of Conformational Changes in Biological Macromolecules, Sadron, C., Ed., D. Reidel Publishing Co., Dordrecht, Netherland, p 129.
Ware, W. R. (1971), Transient Luminescence Measurements in Creation and Detection of the Excited States, Lamola, A., Ed., New York, N.Y., Marcel Dekker, p 213.
Weber, G. (1958), *J. Chim. Phys. Phys. Chim. Biol. 55,* 878.
Winer, A. D., and Schwert, G. W. (1958), *Biochim. Biophys. Acta 29,* 424.

Effect of Polylysine on the Activation of Prothrombin. Polylysine Substitutes for Calcium Ions and Factor V in the Factor Xa Catalyzed Activation of Prothrombin†

Christine N. Vogel, Ralph J. Butkowski, Kenneth G. Mann, and Roger L. Lundblad*

ABSTRACT: Polylysine has been demonstrated to dramatically accelerate the rate of the factor Xa catalyzed activation of both prothrombin and prethrombin 1. Under the present experimental conditions (pH 8.0, 23 °C), no detectable activation of prothrombin or prethrombin 1 occurs with either factor Xa or polylysine alone. The activation of prethrombin 2, the direct precursor of α-thrombin, by factor Xa is not stimulated by polylysine. The activation of either prothrombin or prethrombin 1 by factor Xa in the presence of polylysine is partially inhibited by the presence of 5 mM $CaCl_2$. Electrophoretic analysis in sodium dodecyl sulfate showed that the products that were formed in the above activation system comigrated with the reaction products derived from prothrombin activated by factor Xa in the presence of calcium ions and phospholipid. It is suggested that polylysine stimulates the factor Xa-catalyzed activation of prothrombin by replacing the combination of calcium ions and factor V.

The activation of partially purified equine prothrombin by polylysine was first described by Miller (1960). Subsequent work from that laboratory showed that a variety of polymeric amines could also activate these prothrombin preparations (Miller et al., 1961). Subsequently Aronson and Menáché (1968) showed that the activation of more highly purified prothrombin preparations by polylysine was dependent on the addition of factor Xa.[1]

The results from a number of laboratories have shown that the activation of prothrombin is a complex process involving one or two intermediate steps (Mann et al., 1971; Stenn and Blout, 1972; Heldebrant and Mann, 1973; Engel and Alexander, 1973; Owen et al., 1974; Kisiel and Hanahan, 1974; Silverberg and Nemerson, 1975). In view of these recent observations, the effect of polylysine on the factor Xa catalyzed activation of prothrombin and prothrombin activation intermediates was subjected to further investigation.

The present results suggest that polylysine can substitute for calcium ions and factor V in the factor Xa catalyzed activation of prothrombin.

Materials and Methods

Poly(L-lysine) hydrobromide samples of various molecular weight ranges were obtained from Miles-Yeda Laboratories, Inc. The molecular weights given in the text for these samples were taken from the manufacturer's data sheet as obtained by ultracentrifugal analysis. As these materials are quite hydroscopic, stock solutions of 10 mg/ml in distilled water were prepared utilizing the entire contents of a vial and stored at −20 °C until use. Bovine fibrinogen was purchased from Sigma Chemical Corp., while *p*-tosyl-L-arginine methyl ester (TosArgOMe)[2] was a product of Schwarz-Mann. Crude Russell's viper venom (Lot No. 04158-2) was obtained from Pierce Chemical Co. and used without further purification. Sodium [^{3}H]borohydride (6.4 Ci/mmol) was purchased from Amersham Searle. All other chemicals were of reagent grade and used without further purification.

Bovine prothrombin was prepared as described by Bajaj and Mann (1973). Prethrombin 1[3] and prethrombin 2 were prepared as previously described (Heldebrant et al., 1973). Prothrombin and the intermediates of prothrombin activation was dialyzed against 0.01 M NH_4HCO_3 and lyophilized prior to use. Bovine factor X_1 was purified as described by Fujikawa and co-workers (Fujikawa et al., 1972a) and was a generous gift of Drs. K. Fujikawa and E. W. Davie. Factor Xa was obtained by the activation of purified bovine factor X_1 by Russell's viper venom (Fujikawa et al., 1972b).

