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Structure of the non-covalent complex of prothrombin kringle 2 with PPACK-thrombin

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Abstract

Prothrombin fragment 2 (the second kringle) has been co-crystallized with PPACK (D-Phe-Pro-Arg)-thrombin and the structure of the non-covalent complex has been determined and refined ($R = 0.16$) at 3.2 Å resolution using X-ray crystallographic methods. The kringles interact with thrombin at a site that has previously been proposed to be the heparin binding region. The latter is a highly electropositive surface near the C-terminal helix of thrombin abundant in arginine and lysine residues. These form salt bridges with acidic side chains of kringle 2. Somewhat unexpectedly, the negative groups of the kringle correspond to an enlarged anionic center of the lysine binding site of lysine binding kringles such as plasminogen K1 and K4 and TPA K2. The anionic motif is DGDEE in prothrombin kringle 2. The corresponding cationic center of the lysine binding site region has an unfavorable Arg71Phe substitution but Lys35 is conserved. However, the folding of fragment 2 is different from that of prothrombin kringle 1 and other kringles: the second outer loop possesses a distorted two-turn helix and the hairpin β-turn of the second inner loop pivots at V64 and D70 by 60°. The Lys35 is located on a turn of the helix, which causes it to project into solvent space in the fragment 2-thrombin complex, thereby devastating the cationic center of the lysine binding site. Since fragment 2 has not been reported to bind lysine, it most likely has a different inherent folding conformation for the second outer loop, while the movement of the V64-D70 β-turn is most likely a conformational change accompanying complexation, both of which reveal a new heretofore unsuspected flexibility in kringles. The fragment 2-thrombin complex is the first cassette module-catalytic domain interaction determined for a multi-domain blood protein and only the second domain-domain interaction to be described among such proteins, the other being Ca^{+2} prothrombin fragment 1 (Gla-domain and kringle 1). It has been possible to propose a reasonable model for the four domain prothrombin structure using these two double domain structures.

Key words: Fragment 2; Thrombin complex; Prothrombin kringle 2; α-Thrombin

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Abbreviations: Gla, γ-carboxyglutamic acid; F1, fragment 1, prothrombin$_{1-156}$; F2, fragment 2, prothrombin$_{156-274}$; Gla domain, F1$_{1-48}$; kringle, F1$_{66-144}$ or facsimile; PPACK, D-Phe-Pro-Arg chloromethylketone; TPA, tissue-type plasminogen activator.
Note: Three hundred is added to the numbering of the kringle of F2 beginning with the first disulfide (Fig. 1) to differentiate kringle and thrombin residues.

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1. Introduction

Prothrombin, the most abundant vitamin K-dependent blood protein of coagulation, circulates in the plasma at concentrations between 1 and 2 μM. It is synthesized in the liver as a pre-propeptide consisting of 622 amino acids. The 43 amino acid pre-propeptide region directs the translated, immature polypeptide through a series of post-translational modifications whereby: (1) the ten glutamic acids in the first 33 residues of the mature prothrombin molecule are carboxylated to Gla residues by a vitamin K-dependent carboxylase and (2) the molecule is N-glycosylated at three sites.

The *N*-terminal region of prothrombin preceding prethrombin 2, the immediate inactive precursor of α-thrombin, can be divided into two homologous segments with molecular weights of ~ 23 000 (F1) and 14 000 (F2), respectively. Based on the relatively high degree of ~40% homology between the C-terminal residues of F1 and F2 (Magnusson, 1975) (Fig. 1), it has been suggested that a partial gene duplication may have occurred during the evolution of prothrombin. Prothrombin F1 consists of a Gla domain and a three disulfide, triple loop kringle module (Fig. 1). It is obtained by the proteolytic cleavage of prothrombin at Arg156 by thrombin.

Kringles are a common motif occurring in many proteins involved in blood coagulation and fibrinolysis (Patthy, 1985; Tulinsky, 1991). The function of some is to bind to fibrin (Lerch, 1980) and although the function of the Gla domain of F1 is to bind to phospholipid (Stenflo, 1974; Bajaj, 1975; Nelsestuen, 1976) the F1 kringle has no known function. The ten Gla residues in prothrombin F1 can bind Ca^{2+} ions, whereupon there is a fluorescence quenching event implying a conformational change (Nelsestuen, 1976; Prendergast, 1977). The change has also been observed by circular dichroism (Bloom, 1978) and has since been established to be a folding transition by crystallographic studies of bovine F1 in the absence (Park, 1986; Tulinsky, 1988a; Seshadri, 1991) and in the presence of Ca^{2+} (Soriano-Garcia, 1992) or Sr^{2+} ions (unpublished results of this laboratory). Calcium ion binding and the associated conformational change are an essential requirement for the binding of prothrombin and

