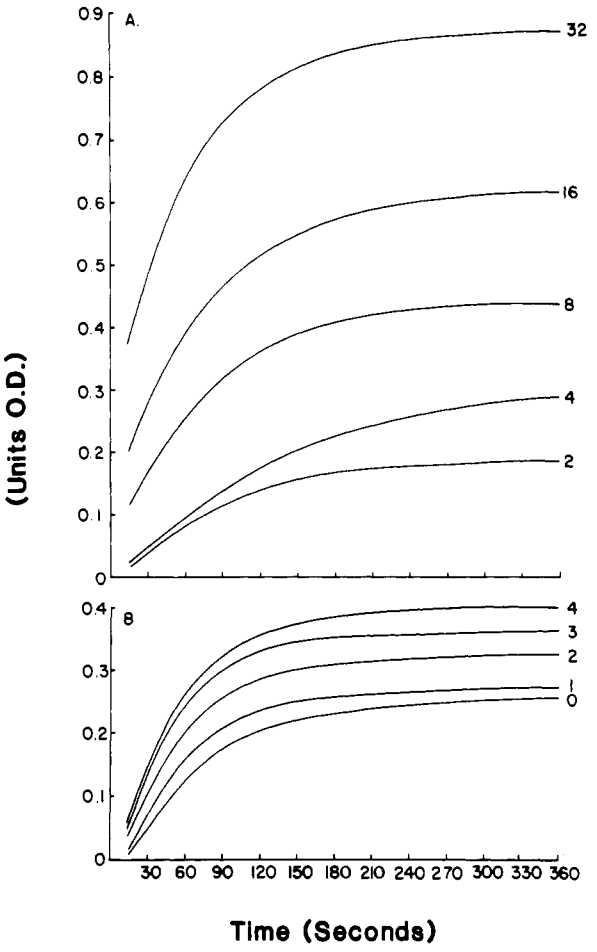

FIGURE 1: Effect of poly(L-lysine) (M_r 25000) on the time dependence of turbidity increase during clotting of thrombin-induced fibrin gels in the absence (panel A) and the presence of 5 mM $CaCl_2$ (panel B). PLL concentration (micrograms per milliliter) is indicated along the right margin. Other clotting conditions included pH 7.4 (0.05 M Tris), ionic strength 0.15, thrombin 1 NIH unit/mL, and fibrinogen 1 g/L. Fibrinogen was added to buffered thrombin solution at time zero.

