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Investigation of the Aggregation and Activation of Prothrombin Using Quasi-Elastic Light Scattering†

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ABSTRACT: The technique of quasi-elastic light scattering was used to measure the translational diffusion coefficient, D, of purified human prothrombin in buffered aqueous solutions and to monitor for the first time the fragmentation of this protein as it is converted to thrombin. The values of $D_{20,w}$, measured at two different concentrations, are 4.72×10^{-7} cm^2/s at 2 mg/cm^3 and 4.51×10^{-7} cm^2/s at 5 mg/cm^3; the corresponding molecular weights ($\overline{M}_w$ of 92 000 and 120 000), obtained by combining sedimentation velocity measurements with the diffusion data, confirm the presence of molecular aggregates of prothrombin in these solutions. These results, as well as analysis of the intensity-intensity autocorrelation functions from two-component systems with various dimer conformations, indicate the presence of end-to-end dimers in these prothrombin solutions. The values obtained for D indicate a dimer weight fraction of 0.4 to 0.5 in the 2 mg/cm^3 solution and 0.6 or greater in the 5 mg/cm^3 solution. The fragmentation of prothrombin was monitored in a nonphysiologic activation system, containing taipan snake venom, dihexanoylphosphatidylcholine, and $CaCl_2$. At a temperature of 15 °C, conversion to thrombin proceeded very slowly and was still incomplete after 90 h. A method for determining the percentage of converted prothrombin is an activated system containing aggregates from the average value of D and light scattering data is discussed.

The physical properties of prothrombin (factor II) and its conversion to thrombin (factor IIa) have been investigated extensively during the past few years. Most preparations of this protein have shown a tendency to form aggregates in various solution environments. Cox and Hanahan (1970) observed that bovine prothrombin dimerized at concentrations less than 4 mg/cm^3 and formed larger aggregates at higher concentrations. They also found from diffusion and sedimentation studies that the frictional ratio of the protein increased with concentration and degree of aggregation and that the aggregated form was more asymmetric than its monomer. Similarly, Kisiel and Hanahan (1973) have shown that the weight-average molecular weight of human prothrombin in solutions of moderately high salt concentrations was also a function of pH. To date,

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‡ Recipient of National Institutes of Health Career Development Award Grant GM00071.

however, there has been no definitive study of the hydrodynamic properties of these dimer forms in buffered aqueous solutions.

The purpose of this communication is twofold: first, to report the results of our studies on the hydrodynamic properties of human prothrombin dimers using quasi-elastic light scattering and sedimentation measurements; and secondly, to show how this new light-scattering technique can be used to monitor an important blood clotting reaction, the conversion of prothrombin to thrombin. The complete activation of prothrombin by factor Xa, factor V, phospholipid, and Ca^{2+} has been shown to result in a sequential fragmentation of this protein (Seegers, 1967; Mann et al., 1971, 1974). By measuring the increase in the average translational diffusion coefficient of the solute species with time during activation, the overall process of prothrombin fragmentation can be followed. As an example we present kinetic data on a system of human prothrombin, activated by taipan snake venom (TSV)[1] and dihexanoylphosphatidylcholine (DHL) in a buffered aqueous solution.

Experimental Section

Materials. Two lots of purified human prothrombin were used for these studies, generously supplied by Dr. Walter Kisiel at the University of Arizona College of Medicine. The human prothrombin was purified using barium citrate adsorption, ion-exchange chromatography, and preparative polyacrylamide gel electrophoresis. Lot 1 human prothrombin was dissolved in a 0.1 M Tris-H_3PO_4, pH 6.0 buffer. Using an $E^{1\%}$ of 13.8, the protein concentration was approximately 2 mg/cm^3. The factor II activity of this lot was 14.0 ortho U/cm^3;[2] contaminating factor X activity was 0.02 ortho U/cm^3. Factor Xa activity in this preparation was estimated to be less than 10^{-5} ortho U/cm^3. When 100 μg of this protein preparation was applied to sodium dodecyl sulfate gel, a trace amount of intermediate I was observed. Lot 2 human prothrombin was dissolved in 0.1 M Tris-HOAc/5 mM benzamidine-HCl, pH 6.0 buffer to a concentration of 5 mg/cm^3. The activity of this lot was assayed to be 40 ortho U/cm^3. When assayed at 3.3 mg/cm^3, no factor Xa activity was detectable. Sodium dodecyl sulfate gel electrophoresis on this preparation showed the protein to be homogeneous and to contain no discernible levels of factor Xa or thrombin. In addition, 0.33 mg of both preparations would not clot at 0.4% solution of fibrinogen at 37 °C in 2 h. TSV (oxyuranus scutellatus) was obtained from Dr. E. Natelson of the Special Hematology Laboratory of the Methodist Hospital. TSV was dissolved in 0.1 M Tris-NaH_2PO_4, pH 6.0 buffer to a concentration of 20 μg/cm^3. DHL was obtained from Dr. Joel Morrisett of the Division of Atherosclerosis and Lipoprotein Research at the Baylor College of Medicine. The phospholipid was also dissolved in the same buffer as TSV to a concentration of 300 μg/cm^3 which is below its critical micelle concentration (Roholt and Schlamowitz, 1961).