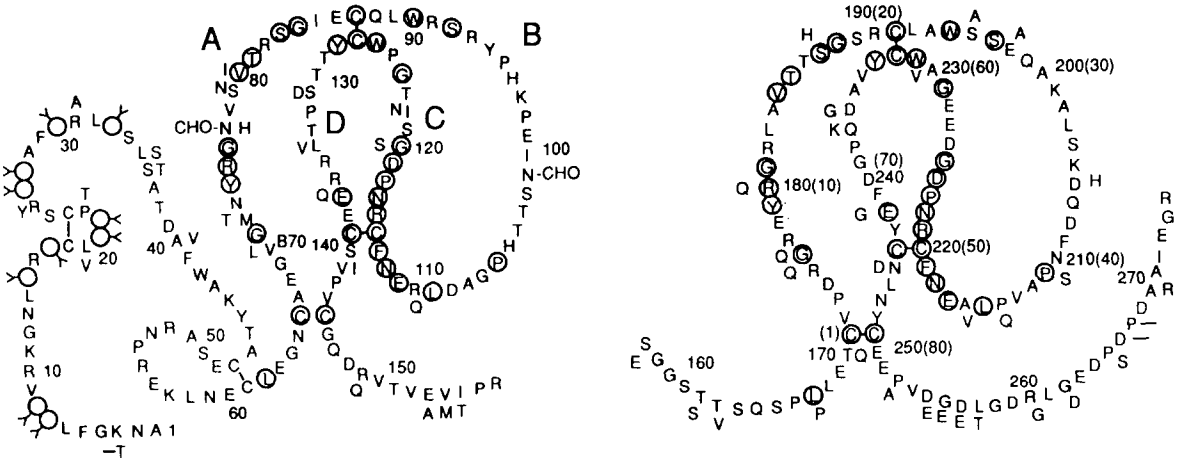

Fig. 1 Sequences of bovine prothrombin fragment 1 (left) and fragment 2 (right). Alternate residues of human counterparts indicated to side of bovine sequences. Conserved residues between F1 and F2 circled; Gla residues designated by open circles and a 'Y'; deletions indicated with hyphen; loops of kringles designated A, B, C, D; CHO is site of carbohydrate attachment; sequential numbering of a single kringle beginning with first disulfide given for F2 in parentheses; other numbering in figure corresponds to that of prothrombin.

F1 to membranes that contain acidic phospholipids. The membrane binding capability in the presence of Ca^{2+} ions is also thought to be important for the effective presentation of prothrombin as a substrate for its subsequent activation to thrombin in the physiologic prothrombinase complex (Mann, 1987), which additionally encompasses Factor Xa and membrane bound Factor Va.

The second kringle and interkringle peptide regions of prothrombin correspond to prothrombin F2 (Fig. 1). This kringle is identical in loop lengths with kringle 1 of prthrombin and has been said to bind Ca^{2+} ions and to the heavy chain of Factor Va (Esmon, 1974; Bajaj, 1975). It also associates with α-thrombin, possessing a K_d in the nanomolar range (Myrmel, 1976) where thrombin most likely mimics prethrombin 2 in the complex producing a prethrombin1-like fragment (F2 linked to prethrombin 2). The binding of F2 partially protects thrombin from inactivation by antithrombin III (Walker, 1979), which is specific for thrombin since Factor Xa is not protected in a similar manner. Prothrombin F2 also acts as a competitive inhibitor of protein C activation (Jakubowski, 1986) and bovine F2 enhances esterolytic activity of both human and bovine α-thrombin but human F2 does not (Myrmel, 1976).

We report here the X-ray crystallographic structure of human F2 complexed with human PPACK-α-thrombin. The binding interaction between F2 and thrombin in the structure of the complex is clear and occurs in the putative heparin binding site (Church, 1989; Bode, 1992; Karshikov, 1992). The folding of the F2 kringle differs significantly from that of the kringle of F1 and other similarly sized kringles of known structure and is consistent with at least a partial conformational change in F2 that optimizes the binding interaction with thrombin. The binding interaction is principally ionic with an aromatic stacking component; the former involves an enlarged anionic center of the lysine binding site of lysine-binding kringles (Tulinsky, 1988b) and a group of arginine residues located near the C-terminal helix of thrombin (Rydel, 1990; Bode, 1992). The binding interaction is also most likely related to the protection afforded thrombin against antithrombin III inactivation.