Fibrinogen-clotting activity and esterase activity were measured as previously described (Lundblad, 1971). Fibrinogen-clotting activity is expressed in terms of NIH units using

† From the Dental Research Center and the Departments of Pathology and Biochemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, and the Hematology Research Section, Mayo Clinic and Foundation, Rochester, Minnesota, 55901. *Received October 16, 1975.* Supported in part by Public Health Service Grants DE-02338, HL-17430, and RR-5333 from the National Institutes of Health.

* To whom correspondence should be addressed at the University of North Carolina.

[1] The nomenclature used for the various coagulation factors is that adopted by the International Committee on Thrombosis and Hemostasis.

[2] Abbreviations used are: TosArgOMe, α-*N*-*p*-toluenesulfonyl-L-arginine methyl ester; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

[3] The nomenclature used for the polypeptide fragments derived from prothrombin activation is that recently developed by the International Committee on Thrombosis and Hemostasis (Paris, July, 1975). The reader is referred to a recent review (Mann, 1976) for a discussion of the nomenclature.

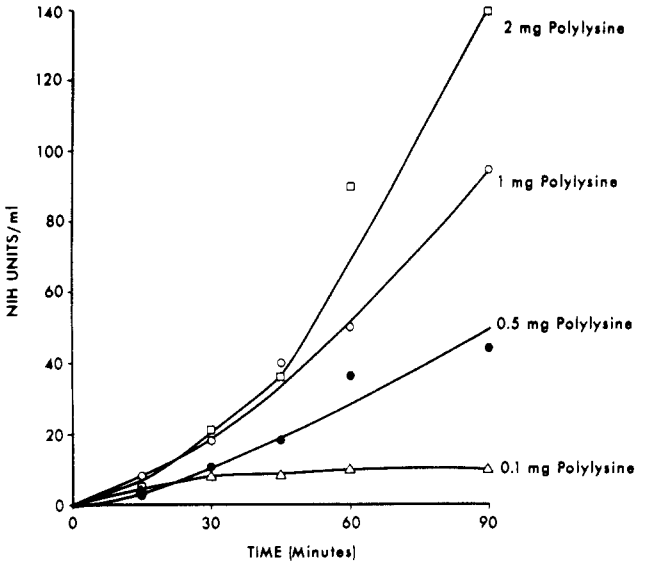

FIGURE 1: The effect of polylysine concentration on the activation of prothrombin by factor Xa. The reactions were performed as described under Table I and contained 10 μl of factor Xa (0.2 mg/ml), 2.0 mg of prothrombin, and either 2.0 mg of polylysine (□—□), 1.0 mg of polylysine (○—○), 0.5 mg of polylysine (●—●), or 0.1 mg of polylysine (△—△) in a final volume of 1.0 ml.

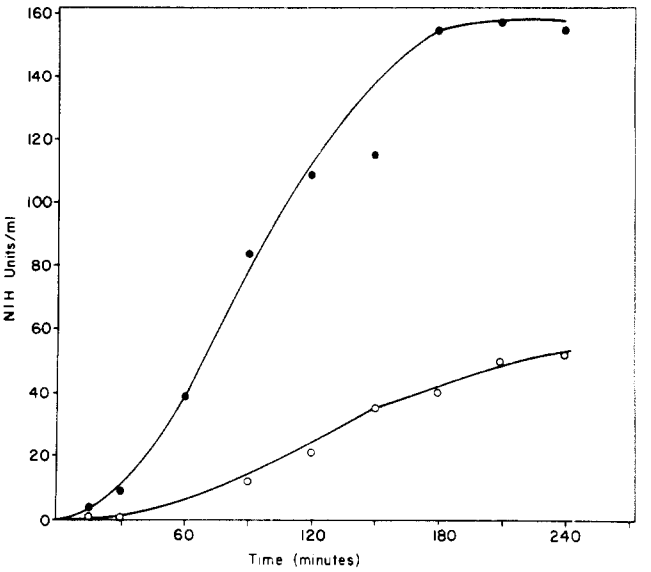

FIGURE 2: The effect of factor Xa concentration on prothrombin activation in the presence of polylysine. The reactions were performed as described under Table I and contained 2.0 mg of polylysine, 2.0 mg of prothrombin, and either 5 μl of (○—○) or 10 μl of (●—●) factor Xa (0.2 mg/ml).