Quasi-Elastic Light Scattering. The translational diffusion coefficient of a macromolecule in a solution has been shown to be readily accessible from measurements of the spectrum of scattered light or its inverse Fourier transform, the correlation function (Pecora, 1964; Cummins and Swinney, 1970; Cummins, 1974). For a monodisperse solution of particle scatterers, the homodyne correlation function arising from the Brownian motion of these particles has been shown to decay exponentially. The decay rate Γ is equal to twice the product of the translational diffusion coefficient D of the particle and the square of the scattering wave vector $\bar{\mathbf{K}}$. This latter parameter is related to the refractive index n, the scattering angle θ, and the reciprocal of the wavelength in vacuum λ_0: $[\bar{\mathbf{K}}] = (4\pi n/\lambda_0) \sin(\theta/2)$. The hydrodynamic radius, R, of the particle can be directly obtained from D, which in turn is defined by the Stokes-Einstein equation: $D = kT/(6\pi\eta R)$. The details of the experimental apparatus and the general procedure for preparing the scattering cells and solutions have been extensively described elsewhere (Morrisett et al., 1974).

Analytical Ultracentrifugation. All experiments were performed at temperatures between 17 and 20 °C, using a Beckman Model E analytical ultracentrifuge, equipped with RTIC temperature control. The sedimentation velocity measurements were obtained from observations of the refractive index gradient using schlieren optics and the results were recorded on metallographic plates. The sedimentation equilibrium data were obtained on the prothrombin preparations in the above buffers using Rayleigh interference optics and were recorded on II-G spectroscopic plates. Initial protein concentrations were 0.2 (lot 1) and 0.5 mg/cm^3 (lot 2) and the operating speed (using mechanical speed control) was 21 740 rpm.

Results and Discussion

Prothrombin Dimer Characterization. Measurements of the translational diffusion coefficient on the samples of lot 1 human prothrombin were taken over the temperature range of 9–26 °C as a function of scattering angle (θ = 20–90 °C). During these experiments aggregation of the prothrombin was found to be time and temperature dependent. Below 20 °C the solutions were stable for over a week, while at higher temperatures, progressive aggregation was evident after 24 h. For this reason the diffusion properties of the prothrombin molecule and its conversion to thrombin were measured at temperatures below 20 °C. The decay rate was measured as a function of scattering angle. For a solution of monodisperse species, a linear relationship should exist between the decay rate Γ, and the square of the scattering wave vector. This relation should also hold for a system containing monomers and dimers (see Discussion), as long as their size falls within the Rayleigh scattering region (scatterer dimensions less than $\lambda/20$). The correlation function from solutions of human prothrombin was described fairly accurately by a single exponential between 2 and 3 correlation times ($\tau_c = 1/\Gamma$). The decay rate Γ was found to increase linearly with the square of the scattering wave vector for the range of scattering angles studied. The average translational diffusion coefficient compiled from over 40 correlation runs of lot 1 gave $D_{20,w} = 4.72 \pm 0.23 \times 10^{-7}$ cm^2/s. Similarly, limited measurements on lot 2 gave $D_{20,w} = 4.51 \times 10^{-7}$ cm^2/s. These results are in good agreement with values of D for bovine prothrombin reported by Cox et al. (1970): 4.80×10^{-7} cm^2/s for protein concentrations of 2 mg/cm^3 and 4.60×10^{-7} cm^2/s for concentrations of 5 mg/cm^3. No appreciable increase of the diffusion coefficient in a sample of lot 1 was observed upon a twofold dilution. Correlation functions could not be obtained on concentrations of this protein below 1 mg/cm^3 because of low signal levels.