2. Kringle-thrombin interaction

The structure of the F2-thrombin complex is shown in Fig. 2 from which it will be seen that the F2 kringle makes extensive contacts with thrombin. The kringle interacts at a thrombin site that has been proposed to be the heparin binding region (Church, 1989; Bode, 1992). The latter was confirmed to be a highly electropositive surface in electrostatic potential energy calculations (Karshikov, 1992) near the C-terminal helix of the B-

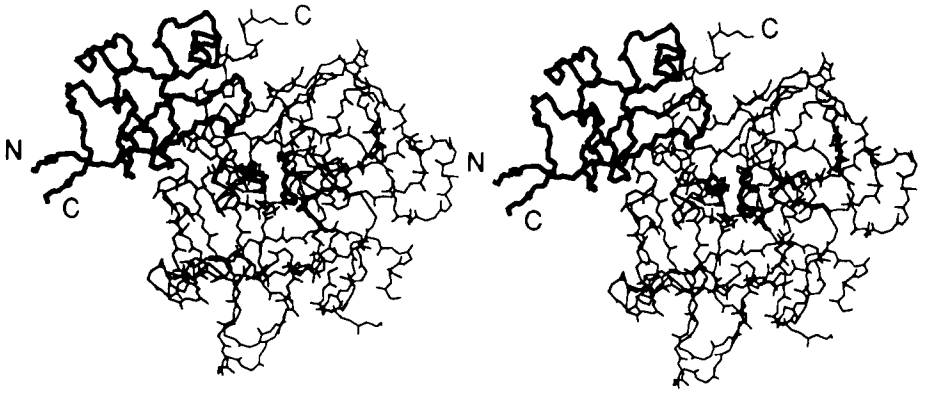

Fig. 2 Stereoview of the folded structure of fragment 2-thrombin. F2 kringle and catalytic triad of thrombin shown in bold; only $C_\alpha CN$ of F2 shown; C– and N– termini of kringle and C-terminus of thrombin B-chain designated.

chain of thrombin that is abundant in arginine and lysine residues. Not surprisingly then, these positively charged residues form salt bridges with acidic side chains of the kringle (Fig. 3), some of which are hydrogen bonded. Somewhat unexpectedly, however, the negatively charged groups of the kringle correspond to an enlarged anionic center of the lysine binding site of lysine binding kringles, such as kringles 1 and 4 of plasminogen and kringle 2 of TPA (Tulinsky, 1988b; Mulichak, 1991; Wu, 1991; de Vos, 1992). The anionic motif is Asp-X-Asp in lysine binding kringles, but is AspGlyAspGluGlu in F2. The salt bridges are particularly intense between Arg93, Asp354 and Asp356[1] at one end of the interdomain contact and Arg175, Glu357 and Glu358 at the other end (Fig. 3).

The total number of contacts less than 4.0 Å between thrombin and F2 in the interdomain interaction is ~102. If the putative heparin binding site of thrombin does in fact become established to be authentic, the utilization of it by F2 will be yet another example of the multiple functional diversity displayed by the various binding regions of the thrombin molecule (Tulinsky, 1992). Moreover, the utilization of the anionic center of lysine binding kringles by F2 in binding to thrombin is an example of a similar diversity being exhibited by kringles. Another is the recent report of heparin binding to the kringle of urokinase, where the electrical polarity of the anionic center between residues 52–60 is reversed by the presence of four arginine residues in urokinase (Stephens, 1992).

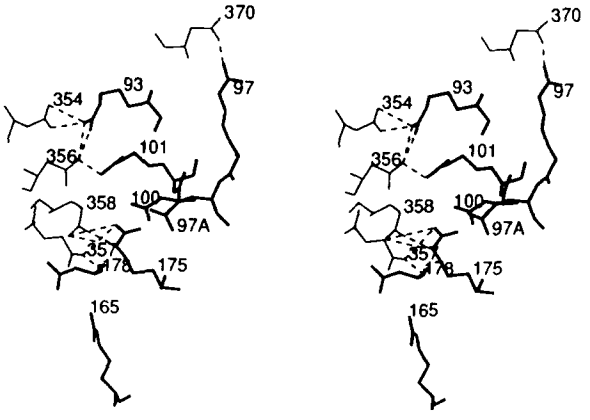

Fig. 3 Stereoview of principal electrostatic interaction between fragment 2 and thrombin. Positively charged residues of thrombin in bold; ion pair interactions designated with broken lines.