NIH lot B3 as the standard. Protein concentration was estimated by the ninhydrin reaction (Moore, 1968) after alkaline hydrolysis (Fruchter and Crestfield, 1966) using crystalline bovine serum albumin as the standard.

The prothrombin activation reactions were performed under ambient conditions in either 0.05 M Tris, pH 8.0, or 0.05 M sodium phosphate, pH 8.0. The prothrombin was added as the dry material after lyophilization as described above. Polylysine and factor Xa were added as solutions in 0.05 M Tris, pH 8.0. Each reaction mixture had a final volume of 1.0 ml and, unless otherwise indicated, contained 2.0 mg of either prothrombin, prethrombin 1, or prethrombin 2. For the purposes of electrophoretic analysis, samples of the activation mixtures were acidified by adding an equal volume of glacial acetic acid, dialyzed against 0.2 M acetic acid, and lyophilized. Subsequent electrophoretic analysis of the samples were performed as described by Swank and Munkres (1971). The electrophoresis gels were stained with Coomassie blue and scanned for absorbance at 560 nm.

Prothrombin with tritium incorporated into its terminal sialic acid residues (sialyl[^{3}H]prothrombin) was prepared using a periodate oxidation followed by borotritiide reduction (van Lenten and Ashwell, 1971; Butkowski et al., 1974). Sialyl[^{3}H]prothrombin (1 mg/ml) was activated in 0.016 M imidazole–0.145 M NaCl, pH 7.4, with factor Xa (5 units/ml) in the presence of 6 mM $CaCl_2$ and phospholipid as described (Bajaj and Mann, 1973). A sample obtained at 5 min of activation was acidified as described above, dialyzed against 0.2 M acetic acid, and lyophilized. The lyophilized activation mixture was then used for the coelectrophoresis experiments. Radioactivity was monitored as previously described (Mann et al., 1973).

Results

Our initial experiments indicated that highly purified prothrombin that would not activate in the presence of 25% trisodium citrate also would not activate with polylysine at an equal weight ratio at pH 8.0 (Aronson and Menaché 1968). This would suggest the necessity of either factor VII (Spaet and Cintron, 1963), or factor X (Alexander, 1959), or both (Radcliffe and Barton, 1973) for the phenomena observed by Miller. Aronson and Menaché have previously presented evidence indicating that a preparation of thrombokinase[4] could activate purified prothrombin in the presence of polylysine. We have observed similar results using highly purified preparations of factor Xa. It was observed that polylysine greatly stimulates the rate of conversion of prothrombin to thrombin in the presence of factor Xa. Over the time period of these experiments, there was no detectable conversion of prothrombin to thrombin by factor Xa alone, polylysine alone, or factor X in the presence of polylysine.

The initial experiments were performed as described by Miller (1960) in that an equal weight ratio of polylysine (mol wt = 6900) to prothrombin was used. This represents an approximate tenfold molar excess of polylysine to prothrombin. The effect of polylysine concentration on the time course of prothrombin activation at a constant level of factor Xa was examined to determine if this high molar ratio was necessary. The results of this experiment are shown in Figure 1. It is observed that the rate of activation is insignificant at less than an equivalent molar amount. The rate of activation increased as one increased the ratio of polylysine to prothrombin. Although the extent or magnitude of the association of polylysine with prothrombin has not been ascertained, it would appear that such an association does exist as the initial reaction mixture of prothrombin and polylysine is quite turbid and clarifies during the course of the reaction as thrombin is evolved. This turbidity was not observed when albumin was substituted for prothrombin under the same reaction conditions. The time courses shown in Figure 1 are sigmoidal exhibiting a distinct lag phase. Such a lag phase has been observed by other investigators in several different prothrombin activation systems (Stenn and Blout, 1972; Silverberg and Nemerson, 1975). Figure 2 shows that effect of factor Xa concentration on the conversion of prothrombin to thrombin in the presence of po-

[4] It is assumed that thrombokinase is functionally, if not chemically, identical with factor Xa.