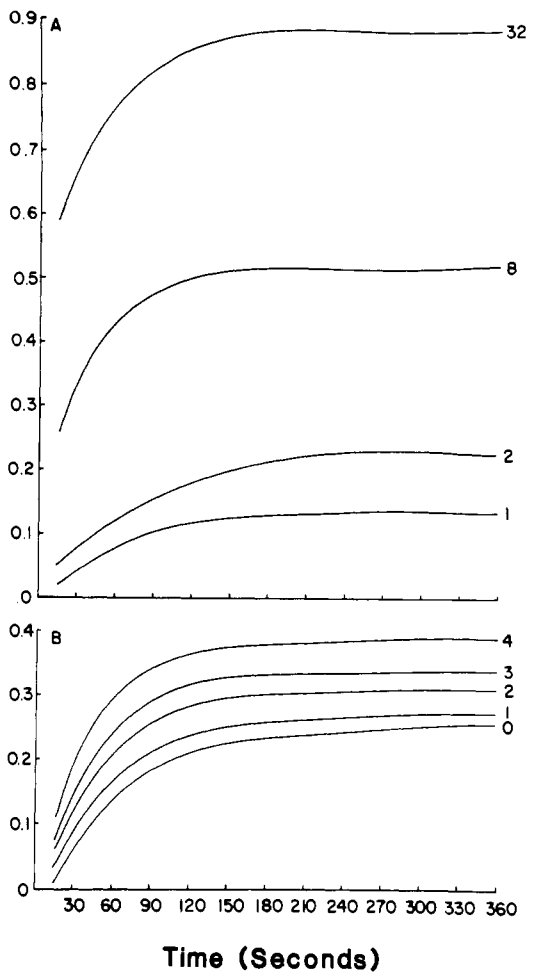

FIGURE 2: Effect of poly(L-lysine) (M_r 90000) on the time dependence of turbidity increase during clotting of thrombin-induced fibrin gels in the absence (panel A) and the presence (panel B) of 5 mM $CaCl_2$. PLL concentration (micrograms per milliliter) is indicated along the right margin. Other conditions were the same as in Figure 1.

on fibrin assembly and structure.

Materials and Methods

Human fibrinogen was purchased from Kabi Vitrum, dissolved in water, exhaustively dialyzed against 0.3 M NaCl, divided into 1-mL lots, and stored at −80 °C. Clottability was 95% by the method of Laki (1951). Fibrinogen concentration was determined spectrophotometrically from the absorbance at 280 nm using an extinction coefficient of 1.6 mL/(mg·cm). Plasmin(ogen)-free human thrombin (4300 NIH units/mL) was purchased from Sigma Chemical Co., dissolved in water, diluted with 0.10 M NaCl to a final concentration of 20 NIH units/mL, divided into 1-mL lots, and frozen at −80 °C. Poly(L-lysine) (M_r 25000, 90000, and 240000), poly(L-glutamic acid) (M_r 60000), poly(L-aspartic acid) (M_r 20000), poly(L-arginine) (M_r 40000), and poly(L-histidine) (M_r 9300) were purchased as lyophilized powders (Sigma Chemical Co.) and dissolved in 0.1 M NaCl. Polymer charge at a specific pH was determined from the degree of ionization of the polymer as previously described (Pederson, 1971).

Fibrin gels for turbidity measurements were formed directly in 1-cm polystyrene cuvettes by mixing purified fibrinogen solutions (1 g/L) with buffered solutions of thrombin (final concentration 1 NIH unit/mL). Gels were formed at pH 7.4, 0.05 M Tris, 5 mM $CaCl_2$, and the ionic strength was adjusted to 0.15 with NaCl.

Turbidity measurements were made at 25 °C with a Cary 210 spectrophotometer. Kinetic measurements were made at the HeNe laser line 632.8 nm. Fibrinogen was added to the clotting solution at time zero, and turbidity was monitored for 10 min, after which time gelation was allowed to go to completion unobserved. After 24 h, the gels were scanned from 400 to 740 nm and the mass/length ratios of the fibrin fibers determined according to the equation (Carr & Gabriel 1980):

$$\tau = [(88/15)\pi^3 n(dn/dC)^2 C\mu]/N\lambda^3$$

where n is the solution refractive index, dn/dC is the refractive index increment, λ is the wavelength, C is the concentration of fibrinogen in grams per milliliter, N is Avogadro's number, and μ is the mass/length ratio. For clear gels, μ was determined from the slope of a plot of τ versus $1/\lambda^3$. For more turbid gels where the radius of the fibers is no longer small compared to the incident wavelength, μ was obtained from the inverse of the intercept of a plot of $C/\tau\lambda^3$ versus $1/\lambda^2$ (Carr & Gabriel, 1980).

Results

The effects of positively charged PLL (M_r 25000, 90000, and 240000) on the kinetics of fibrin assembly are shown in Figures 1, 2, and 3, respectively. These figures present the kinetics of turbidity increase, resulting from fibrin fiber formation, during thrombin-induced clotting of purified fibrinogen solutions in the presence of increasing PLL concentration.