The electrostatic forces producing the F2-thrombin interaction have been investigated by calculating the electrostatic potentials of F2 and thrombin separately and in the complex. The program DELPHI of the suite of Biosym programs was used for the calculation using 0.15 for the ionic strength of the solvent. The electrostatic potentials of the kringle and thrombin that have been calculated separately reveal that the positive potential extending from the C-terminal helix of thrombin is remarkably complementary to the negative potential recesses of the lysine binding-like anionic center of F2. The electrostatic potential of the intact complex shows that the potential of the interface region between the two domains is essentially compensated and neutralized.

3. Conformational changes

The C_α-structure of thrombin in F2-thrombin and that of thrombin in the PPACK-thrombin complex (Bode, 1992) have been compared in order to ascertain structural changes accompanying complex formation. The structure of PPACK-thrombin used was that reported by Qiu (1992), which was measured and refined comparably to the F2-thrombin complex. Using the PPACK-thrombin structure, the root mean squared (RMS) difference in positions is 0.7 Å; removing 35 differences $> 1\sigma$ (~13%) reduces this to 0.3 Å, which is within the error of the structure determination. Minor changes are observed in the conformations of two of the side groups in the interdomain region (Arg93 and Arg175); however, the guanidinium groups appear to be in similar positions as those of PPACK-thrombin. These, along with Arg101, are the dominant interactions with the kringle module (Fig. 3). The largest deviations in thrombin occur near the poorly defined C-terminal of the A-chain. Thus, thrombin essentially accommodates the fragment 2 complex formation with little change in its folded structure.

From the sequence of F2 (Fig. 1), it will be seen that the A and C loops have a fairly high degree

of conservation with those of K1 of F1 (15 of 31, 50%), but the same is not so for the B and D loops (9 of 38, 24%). This is also generally true of kringle modules found in other protein systems (Tulinsky, 1988a). Moreover, the lengths of the loops of the F1 and F2 kringles are identical. Since the folding of kringle 1 of F1 (Seshadri, 1991), plasminogen kringle 1 (unpublished results of this laboratory) and kringle 4 (Mulichak, 1991) are the same, it is somewhat surprising that the folding of kringle 2 in F2-thrombin differs from these kringles (Fig. 4). Kringle 2 of TPA also has a different folding (Byeon, 1991; de Vos, 1992) but this has been attributed to its different sized-loop structure.

The folding of F2 is compared with kringle 1 of F1 in Fig. 4, from which it can be seen that the second outer B-loop assumes a completely different conformation possessing a distorted two-turn helix (Ala328-Lys335) and the hairpin β-turn of the second inner loop pivots as a unit $\sim 60°$ at Val364 and Asp370. These features lead to 30 contacts less than 4.0 Å in the interface of the complex, which appear to be as important as the electrostatic interaction in maintaining the structure of the complex. The β-turn region of TPA kringle 2 shows a greater twist of the β-strand between 364–370 compared to K1 of F1 and plasminogen kringles 1 and 4, but not the hinged pivot motion as found in F2-thrombin. This movement in F2 is most likely a kringle conformational change accompanying complexation and the two-turn helix is similar to that recently observed by NMR in the urokinase

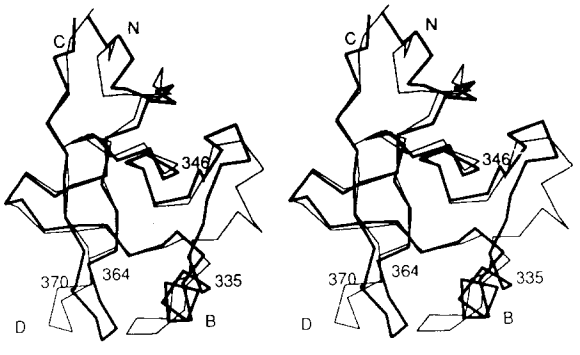

Fig. 4 Stereoview comparing the C_α-folding of kringles 1 and 2 of prothrombin. Kringle 2 of F2 shown in bold; B and D loops (Fig. 1) of kringle indicated.