TABLE I: Effect of Polylysine on the Factor Xa Catalyzed Activation of Prothrombin and Prothrombin Derivatives.[a]

Reaction Conditions[a]	Incubation Time (min) 0	15	30	60
Prothrombin + polylysine + factor Xa	20[b]	720	1100	1560
Prothrombin + polylysine	0	0	0	0
Prothrombin + factor Xa	0	0	0	0
Prethrombin 2 + factor Xa	0	50	69	100
Prethrombin 2 + polylysine + factor Xa	0	25	60	105
Prethrombin 1 + factor Xa	0	0	10	10
Prethrombin 1 + polylysine + factor Xa	36	330	625	1040

[a] The reactions were performed as described under Table I. The polylysine and prothrombin (or prothrombin derivative) were present at a concentration of 2.0 mg in 1.0 ml of 0.05 M Tris, pH 8.0. Factor Xa (10 μl; 1.0 mg/ml) was added to initiate the reaction and portions were removed and assayed against fibrinogen at the indicated times. [b] NIH U/ml.

TABLE II: Effect of Calcium Ions on the Factor Xa–Polylysine Activation of Prothrombin and Prethrombin 1.

Reaction Conditions[a]	Incubation Time (min) 0	15	30	45	60
Factor Xa, polylysine, prothrombin	<1[b]	425	700	800	850
Factor Xa, polylysine, prothrombin, Ca^{2+}	<1	425	550	560	675
Factor Xa, polylysine, prethrombin I	32	225	300	340	480
Factor Xa, polylysine, prethrombin I, Ca^{2+}	<1	54	120	140	170

[a] The reactions were performed as described under Table I. Calcium chloride, when present as indicated, was at a concentration of 5 mM. [b] NIH U/ml.

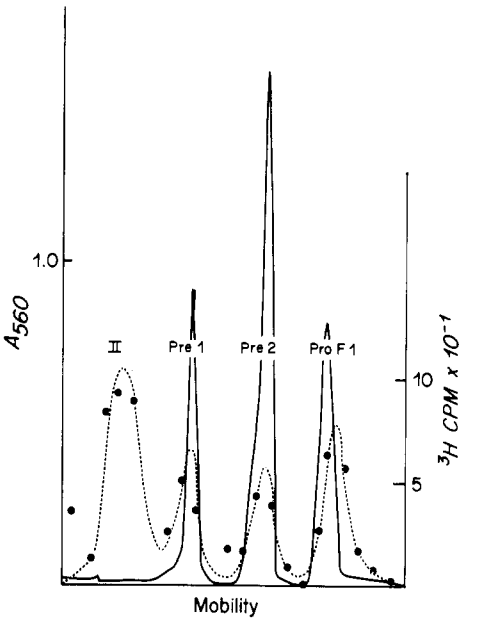

FIGURE 3: Coelectrophoresis in sodium dodecyl sulfate of a 10-min polylysine–factor Xa activation sample with a sample obtained during the activation of [^{3}H]prothrombin. The polylysine activation was performed as described for Figure 1, and the [^{3}H]prothrombin was activated for 3 min in 0.016 M imidazole, 0.145 M NaCl, pH 7.4, with 5 units/ml of factor Xa in the presence of 6 mM $CaCl_2$ and 40 μM phospholipid (see Methods). The gel was stained with Coomassie blue and scanned at 560 nm, then sliced laterally and each slice counted for tritium incorporation. Tritium incorporation is plotted vs. position within the gel (mobility) and is shown from the top to the point in the gel where no more incorporation was observed. The specific ratioactivity of the [^{3}H]prothrombin was 2×10^6 cpm/mg, hence the level present in these gels is not detectable by staining with Coomassie blue. Prothrombin, II; prethrombin 1, Pre 1; prethrombin 2, Pre 2; and prothrombin fragment 1, Pro F1.

