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REVIEW ARTICLE

Meizothrombin, a Major Product of Factor X_a-Catalyzed Prothrombin Activation

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Key words

Meizothrombin – Thrombin – Prothrombin activation

Introduction

The factor X_a-catalyzed conversion of the zymogen prothrombin into the active serine protease thrombin is one of the crucial steps of blood coagulation (1). Thrombin is the enzyme responsible for the conversion of fibrinogen into fibrin, a reaction that is followed by fibrin polymerization and clot formation. Thrombin also activates the transglutaminase factor XIII to factor $XIII_a$, an enzyme which stabilizes the fibrin clot. Apart from its role in the formation and stabilization of the fibrin clot, thrombin also has an important function in the regulation of the overall hemostatic process. Thus, thrombin accelerates its own rate of formation by activating the blood coagulation factors V and VIII and by stimulating blood platelets. Potential negative feedback control is exerted through the thrombin-dependent activation of protein C, producing an enzyme with anticoagulant properties that inactivates factors V_a and $VIII_a$ and that may also stimulate the fibrinolytic pathway. Considering the key role of thrombin in hemostatic plug formation, it is not surprising that the activation of prothrombin is one of the most intensively studied coagulation reactions (2) and that the product of this reaction, thrombin, is one of the best characterized coagulation enzymes (3).

Thrombin is not the only catalytically active product that is formed during prothrombin activation. In recent years it has been shown that during factor X_a-catalyzed prothrombin activation in addition to thrombin substantial amounts of another enzymatically active product i.e. meizothrombin can be formed (4, 5). Meizothrombin differs significantly from thrombin in many of its properties and therefore, it may have different functions during the hemostatic process. In view of the pivotal role of thrombin in blood coagulation it will not be surprising that further studies of another enzymatically active prothrombin derivative with different catalytic properties may add to our understanding of the mechanisms of the reactions leading to thrombus formation. This paper is aimed at reviewing our present knowledge of meizothrombin formation during factor X_a-catalyzed prothrombin activation and at discussing current data regarding the functional properties of meizothrombin as compared to thrombin.

Peptide Bond Cleavages During Prothrombin Activation

Before introducing the pathways via which prothrombin can be activated and converted into thrombin or meizothrombin it will be helpfull to discuss some properties of the prothrombin molecule that are relevant for its activation by limited proteolysis. Prothrombin is a single chain glycoprotein with a molecular weight of about 72,000 that consists of three domains with different functions (6). The fragment 1 domain contains the γ-carboxyglutamic acid residues essential for the Ca^{2+}-dependent binding of prothrombin to procoagulant membranes, the fragment 2 region is thought to be involved in the interaction with the protein cofactor V_a and the carboxyterminal (prethrombin 2) domain is highly homologous to trypsin and is converted to thrombin after further activation. In the zymogen molecule there are several peptide bonds that are susceptible to proteolytic cleavage (Fig. 1). It appears that two of these bonds are cleaved by factor X_a and these are at Arg273 – Thr274 (site A) and at Arg322 – Ile323 (site B)[1]. The other peptide bonds (bonds 1 through 6) are target sites for cleavage by thrombin and presumably also meizothrombin.

In order to convert prothrombin into thrombin both site A and site B have to be cleaved by factor X_a (7). Cleavage at site A by factor X_a yields the activation peptide fragment 1.2 and the zymogen intermediate, prethrombin 2. Cleavage at site B results in exposure of the active site and depending on whether or not site A has already been cleaved, the active enzymes thrombin (M_r = 36,000) or meizothrombin (M_r = 72,000) can be formed. Since the Arg322 – Ile323 bond (site B) lies within a disulfide bridge, both thrombin and meizothrombin are two-chain enzymes, the active site of which is located on the chain originating from the carboxyterminal part of the prothrombin molecule. This so-called thrombin B-chain (M_r = 31,000) is highly homologous to trypsin and contains the serine, histidine and aspartic acid residues that comprise the catalytic triad of serine proteases. The light chain of thrombin (A-chain) contains 49 aminoacid residues and has a molecular mass of about 5,000 dalton, whereas the second chain of meizothrombin consists of the complete aminoterminal part of

The nomenclature used for proteolytically derived products of prothrombin is that recommended by the International Committee on Thrombosis and Haemostasis (Jackson C M. Thromb Haemostas 1977; 38: 567–77).

Abbreviations
S2238, D-phenylalanyl-L-pipecolyl-L-arginine-p-nitroanilide-dihydrochloride; DFP, diisopropylphosphofluoridate; meizothrombin-desF1, meizothrombin des fragment 1; DAPA, dansylarginine-N-(3-ethyl-1,5-pentanediyl) amide; PS, phosphatidylserine; PC, phosphatidylcholine.