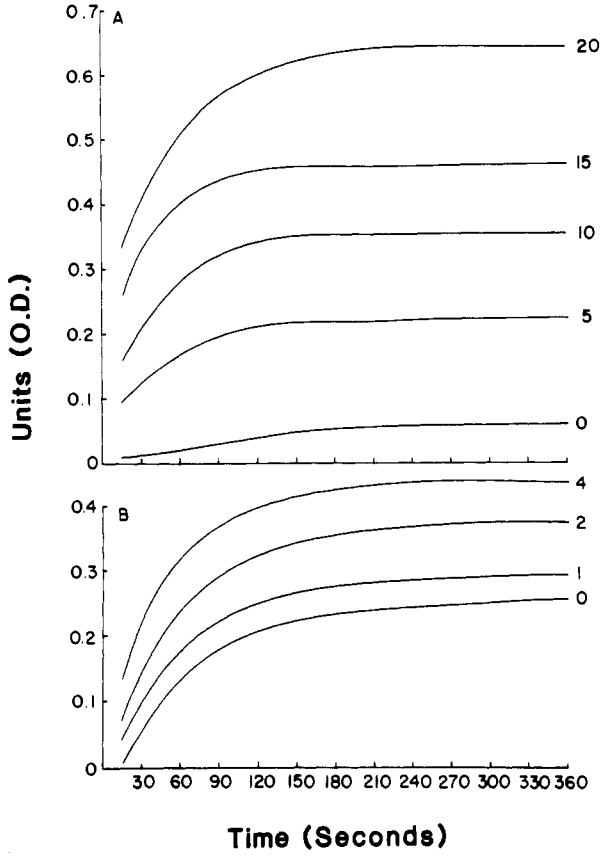

FIGURE 3: Effect of poly(L-lysine) (M_r 240 000) on the time dependence of turbidity increase during clotting of thrombin-induced fibrin gels in the absence (panel A) and the presence (panel B) of 5 mM $CaCl_2$. PLL concentration (micrograms per milliliter) is indicated along the right margin. Other conditions were the same as in Figure 1.

Kinetic data shown in the upper panels are for gels which contained no added calcium while gels in the lower panel contained 5 mM $CaCl_2$. The addition of PLL leads to an increase in the initial fibrin polymerization rate (steeper slope) and results in a higher fibrin fiber mass/length ratio as reflected by a higher final gel turbidity. The effect of PLL on gel turbidity is dependent on PLL concentration but independent of PLL molecular weight (Figures 1–3). The addition of 5 mM calcium further amplifies the early turbidity rise, shortens the time required to reach final turbidity, and leads to an additional increase in final gel turbidity. Since calcium greatly increases the fibrin fiber thickness and thus the turbidity, the effect of increasing PLL concentration is partially masked in the presence of calcium. The kinetic effects of poly(L-arginine), another positively charged polymer, parallel those seen with PLL. The effect of the more highly charged PLArg on the fiber thickness (data not shown) indicates that PLArg is more potent than PLL in enhancing gel assembly kinetics. The effects of PLH, less positively charged than PLL at pH 7.4, are less dramatic than those seen with PLL. The negatively charged polymers poly(L-glutamate) (PLG) and poly(L-aspartic acid) (PLA) at concentrations greater than 50 μg/mL do not affect fibrin assembly as measured under the described conditions.

The effects of charged polymers on gel fiber structure are summarized in Table I. Negatively charged polymers, PLG and PLA, did not affect fibrin structure. Concentrations of PLG as high as 48 μg/mL and of PLA as high as 64 μg/mL did not alter fiber mass/length ratios. Conversely, each of the positively charged polymers induced larger and more massive

Table I: Fiber Mass/Length Ratios (μ) for Fibrin Gels Formed in the Presence of Charged Polymers[a]

polymer	M_r	isoelectric point	concn (μg/mL)	$\mu \times 10^{13}$ (daltons/cm)
PLA[b]	20 000	2.98	0	1.19
			4	1.30
			48	1.22
PLG[c]	60 000	3.08	0	1.34
			4	1.31
			48	1.37
PLL[d]	25 000	9.47	0	1.57
			4	2.45
			16	5.05[e]
PLL	240 000	9.47	0	1.51
			4	2.59
			16	5.53[e]