Sedimentation velocity experiments were performed on both lots of human prothrombin in the above stated buffers after filtering through 0.22-μm Millipore filters. The sedimentation coefficients were reduced to standard conditions of H_2O and

[1] Abbreviations used: TSV, taipan snake venom; DHL, dihexanoylphosphatidylcholine; Tris, tris(hydroxymethyl)aminomethane.

[2] Based upon the one-stage prothrombin assay (Hjort et al., 1955); one ortho factor II unit is arbitrarily defined as the amount of factor II activity in 1 cm^3 of ortho coagulation control human plasma.

20 °C temperature. The average sedimentation coefficients $s_{20,w}$ were 5.37 S and 6.70 S of lots 1 and 2, respectively. The diffusion and sedimentation coefficients were then combined in the Svedberg equation (Tanford, 1961) to determine the weight-average molecular weight of the protein, assuming a partial specific volume of $\bar{v} = 0.70$ cm^3/g (Lamy and Waugh, 1953). The values obtained for the molecular weights at protein concentrations of 2 and 5 mg/cm^3 were 92 000 and 120 000, respectively. These results also confirm the findings of Cox et al. (1970) that the sedimentation coefficient of prothrombin is concentration dependent.

In addition, two separate experiments were carried out to determine directly the molecular weights of the prothrombin samples. At sedimentation equilibrium, the logarithm of the fringe displacement, ln f, should be linearly related to the square of the radial position (r^2) for a homogeneous population according to the equation:

$$\ln f = \ln f_a + \frac{M(1 - \bar{v}\rho)\omega^2(r^2 - r_a^2)}{2RT} \quad (1)$$

where ω is the angular velocity, R is the gas constant, T is the absolute temperature, ρ is the density, M the molecular weight, and the subscript a indicates the meniscus position. The slope of the linear plot leads to the determination of M. A sample of lot 1, diluted to a mean concentration of 200 μg/cm^3, gave a linear plot of ln f vs. r^2, yielding a value of 69 000 for M. However, persistent aggregation was evident in samples of lot 2, diluted to 500 μg/cm^3. A plot of ln f vs. r^2 was nonlinear, with the molecular weight ranging from 70 000 to 140 000.

The results obtained from the quasi-elastic light scattering, as well as the ultracentrifugation experiments, revealed that these prothrombin preparations contained aggregates of the protein. Using the theory of the hydrodynamic properties of rigid macromolecules to calculate shape parameters, the intensity-intensity autocorrelation function was calculated for a two-component system, containing monomers and dimers of this protein. For such a system of Rayleigh scatterers, it can be shown (Gallagher, 1972) that the intensity-intensity autocorrelation function has the following form:[3]

$$C(\tau) = (c_m M_m)^2 \exp[-2|\bar{K}|^2 D_m \tau] + 2(c_m M_m c_d M_d) \exp[-|\bar{K}|^2 (D_m + D_d)\tau] + (c_d M_d)^2 \exp[-2|\bar{K}|^2 D_d \tau] \quad (2)$$

where c_m, M_m, D_m, and c_d, M_d, D_d are the weight concentration, molecular weight, and translational diffusion coefficients of the monomer and dimers, respectively. In addition, calculations were performed to see how the z-average diffusion coefficient, $\bar{D}_z$, and the weight-average molecular weight, $\bar{M}_w$, varied as a function of these parameters:

$$\bar{D}_z = \frac{\Sigma c_i M_i D_i}{\Sigma c_i M_i} \quad (3a)$$

$$\bar{M}_w = \frac{\Sigma c_i M_i}{\Sigma c_i} \quad (3b)$$

[3] The autocorrelation functions were obtained on a single-clipped correlator using a clipping level of zero. The average number of counts per decay time interval was much less than one (typically 0.001 to 0.01). Under such conditions, the autocorrelation function is approximately equal to that derived from full correlation techniques (Jakeman, 1974; Koppel, 1971). The intensity-intensity autocorrelation function for Gaussian statistics is then equal to the square of the field-field autocorrelation function and is proportional to the second power of the molecular weight and concentration of the scatterer.