lylysine. The rate of evolution of thrombin is markedly increased by increasing the factor Xa concentration and the extent of the lag phase is also reduced. Although not shown in Figure 2, the two experiments, when assayed after 24 h of incubation, contained the same amount of thrombin (1800–1900 NIH U/ml) that represents approximately 60–80% conversion of the prothrombin initially present to thrombin. Both fibrinogen-clotting activity and esterase activity are evolved at similar rates under these activation conditions. A study of the pH dependence of the reaction showed that the activation proceeded optimally at pH 8.0 in either Tris or sodium phosphate. The pH of the final reaction was the same as the solution before the addition of the final reactant, polylysine.

In the hopes of gaining some insight into the mechanism of action of polylysine in this reaction similar experiments were performed using various prothrombin activation intermediates. Table I shows that polylysine can stimulate the activation of prothrombin and prethrombin 1 to thrombin in the presence of factor Xa but does not stimulate the activation rate of prethrombin 2. This latter observation appears to preclude the possibility that polylysine exerts its stimulatory effect directly on factor Xa during the activation of prothrombin. If this was the situation, then one would expect to see an effect of polylysine on the activation of prethrombin 2, as well as prothrombin and prethrombin 1.

In a further attempt to determine the mechanism of action of polylysine in the conversion of prothrombin to thrombin, the effect of calcium ions on the activation of prothrombin to thrombin by factor Xa in the presence of polylysine was evaluated. In the presence of plasmatic cofactors, calcium ions are required for the binding of phospholipid (Gitel et al., 1973; Bajaj et al., 1975), as well as for the effect of factor V on prothrombin activation (Esmon and Jackson, 1974; Mann and Fass, 1974; Bajaj et al., 1975). In contrast to more conventional stimulators of prothrombin activation, the presence of calcium ions partially inhibits the rate of thrombin formation from both prothrombin and prethrombin 1 in the polylysine system, as shown in Table II.

It seemed likely that the factor Xa catalyzed conversion of prothrombin to thrombin in the presence of polylysine yielded the same reaction products as those observed in other activation systems (Fass and Mann, 1973; Heldebrant et al., 1973; Bajaj and Mann, 1973; Butkowski et al., 1974). In order to validate this hypothesis, a 10-min activation sample for the factor Xa–polylysine system was coelectrophoresed with an activation mixture obtained from a factor Xa, Ca^{2+}, phospholipid, sialyl[^{3}H]prothrombin activation system. Figure 3 demonstrates that the products obtained from the activation of prothrombin by factor Xa in the presence of polylysine coelectrophorese with those obtained from the activation of prothrombin by factor Xa in the presence of calcium ions and phospholipid.

The activation of prothrombin and of the various thrombin-producing intermediates was further examined by disc gel electrophoresis in the presence of sodium dodecyl sulfate. The results of this study are shown in Figure 4. Figure 4A presents a zero time to 70-min time course of prothrombin activation in the factor Xa–polylysine system. The presence of the acti-