[a] Clotting conditions: fibrinogen 1 g/L, thrombin 1 NIH unit/mL, pH 7.4 (Tris 0.05 M), 0.15 M NaCl, 5 mM $CaCl_2$. Gels were allowed to form for 24 h before being scanned. [b] Poly(L-aspartic acid). [c] Poly(L-glutamic acid). [d] Poly(L-lysine). [e] μ calculated from a plot of $C/\tau\lambda^3$ versus $1/\lambda^2$.

Table II: Concentrations of Positively Charged Polymers Capable of Precipitating Fibrinogen[a]

polymer	M_r	isoelectric point	concn (μg/mL)
PLA[b]	40 000	10.76	8
PLL[c]	25 000	9.47	32
	90 000	9.47	32
PLH[d]	9 300	7.64	32

[a] Conditions: fibrinogen 1 g/L, pH 7.4 (Tris 0.05 M), 0.15 M NaCl, temperature 28 °C. [b] Poly(L-arginine). [c] Poly(L-lysine). [d] Poly(L-histidine).

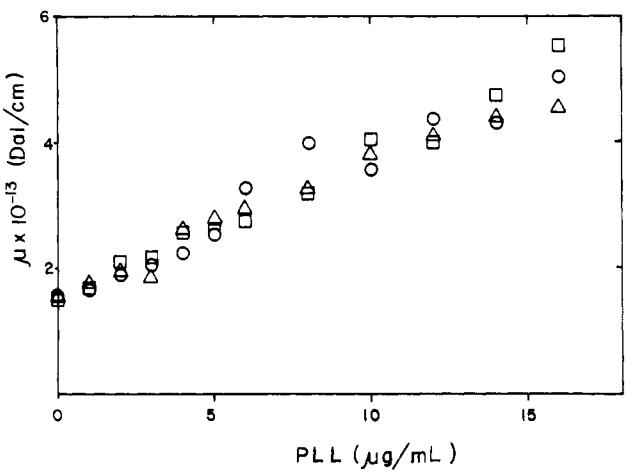

FIGURE 4: Effect of poly(L-lysine) of varying molecular weights on fibrin gel fiber mass/length ratios: M_r 25 000 (○); M_r 90 000 (△); M_r 240 000 (□). Other conditions were the same as in Figure 1.

fibrin fibers. The magnitude of each polymer's effect correlated with its degree of positive charge. When present in concentrations as low as 16 μg/mL, PLH (isoelectric point 7.64) produced a 26% increase in gel fiber mass/length ratio. PLL (isoelectric point 9.47) produced a 220% increase in fiber mass/length ratio when present at the same concentration. PLArg (isoelectric point 10.76) produced a 22% increase in the gel fiber diameter at 4 μg/mL and caused fibrinogen precipitation at higher concentrations.

The ability of positively charged synthetic polymers to precipitate fibrinogen appeared to be charge dependent (see Table II). Precipitation was observed as an increase in turbidity. Lower concentrations of the more highly charged PLArg will precipitate fibrinogen. In the presence of 5 mM $CaCl_2$, even smaller concentrations of charged polymer are

required to precipitate fibrinogen.

The effects of different molecular weights of PLL on fibrin mass/length ratio are illustrated in Figure 4. The final concentration of added poly(L-amino acid) is plotted against the final gel fiber mass/length ratio. Gel fibers increase in size as increasing amounts of PLL are added, but the effect of PLL is independent of the molecular weight of the added species.