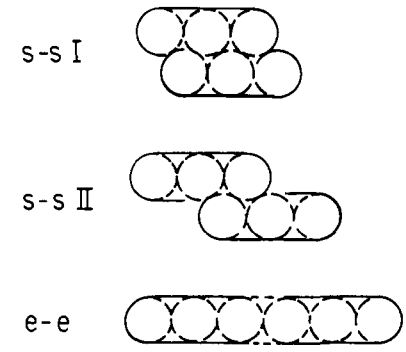

FIGURE 1: Models of side-to-side and end-to-end conformations for prothrombin dimers (see footnote *a*, Table I).

TABLE I: Ratios of the Frictional Coefficients of Dimers to Monomers of Prothrombin (f_d/f_m) and Corresponding Translation Diffusion Coefficients for the Three Dimer Structures, Corrected to 20 °C and Standard Solvent, H_2O.[a]

Dimer Model	f_d/f_m	$D_{d,20;w}$ ($\times 10^{-7}$ cm^2/s)
s-s I	1.215	5.14
s-s II	1.312	4.76
e-e	1.509	4.14

[a] The side-to-side models of the dimer and the f_d/f_m values were obtained from Squire et al. (1968). Their calculations were based on the results of Kirkwood (1954) and Bloomfield et al. (1967) for frictional coefficients of a structure composed of n identical subunits.

where the subscript i refers to the form of the macromolecule. A molecular weight value of 68 500 was used for the isolated prothrombin macromolecule, obtained from the studies of Harmison et al. (1961) on bovine prothrombin. This is in agreement with our sedimentation equilibrium data on lot 1 human prothrombin. Single molecules of the protein were assumed to have a diffusion coefficient of 6.24×10^{-7} cm^2/s and mean axial ratio of 3.5 (Lamy and Waugh, 1953), based on an equivalent hydrodynamic form of a prolate ellipsoid. The monomer and dimer forms were assumed to have the same partial specific volume and degree of hydration. The ratio of the diffusion coefficients of the dimer and monomer, resulting from the shape portion of the frictional coefficient, was obtained from the following expression (Tanford, 1961):

$$\frac{D_d}{D_m} = \frac{f_m}{f_d} = 2^{-1/3} \frac{(f/f_0)_m}{(f/f_0)_d} \quad (4)$$

Three types of dimer structures were considered: two different side to side (s-s) placements and one end-to-end (e-e), as shown in Figure 1. The ratio of the frictional coefficients of the dimer to monomer f_d/f_m for the two s-s forms was obtained from the results of Squire et al. (1968) on serum albumin. This protein is similar to prothrombin in its hydrodynamic properties. The ratio f_d/f_m for the e-e form was obtained using the Perrin equation (Tanford, 1961) for a prolate ellipsoid assuming an axial ratio of seven for the dimer. Calculated values of these parameters are given in Table I.

Based on the above assumptions and the results obtained on the hydrodynamic modeling of the dimer form of prothrombin, it is seen that only the e-e form of the dimer is consistent with the diffusion and molecular weight values obtained in this study. Autocorrelation functions, free of statistical noise, were computed at $\theta = 90°$ for solutions containing weight fractions of 0.4, 0.5, and 0.6 of e-e dimer. The values of $\bar{D}_z$ and $\bar{M}_w$ for

TABLE II: $\bar{M}_w$ and D Values for Model Systems of Prothrombin with Different Weight Fractions of e-e- Dimers.