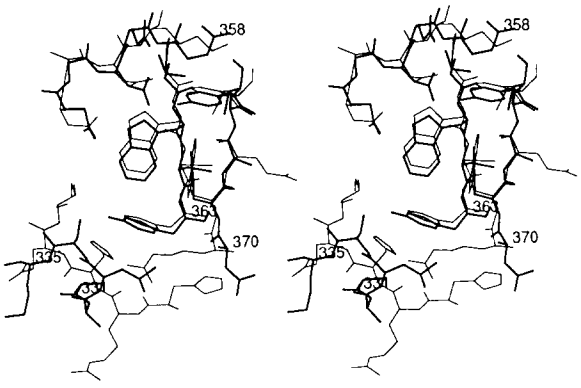

Fig. 5 Stereoview comparing the lysine binding site of plasminogen kringle4 with the same region in kringle 2 of F2. Kringle 2 shown in bold; anionic center of kringle4 at top, cationic center at bottom.

kringle Li (1992), *both revealing a new flexibility heretofore unknown in kringles.* Residues Gly368-Asp369 of F2 run parallel to Tyr94-Asn95 of thrombin at ~ 3.5–4.0 Å and Pro368 is sandwiched in between Ile90 and Pro92. Except for the pivoting movement, and the differently folded B-loop, the folding of F1 and F2 kringles is practically the same. This is especially the case for the first inner loop (Fig. 5), which comprises the anionic center of lysine binding kringles that produces the principal electrostatic interaction with thrombin in the F2 complex by making 18 contacts less than 4.0 Å with the putative heparin binding site.

The same is not true of the region of F2 corresponding to the cationic center of lysine binding kringles, which neither possesses the requisite positively charged residues appropriately located nor the folding conformation of such kringles. The important positive residues in kringle 4 of plasminogen are Lys35 and Arg71 (Mulichak, 1991; Wu, 1991) and Lys33 in TPA kringle 2 (Byeon, 1991; de Vos, 1992). In fragment 2, position 371 is phenylalanine, which clusters with Trp361, Tyr363 and Tyr373 and position 333 is occupied by leucine. Although Lys335 is conserved in F2, it is on a turn of the newly observed helix of F2 which, as a consequence, causes it to project out into solvent region.

The conformation of the second outer B-loop of the F2 kringle in the thrombin complex is different

from that observed in all other kringles, additionally displaying flexibility of folding in kringles. Its most distinguishing characteristic is a distorted two-turn helix between Ala328-Lys335, similar to the one in the urokinase kringle module (Li, 1992). The kringle helical turns abut the Lys236-Val241 turn of the C-terminal helix of the B-chain of thrombin. A helical turn also occurs in the same second outer loop of TPA kringle 2, but it is located five residues away between 41–45 (de Vos, 1992) and is three dimensionally, positionally very different from that of F2. The remainder of the loop in F2 is in an extended conformation that is again different from other kringles. Since the structure of the F2 kringle alone has yet to be determined, it is not clear whether the different conformation of this loop is inherent to the kringle, as in urokinase, or whether it is also due to a conformational change upon complexation. If the latter, the helical transition might have been induced by the close approach to the C-terminal helix of the B-chain of thrombin and its intense positive electrostatic field (Karshikov, 1992). However, since the F2 kringle could potentially display a lysine binding site if its folding corresponded to that of kringle 4, the fact that it is generally thought not to bind lysine is more consistent with a different native folding conformation. Further support for this inference is the same two-turn helix that has been established in the urokinase kringle by NMR (Li, 1992). Lastly, the outer B-loop of the kringle makes one other impressive contact with thrombin, where His336 intercalates among Tyr89, Trp237 and Val241.

4. A proposed structure for prothrombin

The F2-thrombin complex is the second cassette module-catalytic domain interaction to be determined for a multi-domain blood protein, the other is a factor Xa structure less the Gla domain (Padmanabhan, 1993) and only the third domain-domain interaction to be described among such proteins, the other one being that of Ca^{+2}-prothrombin fragment 1 (Gla domain and kringle) (Soriano-Garcia, 1992). The structure of Ca^{+2} prothrombin fragment 1 consists of two discoid domains: a triangular Gla domain complexed with seven Ca^{+2} ions, principally through the side groups of Gla residues, and an ellipsoidal kringle domain (18 × 28 × 30 Å) (Soriano-Garcia, 1992). The remaining component of prothrombin is prethrombin 1, which probably can be approximated by the structure of the F2-thrombin complex. If these two double domain fragments are oriented reasonably well with respect to one