Discussion

Fibrinogen is a 340 000 molecular weight protein composed of three pairs of peptide chains, α, β, γ chains. Fibrin polymerization is initiated when thrombin cleaves fibrinopeptide A from the amino-terminal end of the Aα chain (Lorand, 1951; Bettleheim & Barley, 1952). Removal of fibrinopeptide A exposes an association site on the E domain of fibrinogen. The E-domain binding site interacts with a complementary region on the D domain of a second fibrin molecule, resulting in a half-staggered overlapping of the monomeric units. Because of a 2-fold rotational symmetry about the minor hemiaxis in the E domain, polymerization can proceed at either end of the dimer. However, rotational symmetry is not present about the major hemiaxis, since each monomer has preferred orientation for monomer binding about this axis. Thus, protofibril polymerization proceeds from both ends in a linear manner rather than as a sheet (Hantgan & Hermans, 1979; Hantgan et al., 1983). The important result of linear polymerization is that a "free" D domain at either end of the forming protofibril is provided for possible interaction with poly(L-amino acids). Poly(L-amino acids) can also perturb fibrin formation during fiber assembly. Once protofibrils reach a critical length, weak lateral association forces become sufficient to cause alignment and stabilization of protofibrils into fibers. The process of fiber formation results in a branching three-dimensional network of fibrin (Hantgan et al., 1983). Fibrin fiber size is influenced by the assembly microenvironment (Jones & Gabriel, 1988; Stewart et al., 1969) including the presence of the positively charged poly(L-amino acids).

The findings of this study emphasize the ability of positively charged polymers to enhance lateral association of fibrin protofibrils during fibrin network formation. The exact mechanism by which positively charged homo poly(L-amino acids) induce thick fibers is not certain, but several mechanisms are possible. Although poly(L-amino acids) are large enough to participate in an excluded volume effect and hence decrease the solubility of the forming protofibrils, the molar concentration of the poly(L-amino acid) at which effects occur is so small that this mechanism seems very doubtful.

Interfibril bridging or linking of one protofibril to another by the poly(L-amino acid) is a second possibility. However, one would predict a molecular weight effect relating to the average end-to-end length of the poly(L-amino acid) to the fiber thickness; the longer the poly(L-amino acid), the greater the possibility of interprotofibril bridging. No such effect could be shown.

The presence of the poly(L-amino acid) could increase the bulk viscosity of fibrinogen solution, providing a third mechanism for poly(L-amino acid) effects. Increased solution viscosity can, through a Stokes law relationship, decrease protofibril diffusion coefficients, thus altering network assembly. Very small changes in the solution viscosity can affect the final clot structure (Gabriel et al., 1983). However, the concentration of the poly(L-amino acid) is very low, so that a rheologic mechanism also seems very unlikely.

Finally, the possibility of a "template effect" by the poly(L-amino acid) to promote fiber formation has not been excluded. The major determinant of this potential effect is dependent on the charge and isoelectric point of the poly(L-amino acid). The high pK_a may facilitate strong interaction between the protofibrils and the poly(L-amino acid). The conformational state of the poly(L-amino acid) is also an important consideration as PLL readily undergoes a temperature- and pH-dependent α-helix to random coil transition (Hermans, 1966), and the polymer side chain exposure to assembling fibrin is different for the two PLL conformations. At higher temperatures, the transition from α-helix to β-structure is favored (Pederson et al., 1971). Of relevance to this study, at physiologic pH and 25 °C, PLL is still mostly in the random coil state. If PLL is bound to the D domain, there would be severe steric hindrance to addition and alignment of the next fibrin monomer. Binding of PLL could disrupt protofibril formation and prevent proper protofibril alignment as fiber formation occurs. If this model is correct, multiple fibrin monomers could interact with the PLL, and a very strong dependence of fibrin structure on PLL concentration, as observed in these experiments, would be expected.

The important findings of this study are the magnitude of the effects at small poly(L-amino acid) concentrations, the direct dependence of the effect on the total charge, the lack of a molecular weight effect, the virtual absence of effects of negatively charged polymers, and the documentation of fibrin(ogen) precipitation at low poly(L-amino acid) concentrations. The dependence on charge, the lack of a molecular weight effect, and the very low synthetic polymer concentrations at which effects are noted virtually eliminate excluded volume as the mechanism involved (Minton, 1981; Wilf et al., 1985).