Dimer Wt Fraction	Calcd Values from Eq 3a and 3b		D Values ($\times 10^{-7}$ cm^2/s) from Fits to Single Exp.	
	$\bar{M}_w$	D_2 ($\times 10^{-7}$ cm^2/s)	Least Sq.	Graphical
0.4	95 000	5.04	4.88	4.84
0.5	103 000	4.84	4.69	4.65
0.6	110 000	4.66	4.55	4.51

each of these dimer compositions were calculated. These are presented in Table II. Also shown in this table are D values, resulting from least-square fits to a single exponential of the computed autocorrelation functions between 2 and 3 correlation times (corresponding to 30–50 equally spaced delay times τ). The coefficient of determination (a measure of the goodness of fit of the data to the assumed equation) was 0.99 for the three dimer compositions. Table II also shows D values obtained from graphical fits of the experimental data to the assumed equation. It was found that the calculated autocorrelation function from a system with dimer weight fraction of 0.4 could be directly superimposed over experimental data taken on samples of lot 1. A comparison of computed and experimental data at 26 °C gave values for τ_c and $D_{20,w}$ equal to 14.08 μs and 14.11 μs and 4.88×10^{-7} cm^2/s and 4.87×10^{-7} cm^2/s, respectively, in good agreement with the proposed presence of e-e dimers of prothrombin in these preparations. These results are in excellent agreement with a theoretical study by Koppel (1972) which showed that the computer-generated data of a two-component system, complete with statistical noise, deviated only slightly from the best single exponential fit of the same data. The discrepancy between the values of D calculated from eq 3a and those obtained from eq 2 arises from force-fitting the computed data to a single exponential which creates a systematic error in the determination of D (Koppel, 1972). In this study the error is typically 3% for fits obtained between 2 and 3 correlation times and decreases further for shorter delay time ranges. As a direct consequence of this analysis, it is found that, for the case of a two-component system of Rayleigh scatterers, a plot of Γ vs. $|\bar{K}|^2$ yields approximately a straight line which is in good agreement with the findings of this study.

From the above analysis, it is found that solutions of 2 mg/cm^3 prothrombin contain on the average a 0.4 to 0.5 weight fraction of dimers. Limited data from 5 mg/cm^3 solutions indicate a dimer weight fraction of 0.6 or greater. The presence of higher dimer concentration in the latter preparation is also supported by a fit of the sedimentation equilibrium data to the sum of exponentials (Rohde et al., 1975).

$$f_{ij} = f_{aj} \exp\left[\frac{M_i(1 - \bar{v}\rho)\omega^2(r_i^2 - r_a^2)}{2RT}\right] \quad (5)$$

where f_{ij} is the absolute concentration at the radial position r_i due to the jth component, and f_{aj} is the meniscus concentration of the sedimenting species j. Assuming the presence of only one species, a fit was obtained with an average error of 19.5 μm. However, when the same data were analyzed assuming two species are present, monomers and dimers, the value dropped to 9.6 μm which is more in agreement with the average error observed in data collection. An equilibrium constant K of association was then computed for a prothrombin system containing monomers and dimers and it was found to be 1.24×10^5 M^{-1} with the relative error of $\Delta K/K = 0.39$. This value of K predicts a dimer weight fraction of 0.78 when the total concentration of prothrombin increases to 5 mg/cm^3.

The hypothesis of e-e dimerization of prothrombin is also consistent with structural studies of the protein by other authors (Magnusson et al., 1974; Mann et al., 1974). These indicate that over 60% of the carbohydrate groups in prothrombin are attached at neighboring sites, located in the "pro" part of the prothrombin molecule. This carbohydrate localization gives rise to a region of higher hydrophilicity than the remainder of the protein, where the hydrophilic amino acids (less than 10%) are well distributed along the chain. Since prothrombin assumes an ellipsoidal shape in aqueous solutions, its conformation is expected to be such that the carbohydrate groups would be placed near the poles of the ellipsoid, where the local surface to volume ratio is greatest. This conformation should be thermodynamically favored since it minimizes the hydrophobic fraction of the macromolecular surfaee. In the case of dimer formation, it is therefore expected on the basis of similar considerations that an e-e placement, with the hydrophilic groups at the outer poles, would be favored.

Prothrombin Activation. The technique of quasi-elastic light scattering was also used to monitor the fragmentation of the prothrombin molecule to thrombin in a monomer-dimer population of this protein (lot 1). As previously shown, the intensity-intensity autocorrelation function is very sensitive to the presence of large scatterers. Thus, in order to follow this conversion, it was necessary to activate the prothrombin with species that were significantly smaller in size, with respect to the initial prothrombin macromolecule, as well as the conversion products. The nonphysiologic system of dihexanoylphosphatidycholine (DHL)[4] (Roholt and Schlamowitz, 1961) and taipan snake venom (TSV) (Pirkle et al., 1972) satisfies these size requirements and was also found to promote activation in the presence of Ca^{2+} ions.