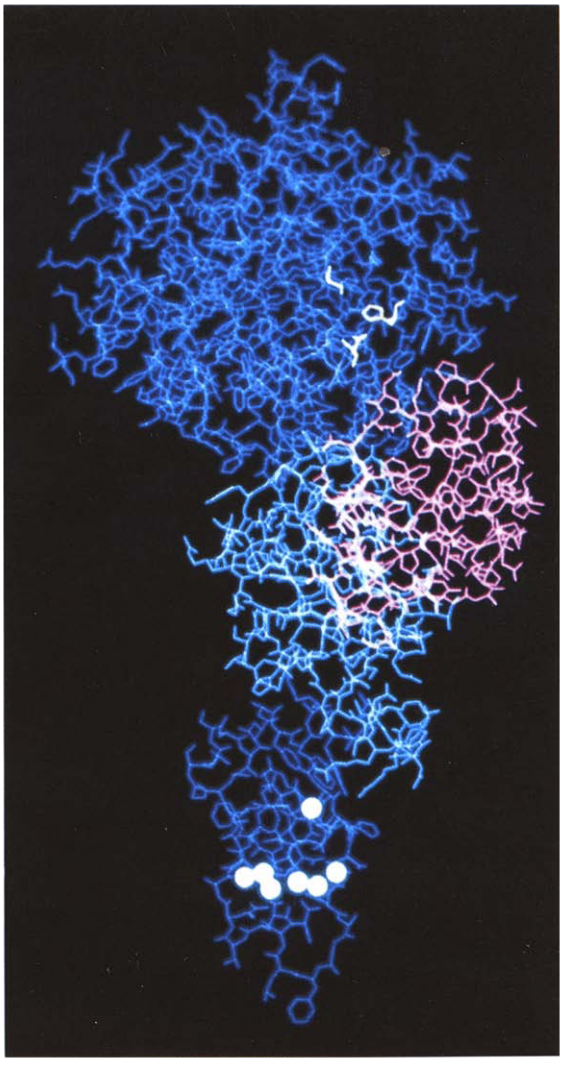

Fig. 6 Model of prothrombin based on the structures of Ca^{+2} fragment 1 and the fragment 2-thrombin complex. Gla domain, dark blue (bottom); kringle 1, light blue; kringle 2, pink; thrombin, blue (top); catalytic triad, white in thrombin domain; Ca^{+2} ions, white spheres in Gla domain.

another, they should mimic the structure of the multi-domain prothrombin molecule.

An approach to orient the fragments was made by examining the electrostatic potential of Ca^{+2} fragment 1 calculated with DELPHI (Soriano-Garcia, 1992). The Gla and kringle domains are, in general, electropositive, whereas the tetradecapeptide disulfide loop connecting the two (Fig.1) is highly electronegative. The kringle of F2 is also electropositive, except at the anionic center corresponding to lysine binding kringles. Therefore, the electropositive F2 kringle of F2-thrombin was docked at the electronegative disulfide loop of F1 in a manner such as to compensate and neutralize charge, and such that the overall length of the resultant four domain structure was about 120 Å (Fig. 6). This is in agreement with 110 Å estimated from light scattering (Lim, 1977) and 130 Å, with a radius of gyration of 35 Å, from low angle solution X-ray scattering (Osterberg, 1980). The model also agrees with the axial ratios predicted from light scattering (2.8 vs. 3.0 for the model). If the Gla domain, which has Phe5-Leu6 and Val9 exposed at an apex of the triangular module, is embedded into a phospholipid surface to the linear array of seven Ca^{+2} ions of the Gla domain, then the active site of thrombin is ~60–70 Å above the phospholipid surface, which is the same as that approximated from fluorescence energy transfer experiments (Husten, 1987). Nelsesteuen (1984) has estimated that 1100 $Å^2$ of membrane surface, is covered by the Gla domain in membrane binding, corresponding to 16 phospholipid molecules. The cross-section of the Gla domain in Ca^{+2} fragment 1 is 25 × 35 Å or 900 $Å^2$.

The gross aspects of the model of prothrombin shown in Fig. 6 correspond quantitatively rather than qualitatively with certain dimensional parameters that are accepted for prothrombin. Although the interactions among the different domains of the model of prothrombin most likely differ in some of their details from that of the actual structure, and the interkringle peptides are not accounted for, and the structure of thrombin rather than prethrombin 2 has been utilized in the modeling, nonetheless, the model of prothrombin thusly arrived at is impressively consistent with well established observations. The model could be served well by the structure determination of fragment 1–2 (fragment 1 + fragment 2).