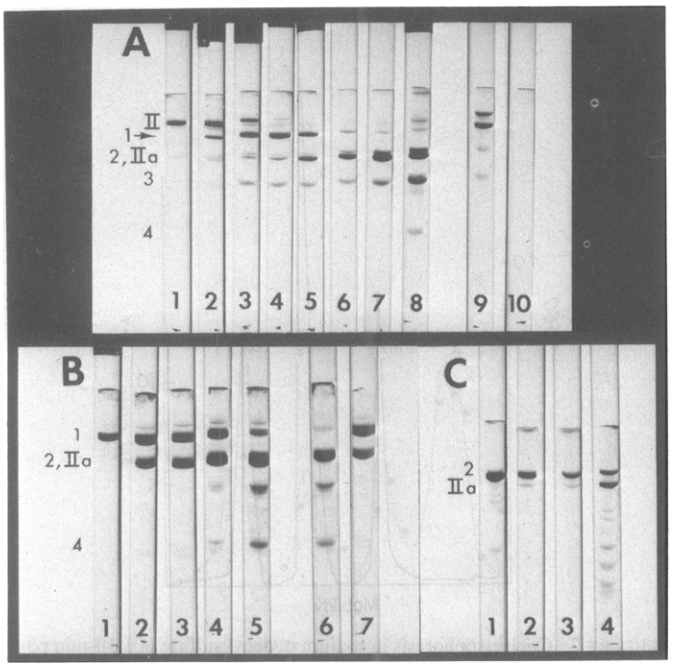

FIGURE 4: Sodium dodecyl sulfate polyacrylamide electrophoretograms of polylysine–factor Xa activation of A, prothrombin; B, prethrombin 1; and C, prethrombin 2. All activations were performed at 2 mg of proteins/ml, as described for Figure 3. (A) Zero time to 70-min activation analysis of prothrombin at 10-min intervals (gels 1–8). A 70-min control is shown without polylysine by gel 9, while gel 10 represents polylysine at a concentration equivalent to that in gels 1–8. Each gel contains 20 μg of protein. (B) Zero time to 70-min activation analysis of prethrombin 1. A higher protein load (60 μg) was used for gels 2–7 in order to visualize prothrombin fragment 2. Gels 1–5 represent 0 time, 10-, 30-, 50-, and 70-min samples, respectively. Gel 6 presents a sample from the same activation mixture taken at 180 min and gel 7 presents a 180-min control. (C) Activation analysis of prethrombin 2 electrophoresed with disulfide bonds reduced (20 μ/gel). Gel 1 presents a zero time; gel 2, 40-, gel 3, 80-, and gel 4, 180-min reaction sample. The controls for prethrombin 2 are not shown, since they are identical to the activation samples. Prothrombin, II; thrombin, IIa; prethrombin 1, 1; prethrombin 2, 2; prothrombin fragment 1, 3; and prothrombin fragment 2, 4.

vation products prethrombin 1, prethrombin 2, prothrombin fragment 1, and to a lesser extent, prothrombin fragment 2, is apparent from the electrophoretograms. Gel 9 in Figure 4A shows a 70-min control activation sample in which polylysine was absent, while gel 10 contained polylysine alone at the same concentration as in gels 1–6. In Figure 4B, samples were taken during the activation of prethrombin 1 from zero time to 70 min (gels 1–5). Gel 6 in Figure 4B shows a 3-h activation sample, while gel 7 contains a control reaction sample after the same time intervals. Figure 4C shows two results of the electrophoresis of samples taken during the activation of prethrombin 2 from zero time (gel 1) to 80 min (gel 3), while gel 4 presents a 3-h activation sample. As would be expected, the prethrombin 2 control, prethrombin 2 and factor Xa, was identical upon electrophoresis to the prethrombin 2–polylysine–factor Xa reaction. The unidentified bands in gels 4, 5, and 6 of Figure 4B and gel 4 of Figure 4C represent degradation products of thrombin that are seen after prolonged time under all activation conditions.

The effect of other polyamines was examined. Table III shows that protamine can also accelerate the factor Xa-catalyzed conversion of prothrombin to thrombin. We have observed that the effect of polylysine is independent of the molecular weight of the polylysine from a value (weight-average molecular weight) of 6900 to 72 000.

TABLE III: The Effect of Protamine on the Factor Xa Catalyzed Activation of Prothrombin.[a]

Reactions Conditions[a]	Incubation Time (min) 0	30	60	90
Prothrombin + factor Xa	0[c]	0	0	0
Prothrombin + polylysine + factor Xa	0	90	150	190
Prothrombin + protamine + factor Xa[b]	0	13	38	64

[a] The reactions were performed as described under Table I at a final concentration of factor Xa of 0.002 mg/ml. [b] Protamine was present as protamine sulfate at a final concentration of 2.0 mg/ml. [c] NIH U/ml.

Discussion

It is clear from the above results that polylysine can function as a cofactor in the conversion of prothrombin to thrombin by factor Xa. This is in agreement with the observations of Aronson and Menáché (1968). There does not appear to be a rigorous requirement for polylysine of a defined character in this reaction: polylysine preparations representing a broad range of molecular size have equivalent function in this reaction. Furthermore, other polyamines such as protamine can function in this reaction. It would appear, based upon the experiments described herein, that polylysine can partially replace (or substitute for) some or all of the cofactors (calcium ions, phospholipid, factor V) that are required for the optimal conversion of prothrombin to thrombin by factor Xa.