The activation was carried out in the same buffer as lot 1 prothrombin so as not to disturb the monomer–dimer equilibrium of the protein. The reaction mixture, containing TSV, DHL, and Ca^{2+} ions, was filtered through 0.1-μm pore size Millipore filters prior to its addition to prothrombin solutions and was judged by ultramicroscope observations to be free of large scatterers. The activated solution contained: 1.33 mg/cm^3 prothrombin, 2 μg/cm^3 TSV, 100 μg/cm^3 DHL, and 0.3 mM $CaCl_2$. The relatively low concentration of $CaCl_2$ was necessary to avoid further aggregation of prothrombin, which had been observed in earlier experiments, where Ca^{2+} alone was added to pure prothrombin in concentrations over 0.3 mM.

Changes in the translational diffusion coefficient of the prothrombin–activator mixtures were recorded over a period of 70 to 90 h. The autocorrelation function obtained from these mixtures was also accurately described by a single exponential function. In Figure 2 the translational diffusion coefficient corrected to standard conditions ($D_{20,w}$) and the relative scattered intensity are plotted as a function of time for an activation experiment performed at 15 °C. The progressive increase in D value for the prothrombin–activator mixture is accompanied by a decrease in relative intensity, indicating a smaller value for the weight-average molecular weight of the activated system. The increase in D over the 90-h period, covered by the data in Figure 2, is consistent with fragmentation of prothrombin, and formation of thrombin. This was

[4] Dihexanoylphosphatidylcholine below its critical micelle concentration (~5 mg/cm^3) is predominantly in its monomer forms and has a molecular weight of 472.

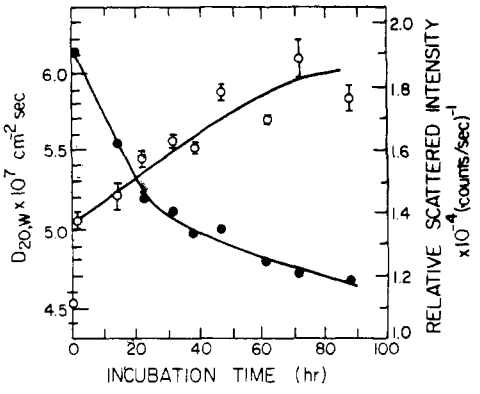

FIGURE 2: $D_{20,w}$ (○) and relative scattered intensity (●) vs. time for a sample of lot 1 human prothrombin in an activation mixture of TSV (2 μg/cm^3), DHL (100 μg/cm^3), and $CaCl_2$ (0.3 mM) at T = 15 °C.

verified by independent clotting experiments, which showed increasing thrombin activity in the system with incubation time. It should be noted, however, that at the end of the 90-h period the average value of D (6.05×10^{-7} cm^2/s) in the system is still considerably lower than the reported diffusion coefficient of pure thrombin ($D_{20,w} = 8.76 \times 10^{-7}$ cm^2/s, Seegers, 1967), indicating the presence of unconverted prothrombin. Based on the average value of D obtained for the activation system after 90 h, the concentrations of prothrombin (monomer + dimer) and thrombin in the system and thus the percentage of converted prothrombin were determined, assuming that no significant change occurs in the dimer weight fraction of prothrombin upon dilution. Initially, three values of the dimer weight fraction for lot 1 prothrombin were considered, 0.4, 0.45, and 0.5, assuming only the formation of monomeric thrombin. The major contribution to the intensity–intensity autocorrelation (hence the z-average diffusion coefficient) was assumed to origihate from the prothrombin (monomer + dimer) and thrombin macromolecules; the remaining individual fragments were neglected because of their significantly lower molecular weights (Mann et al., 1974). The final concentrations of prothrombin and thrombin in the system were obtained by combining the expressions for the z-average diffusion coefficient (eq 3a), the dimer weight fraction