5. Acknowledgement

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6. Note added in proof

Site specific mutagenesis has implicated Leu5 of protein C (Leu6 of prothrombin) as crucial for the hydrophobic component of phospholipid binding of the Gla domain (Zhang, 1993). This residue is strictly conserved in vitamin K-dependent proteins.

7. References

Bajaj, S.P., Butkowski, R.J. and Mann, K.G., 1975, Prothrombin fragments. Calcium binding activation kinetics. J. Biol. Chem. 250, 2150–2156.

Bloom, J.W. and Mann, K.G., 1978, Metal ion induced conformational transitions of prothrombin and prothrombin fragment 1. Biochemistry 17, 4430–4438.

Bode, W., Turk, D. and Karshikov, A., 1992, The refined 1.9 Å X-ray crystal structure of D-Phe-Pro-Arg chloromethylketone-inhibited human α-thrombin: Structure analysis, overall structure, electrostatic properties, detailed active-site geometry, and structure-function relationships. Protein Sci. 1, 426–471.

Byeon, I.L. and Llinas, M., 1991, Solution structure of tissue-type plasminogen activator kringle 2 domain complexed with 6-aminohexanoic acid: An antifibrinolytic drug. J. Mol. Biol. 222, 1035–1051.

Church, F.C., Pratt, C.W., Noyes, C.M., Kalayanamit, T., Sherril, G.B., Tobin, R.B. and Meade, J.B., 1989, Structural and functional properties of human α-thrombin, phosphopyridoxylated α-thrombin, and γ_T-thrombin. J. Biol. Chem. 264, 18419–18425.

deVos, A.M, Ultsch, M.H., Kelley, R.F., Padmanabhan, K., Tulinsky, A., Westbrook, M.L. and Kossiakoff, A.A., 1992, Crystal structure of the kringle 2 domain of tissue plasminogen activator at 2.4A resolution. Biochemistry 31, 270–279.

Esmon, C.T. and Jackson, C.M., 1974, The conversion of prothrombin to thrombin. IV. The function of the fragment 2 region during activation in the presence of factor V. J. Biol. Chem. 249, 7791–7797.

Huston, E.J., Esmon, C.T., and Johnson, A.E., 1987, The active site of blood coagulation factor Xa: Its distance from the phospholipid surface and its conformational sensitivity

to components of the prothrombinase complex. J. Biol. Chem. 262, 12953–12961.

Jakubowski, H.V., Kline, M.D. and Owen, W.G., 1986, The effect of bovine thrombomodulin on the specificity of bovine thrombin. J. Biol. Chem. 261, 3876–3882.

Karshikov, A., Bode, W., Tulinsky, A. and Stone, S.R., 1992, Electrostatic interactions in the association of proteins: An analysis of the thrombin-hirudin complex. Protein Sci. 1, 727–735.

Lerch, P.G., Rickli, E.E., Lergier, W. and Gillessen, D., 1980, Localization of individual lysine-binding regions in human plasminogen and investigation on their complex-forming properties. Eur. J. Biochem. 107, 7–13.

Li, X., Smith, R.A.G. and Dobson, C.M., 1992, Sequential ^{1}H NMR Assignments and secondary structure of the kringle domain from urokinase. Biochemistry 31, 9562–9571.

Lim, T.K., Bloomfield, V.A. and Nelsestuen, G.L., 1977, Structure of the prothrombin- and blood clotting factor X-membrane complexes. Biochemistry 16, 4177–4181.

Magnusson, S., Petersen, T.E., Scottrup-Jensen, L. and Claeys, H., 1975, Complete primary structure of prothrombin: Isolation, structure, and reactivity of ten carboxylated glutamic acid residues and regulation of prothrombin activation by thrombin, in: E. Reich, D.B. Rifkin and E. Shaw (Eds.), Cold Spring Harbor Conferences on Cell Proliferation 2, Proteases and Biological Control, Cold Spring Harbor Laboratory, New York, pp. 123–149.

Mann, K.G, 1987, The assembly of blood clotting complexes on membranes. Trends Biochem. Sci. 12, 229–233.

Mulichak, A.M. Tulinsky, A., and Ravichandran K.G., 1991, Crystal and molecular structure of human plasminogen kringle 4 refined at 1.9–Å resolution. Biochemistry 30, 10576–10588.