The molecular anatomy and function of prothrombin has been the subject of considerable study in the past several years. It is apparent that there are vitamin K dependent tight calcium binding sites in the amino-terminal portion (prothrombin fragment 1) of the prothrombin molecule (Gitel et al., 1973; Bajaj et al., 1975) that contain γ-carboxyglutamic acid (Stenflo et al., 1974; Zytkovicz and Nelsestuen, 1975). The binding of calcium to these sites can lead to the subsequent binding of phospholipid to this portion of the prothrombin molecule (Gitel et al., 1973; Bajaj et al. 1975; Esmon et al., 1974). The amino terminal portion of the prethrombin 1 molecule (prothrombin fragment 2) is thought to be responsible for the binding of factor Va to the prothrombin molecule (Esmon and Jackson, 1974; Mann and Fass, 1974; Bajaj et al., 1975). The accelerating effect of factor Xa on the activation of prethrombin 1, and hence upon intact prothrombin, is dependent on the presence of calcium ions (Esmon and Jackson, 1974; Mann and Fass, 1974; Bajaj et al., 1975). It is therefore clear that there are also functional calcium binding sites in the amino-terminal portion of the prethrombin 1 molecule (Bajaj et al., 1975).

The partial inhibition of the polylysine effect by calcium suggests competition for similar binding sites on the prothrombin molecule. This implies that, in part, polylysine is fulfilling the role of calcium ions in the activation of the prothrombin by factor Xa. As mentioned above, calcium is required for both the binding of phospholipid and factor Va to the prothrombin molecule during the process of activation. There is, however, an independent stimulatory effect of calcium ions on the activation of prothrombin by factor Xa (Milstone, 1964; Mann and Fass, 1974; Bajaj et al., 1975). Although it can not be clearly demonstrated at the current time, it would appear likely that calcium ions bind to the prothrombin molecule thereby inducing conformational changes thereby increasing the susceptibility of the cleavage of the peptide bonds required for the formation of thrombin. It has been assumed

that calcium exerts its primary effect through binding to the γ-carboxyglutamic acid residues in the amino terminal portion of the prothrombin molecule. On the other hand, it has recently been demonstrated that the activation of both normal and "abnormal" (formed in the absence of vitamin K and contains no modified glutamic acid residues) prothrombin by factor Xa in the presence of calcium ions proceeds at identical rates (Esmon et al., 1975). This would suggest that the binding sites for calcium ions resulting in the stimulation of prothrombin activation by factor Xa in the absence of other cofactors are different from those responsible for the binding of phospholipid.

The possibility of polylysine acting as an analogue of factor Va must also be considered. Factor Va, in the presence of calcium ions, will accelerate the activation of both prothrombin and prethrombin 1 but not prethrombin 2. Similar results are seen in the current experiments in that polylysine will greatly accelerate the rate of thrombin production from prothrombin and prethrombin 1. Neither factor Va nor polylysine has any effect on the rate of conversion of prethrombin 2 to α-thrombin. The same is true for calcium ions in that acceleration is seen with prothrombin and possibly prethrombin 1 as substrate, while no effect is observed on the rate of conversion of prethrombin 2. However, the magnitude of the increase in the rate of activation of either prothrombin or prethrombin 1 in the presence of polylysine is far greater than that observed by calcium ions alone (Bajaj et al., 1975). There it would seem that polylysine can substitute for factor Va and calcium ions in the activation of prothrombin.

Based on the observations cited above, it would appear that polylysine is a functional analogue of both calcium ions and factor Va in the conversion of prothrombin to thrombin. In this case polylysine would bind to a site (or sites) in the amino terminal portion of the prothrombin molecule increasing the susceptibility of certain peptide bonds to cleavage by factor Xa resulting in the formation of α-thrombin.