$$\frac{c'_d}{c'_d + c'_m} \tag{6}$$

and conservation of mass

$$c_m + c_d = c'_m + c'_d + c_t + c_f = 1.33 \text{ mg/cm}^3 \tag{7a}$$

$$c_t + c_f = c_t \frac{M_m}{M_t} \tag{7b}$$

In the above expressions c'_m, c'_d, c_t, c_f, M_m, and M_t are respectively the final weight concentrations of prothrombin monomers and dimers, thrombin, and the remaining fragments (intermediates 3 and 4) and the molecular weights of the prothrombin monomer and thrombin. The values for the concentrations were obtained using $D_z = 6.05 \times 10^{-7}$ cm^2/s and M_t = 39 000 (Mann et al., 1974). The relative decrease in the scattered intensity ($\Delta I/I$) that accompanies the fragmentation of prothrombin was also calculated from the expression for the weight-average molecular weight (eq 3b) using the initial and final concentrations of prothrombin, thrombin, and intermediate 3. Here, the intensity contribution of the smaller fragment (intermediate 4) could be neglected and the molecular weight of the larger fragment (intermediate 3) was

TABLE III: Relative Change in Scattered Intensity ($\Delta I/I$) and Percent Conversion of Prothrombin for Various Dimer Weight Fractions of Prothrombin and Thrombin in the Activated System.

Dimer Wt Fractions		%	
Prothrombin	Thrombin	Conversion	$\Delta I/I$
0.50	0	67	0.44
0.45	0	65	0.42
0.40	0	62	0.39
0.40	0.2	67	0.39
0.40	0.3	70	0.39
0.40	0.4	73	0.39

taken to be M_{f_3} = 23 000 (Mann et al., 1974). The concentration of this latter fragment (C_{f_3}) was determined from the following relation

$$C_{f_3} = \frac{C_t M_{f_3}}{M_t} \tag{8}$$

In the first three rows of Table III, the results of this analysis are presented for three different dimer weight fractions of prothrombin in terms of the percent conversion of prothrombin and relative decrease in scattered intensity for the case of the formation of thrombin monomers. It is seen for a dimer weight fraction of 0.4 that the calculated decrease in the scattered intensity is in good agreement with the measured value of 0.38 from Figure 2. Furthermore, the analysis of the translational diffusion coefficient, as well as the scattered intensity data, indicates that on the average only 62% of prothrombin was converted to thrombin in the above nonphysiologic activation system. A similar calculation was also performed for the case, where thrombin monomers and dimers are present in the final activation system. Here, the dimer weight fraction of prothrombin was taken to be 0.4 and three different dimer weight fractions of thrombin were considered. The e-e dimer form of thrombin was chosen based on previous data obtained in this study on prothrombin. The ratio f_d/f_m for the e-e form of thrombin was obtained using the Perrin equation (Tanford, 1961) for a prolate ellipsoid assuming an axial ratio for the dimer equal to twice the mean axial ratio (a/b = 2.8; Seegers, 1967) of thrombin. From eq 4, the translational diffusion coefficient of the thrombin dimer was calculated to be $D_{20,w} = 5.93 \times 10^{-7}$ cm^2/s. In the last three rows of Table III, the results of these calculations are shown as a function of the same parameters for the three dimer weight fraction of thrombin. The values of $\Delta I/I$ for this system are found to be equal to the value previously calculated for the system containing only thrombin monomers. However, it is seen that the percentage of converted prothrombin increases as the dimer weight fraction of thrombin increases. Thus, with initial prothrombin dimer weight fraction of 0.4, the amount of converted prothrombin after 90 h can vary between 62 and 73%, depending on the extent of thrombin dimerization. It should be noted here that the slow and incomplete conversion of prothrombin observed in the presence of dihexanoylphosphatidylcholine is in agreement with a recent binding study by Bull et al. (1972), which showed that phosphatidylcholine alone binds very little to the prothrombin–Ca^{2+} complex.

This study has confirmed the tendency of human prothrombin to aggregate into dimers in aqueous solutions and presents evidence that these dimers have predominantly an end-to-end conformation. In addition, this work demonstrates how the technique of quasi-elastic light scattering in conjunction with standard light scattering methods can be used

as a potential probe for studying the fragmentation process of prothrombin even in a complex monomer–dimer population of this protein. The methodology developed in this study should be applicable as well to other dissociating and associating macromolecular systems with particle sizes in the Rayleigh scattering region.

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