In summary, our data show that positively charged polymers enhance lateral association of fibrin protofibrils during fiber bundling. Certain positively charged proteins found in vivo, e.g., leukocyte cationic protein and the carboxyl terminus of PF4, also produce similar modifications in fibrin. Thus, release of these proteins from cells during clot formation could have an effect on fibrin structure in vivo. Such enhancement could be achieved by several mechanisms. The induction of conformational changes in the fibrin molecule might expose additional sites for hydrogen bonding or cause an enhancement of the previously existing lateral bonding sites. A second possibility would involve incorporation of the charged polymers into the fibrin polymer. The charged polymer would thus serve as a bridge between protofibrils while enhancing fiber thickness. Alternatively, PLL may serve as a nucleation point for fibrin assembly. The ability of synthetic polymers to alter in vivo clot formation and structure may offer a new avenue in our approach to hypercoagulable patients, since thicker fibers appear easier to lyse (Carrell et al., 1983; Carr & Hardin, 1987).

Registry No. PLL, 25104-18-1; PLL, SRU, 38000-06-5; PLG, 25513-46-6; PLG, SRU, 24991-23-9; PLA, 25608-40-6; PLA, SRU, 26063-13-8; PLH, 26062-48-6; PLH, SRU, 26854-81-9; PLArg, 25212-18-4; PLArg, SRU, 24937-47-1; Ca, 7440-70-2; thrombin, 9002-04-4.

References

Bale, M. D., & Mosher, D. F. (1986) *J. Biol. Chem. 261*, 862–868.

Bettleheim, F. R., & Bailey, K. (1952) *Biochim. Biophys. Acta 9*, 578–579.

Carr, M. E. (1986) *J. Lab. Clin. Med. 108*, 556–563.

Carr, M. E. (1988) *Thromb. Haemostasis 59*, 535–539.

Carr, M. E., & Gabriel, D. A. (1980) *Macromolecules 13*, 1473–1477.

Carr, M. E., & Hardin, H. L. (1987) *Blood 70*, 400a.
Carr, M. E., Shen, L. L., & Hermans, J. (1977) *Biopolymers 16*, 1-15.
Carr, M. E., Gabriel, D. A., & McDonagh, J. (1986a) *Biochem. J. 239*, 513-516.
Carr, M. E., Gabriel, D. A., Herion, J. C., & Roberts, H. R. (1986b) *J. Lab. Clin. Med. 103*, 199-203.
Carr, M. E., White, G. C., & Gabriel, D. A. (1987) *Thromb. Res. 45*, 539-543.
Carrell, N., Gabriel, D. A., Blatt, P. M., Carr, M. E., & McDonagh, J. (1983) *Blood 62*, 439-447.
Ferry, J. D., & Morrison, P. R. (1947) *J. Am. Chem. Soc. 69*, 388-399.
Gabriel, D. A., Smith, L. A., Folds, J. D., Davis, L., & Cancelosi, S. E. (1983) *J. Lab. Clin. Med. 101*, 545-552.
Hantgan, R. R., & Hermans, J. (1979) *J. Biol. Chem. 254*, 11272-11281.
Hantgan, R. R., McDonagh, J., & Hermans, J., Jr. (1983) *Ann. N.Y. Acad. Sci. 408*, 344-366.
Hermans, J., Jr. (1966) *J. Phys. Chem. 70*, 510-515.
Janmey, P. A., Lind, S. E., Yin, H. L., & Stossel, T. P. (1985) *Biochim. Biophys. Acta 24*, 151-158.
Jones, M., & Gabriel, D. A. (1988) *J. Biol. Chem. 263*, 7043-7048.
Laki, K. (1951) *Arch Biochem. Biophys. 32*, 317-324.
Latallo, Z. S., Fletcher, A. P., Alkjaersig, N., & Sherry, S. (1962) *Am. J. Physiol. 202*, 675-680.
Leung, L. L. K. (1986) *J. Clin. Invest. 77*, 1305-1311.
Lorand, L. (9151) *Nature 167*, 992-993.
Minton, A. P. (1981) *Biopolymers 20*, 2093-2120.
Pederson, D., Gabriel, D. A., & Hermans, J., Jr. (1971) *Biopolymers 10*, 2133-2145.
Shen, L. L., Hermans, J., McDonagh, J., McDonagh, R. P., & Carr, M. E. (1977) *Thromb. Res. 6*, 255-265.
Shulman, S., Ferry, J. D., & Tinoco, I. (1953a) *Arch. Biochem. Biophys. 42*, 245-256.
Shulman, S., Katz, S., & Ferry, J. D. (1953b) *J. Gen. Physiol. 36*, 759-766.
Steward, U. A., Bradley, M. S., Johnson, C. S., Jr., & Gabriel, D. A. (1988) *Biopolymers 27*, 173-185.
Stewart, G. J., & Niewiarowski, S. (1969) *Biochim. Biophys. Acta 194*, 462-469.
Wilf, J., Gladner, J. A., & Minton, A. P. (1985) *Thromb. Res. 37*, 681-688.