Myrmel, K.H., Lundblad, R.L. and Mann, K.G., 1976, Characteristics of the association between prothrombin fragment 2 and α-thrombin. Biochemistry, 15, 1767–1773.

Nelsestuen, G.L., 1976, Role of γ-carboxyglutamic acids. J. Biol. Chem. 251, 5648–5656.

Nelsestuen, G.L., 1984, Calcium function in blood coagulation, in: H. Sigel (Ed.), Metal Ions in Biological Systems, Marcel Dekker, Inc., New York, pp. 353–380.

Osterberg R., Sjoberg B., Osterberg P., and Stenflo J., 1980, Conformational change of human prothrombin induced by calcium ions: An X-ray scattering study. Biochemistry 19, 2283–2286.

Padmanabhan, K., Padmanabhan, K.P., Tulinsky, A., Park, C.H., Bode, W., Huber, R., Blankenship, D.T., Candin, A.D. and Kisier, W., 1993, Structure of human des(1-45) factor $\bar{X}$a at 2.2 Å resolution. J. Mol. Biol. 232, 947–966.

Park, C.H. and Tulinsky, A., 1986, Three-dimensional structure of the kringle sequence: Structure of prothrombin fragment 1. Biochemistry 25, 3977–3983.

Patthy, L., 1985, Evolution of the proteases of blood coagulation and fibrinolysis by assembly from modules. Cell 41, 657–663.

Prendergast, F.G. and Mann, K.G., 1977, Differentiation of metal ion-induced transitions of prothrombin fragment 1. J. Biol. Chem. 252, 840–850.

Qiu, X., Padmanabhan, K.P., Carperos, V.E., Tulinsky, A., Kline, T., Maraganore, J.M. and Fenton II, J.W., 1992, The structure of the hirulog3-thrombin complex and the nature of the S′ subsites. Biochemistry, 31, 11689–11697.

Rydel, T.J., Ravichandran, K.G., Tulinsky, A., Bode, W., Huber, C., Roitsch, C. and Fenton II, J.W., 1990, The structure of a complex of recombinant hirudin and human α-thrombin. Science 249, 277–280.

Seshadri, T.P., Tulinsky, A., Skrzypczak-Jankun, E. and Park, C.H., 1991, Structure of bovine prothrombin fragment 1 refined at 2.25 Å resolution. J. Mol. Biol. 220, 481–494.

Soriano-Garcia, M., Padmanabhan, K., deVos, A.M. and Tulinsky, A., 1992 The Ca^{2+} ion and membrane binding structure of the Gla domain of Ca-prothrombin fragment 1. Biochemistry 31, 2554–2566.

Stenflo, J., 1974, Vitamin K dependent modifications of glutamic acid residues in prothrombin. Proc. Natl. Acad. Sci. USA 71, 2730–2733.

Stephens, R.W., Bokman, A.M., Myohauen, H.T., Reisberg, T., Tapiovaara, H., Pedersen, H., Grondahl-Hansen, J., Llinas, M. and Vaheri, A., 1992, Heparin binding to the urokinase kringle domain. Biochemistry 31, 7572–7579.

Tulinsky, A., 1991, The structure of domains of blood proteins. Thromb. Haemostasis 66, 16–31.

Tulinsky, A., Park, C.H. and Skrypczak-Jankun, E., 1988a, The structure of prothrombin fragment 1 refined at 2.8 A resolution. J. Mol. Biol. 202, 885–901.

Tulinsky, A., Park, C.H., Mao, B. and Llinas, M., 1988b, Lysine/fibin binding sites of kringles modeled after the structure of kringle 1 of prothrombin. Proteins 3, 85–96.

Tulinsky, A. and Qiu, X., 1993, Active site and exosite binding of α-thrombin. Blood Coag. Fibrinoly. 14, 305–312.

Walker, F.J. and Esmon, C.T., 1979, The effect of prothrombin fragment 2 on the inhibition of thrombin by antithrombin III. J. Biol. Chem. 254, 5618–5622.

Wu, T.P., Padmanabhan, K., Tulinsky, A. and Mulichak, A., 1991, The refined structure of the ε-aminocaproic acid complex of human plasminogen kringle 4. Biochemistry 30, 10589–10594.

Zhang, L. and Castellino, F.J., 1993, The hydrophobic component of the binding energy of human coagulation protein C to acidic phospholipid vesicles contains a major contribution from leucine 5 in the γ-carboxyglutamic acid domain. J. Biol. Chem. in press.