Acknowledgements

The authors are indebted to Drs. K. Fujikawa and E. W. Davie for generous quantities of factor X used in these experiments.

References

Alexander, B. (1959), *Proc. Int. Congr. Biochem., 4th,* 37.

Aronson, D. L., and Menáché, D. (1968), *Biochim. Biophys. Acta 167,* 378.

Bajaj, S. P., Butkowski, R. J., and Mann, K. G. (1975), *J. Biol Chem. 250,* 2150.

Bajaj, S. P., and Mann, K. G. (1973), *J. Biol. Chem. 248,* 7729.

Butkowski, R. J., Bajaj, S. P., and Mann, K. G. (1974), *J. Biol. Chem. 249,* 6562.

Engel, A. M., and Alexander, B. (1973), *Biochim. Biophys. Acta 320,* 687.

Esmon, C. T., and Jackson, C. M. (1974), *J. Biol. Chem. 249,* 7791.

Esmon, C. T., Owen, W. G., and Jackson, C. M. (1974), *J. Biol. Chem. 249,* 7798.

Esmon, C. T., Suttie, J. W., and Jackson, C. M. (1975), *J. Biol. Chem. 250,* 4095.

Fass, D. N., and Mann, K. G. (1973), *J. Biol. Chem. 248,* 3280.

Fruchter, R. G., and Crestfield, A. M. (1966), *J. Biol. Chem. 240,* 3868.

Fujikawa, K., Legaz, M. E., and Davie, E. W. (1972a), *Biochemistry 11,* 4882.

Fujikawa, K., Legaz, M. E., and Davie, E. W. (1972b), *Biochemistry 11,* 4892.

Gitel, S. N., Owen, W. G., Esmon, C. T., and Jackson, C. M. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 1344.

Heldebrant, C. M., Butkowski, R. J., Bajaj, S. P., and Mann, K. G. (1973), *J. Biol. Chem. 248,* 7149.

Heldebrant, C. M., and Mann, K. G. (1973). *J. Biol. Chem. 248,* 3642.

Kisiel, W., and Hanahan, D. J. (1974), *Biochim. Biophys. Res. Commun. 59,* 570.

Lundblad, R. L. (1971), *Biochemistry 10,* 2501.

Mann, K. G. (1976), *Methods Enzymol.* (in press).

Mann, K. G., Heldebrant, C. M., and Fass, D. N. (1971), *J. Biol. Chem. 246,* 6106.

Mann, K. G., and Fass, D. W. (1974), *Mayo Clin. Proc. 49,* 929.

Mann, K. G., Yip, R., Heldebrant, C. M., and Fass, D. N. (1973), *J. Biol. Chem. 248,* 1868.

Miller, K. D. (1960), *J. Biol. Chem. 235,* PC63.

Miller, K. D., Copeland, W. H., and McGarren, J. F. (1961), *Proc. Soc. Exp. Biol. Med. 108,* 117.

Milstone, J. H. (1964), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 23,* 742.

Moore, S. (1968), *J. Biol. Chem. 243,* 6281.

Owen, W. G., Esmon, C. T., and Jackson, C. M. (1974), *J. Biol. Chem. 249,* 594.

Radcliffe, R. D., and Barton, P. G. (1973), *Arch. Biochem. Biophys. 155,* 381.

Seegers, W. H., Walz, D. A., Reuterby, J., and McCoy, L. E. (1974), *Thromb. Res. 4,* 829.

Silverberg, S. A., and Nemerson, Y. (1975), *Biochemistry 14,* 2636.

Spaet, T. H., and Cintron, J. (1963), *Blood 21,* 745.

Stenflo, J., Ferlund, P., Egan, W., and Roepstorff, P. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2730.

Stenn, K. S., and Blout, E. R. (1972), *Biochemistry 11,* 4502.

Swank, R. T., and Munkres, K. D. (1971), *Anal. Biochem.* 39, 462.

van Lenten, L., and Ashwell, G. (1971), *J. Biol. Chem. 246,* 1889.

Zytokovicz, T. H., and Nelsestuen, G. L. (1975), *J. Biol. Chem. 250,* 2968.