The Role of Highly Conserved Single-Stranded Nucleotides of *Xenopus* 5S RNA in the Binding of Transcription Factor IIIA†

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ABSTRACT: The role of highly conserved single-stranded sequence elements of *Xenopus* 5S RNA in the binding of transcription factor IIIA (TFIIIA) was studied. A series of mutant 5S RNA genes were constructed with defined block sequence changes in regions corresponding to each of the single-stranded loops of the transcribed 5S RNA. The interaction of the resulting mutant 5S RNA molecules with TFIIIA was determined both by a direct binding assay and by a competition assay. With one exception, the substitution of highly conserved single-stranded loop sequences had only a modest effect on the binding of TFIIIA. The single exception was loop A, which ironically is not part of the protected site of TFIIIA on 5S RNA. The possible involvement of loop A in the coaxial stacking of the helical domains of 5S RNA, and how this might affect TFIIIA binding, are discussed.

TFIIIA performs two essential functions in the *Xenopus* oocyte. It acts as a positive transcription factor, binding to an internal control region in the *Xenopus* 5S RNA gene and modulating the expression of these genes during oogenesis (Sakonju et al., 1980, 1981; Bogenhagen et al., 1980; Engelke et al., 1980). TFIIIA also binds to 5S RNA in the cytoplasm of immature oocytes, forming a 7S RNP storage particle that stabilizes the 5S RNA for later use in ribosome assembly (Picard & Wegnez, 1979; Pelham & Brown, 1980). Several recent discoveries have intensified the desire to understand how TFIIIA can interact specifically with two different nucleic acid targets. Studies on the structure of TFIIIA have shown that this protein contains approximately 9 mol equiv of Zn^{2+} ions essential for its function as a transcription factor (Hanas et al., 1983; Miller et al., 1985). Analysis of the cDNA sequence (Ginsberg et al., 1984) for the protein revealed the presence of nine imperfectly repeating sequence elements (Brown et al., 1985; Miller et al., 1985), containing highly conserved cysteine and histidine residues. It has been proposed that these residues coordinate to a Zn^{2+} ion, folding each repeat element into a novel structural domain best described as a protein "finger", and it is thought that each finger acts independently in binding to DNA (Miller et al., 1985). The presence of these fingers may provide properties required for the function of TFIIIA as a transcription factor, and finger structures appear to be a common motif in the putative nucleic acid binding domains of a wide range of eukaryotic regulatory proteins (Berg, 1986; Vincent, 1986; Evans & Hollenberg, 1988). Additionally, the protein fingers may have characteristics that facilitate the

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