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[1] Throughout this paper we have used the amino acid numbering for human prothrombin. Human prothrombin consists of 581 residues whereas bovine prothrombin contains 582 residues. This difference is due to a deletion of the fourth residue in the human molecule. Consequently, the numbering of amino acids of human prothrombin differs by one residue with that of bovine prothrombin.

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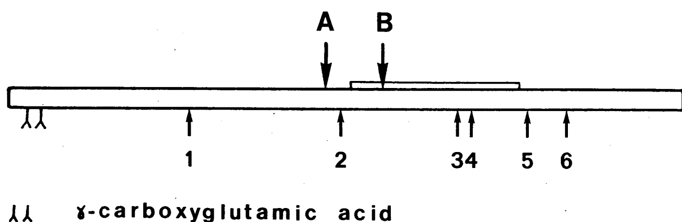

Fig. 1 Proteolytic cleavage sites in prothrombin. Thrombin formation is the result of cleavage of site A (Arg273–Thr274) and site B (Arg322–Ile323) by factor X_a. Cleavage at site B results in active site exposure. The cleavage sites indicated by numbers are target sites for (meizo)thrombin. Cleavage at site 1 (Arg155–Ser156) causes the removal of fragment 1. Proteolysis of peptide bond 2 (Arg286–Thr287) liberates fragment 1. 2. 3. The cleavages 3–6 have been described to occur in α-thrombin by autocatalysis converting it in so-called β- and γ-thrombin. For further details see text

the prothrombin molecule (fragment 1.2-A, M_r = 41,000). A clear description of the reaction products formed during factor X_a-catalyzed prothrombin activation has always been seriously hampered by the fact that there are a number of peptide bonds in prothrombin and in its activation products that are susceptible to proteolysis by thrombin (Fig. 1). The most important ones, at least concerning the proteolysis of prothrombin, are bond 1 (Arg155 – Ser156) and bond 2 (Arg286 – Thr287). Thus, thrombin and meizothrombin will readily cleave prothrombin (or meizothrombin) at site 1 resulting in the removal of the fragment 1 region. Site 2 appears to be less readily cleaved in the bovine molecule than it is in the human molecule. Thus, human meizothrombin-desF1 will rapidly convert into thrombin (with a slightly shorter A-chain) and fragment 2.3 by autocatalytic cleavage at site 2. Recently, Rabiet et al. (8) reported that fragment 1.2.3, which results from cleavage at site 2 instead of site A, is the major activation peptide that is formed during prothrombin activation in human plasma.

To be complete a number of other peptide bonds that are susceptible to cleavage by thrombin are also indicated in Fig. 1. These are most likely only available for proteolysis in thrombin itself and give rise to derivatives of thrombin (so-called β- and γ-thrombin) with different catalytic properties (9). Thus, fully active thrombin (α-thrombin) can be cleaved in its heavy chain (B-chain) at sites 3 and 4, liberating a peptide of twelve aminoacids and β-thrombin. Further proteolysis at sites 5 and 6 liberates another 22 residues and γ-thrombin. The remaining domains in the thrombin B-chain of these products are held together by non-covalent interaction. It is, however, outside the scope of this review to discuss the structural and functional properties of these thrombin derivatives since in this paper we will focus on the enzymatically active products that are formed by factor X_a i.e. meizothrombin and α-thrombin.

Pathways of Prothrombin Activation

From the discussion in the previous paragraph it will be clear that in principle two pathways exist for the factor X_a-dependent conversion of prothrombin into thrombin (Fig. 2). In pathway I site A is cleaved first resulting in the zymogen intermediate prethrombin 2 and the activation peptide fragment 1.2 whereas in pathway II prothrombin is initially cleaved at site B to result in the enzymatically active product, meizothrombin. Thrombin is subsequently formed when factor X_a cleaves the second bond in the activation intermediate (site B in prethrombin 2 or site A in meizothrombin).

Early investigations on product formation during the activation of prothrombin did not produce evidence for the existence of meizothrombin as reaction intermediate (7, 10, 11, 12, 13). In 1974, Esmon et al. (14), therefore, suggested that pathway I describes the most plausible sequence of reactions during factor X_a-catalyzed prothrombin activation. Their proposal was based on the observation that substantial amounts of prethrombin 2 were generated during prothrombin activation whereas meizothrombin was not detected. Until a few years ago this hypothesis was generally held true, although it had been shown with snake venom prothrombin activators (e.g. *Echis carinatus* venom) that site B is in principle available for proteolysis in the native prothrombin molecule (15–19). However, the activator from *Echis carinatus* is an enzyme that is vastly different from factor X_a and is incapable of cleaving prothrombin at site A. More recently a prothrombin activator was purified from the venom of *Notechis scutatus scutatus* (tiger snake) (20) which is remarkably similar to factor X_a. This venom activator appeared to be capable of readily cleaving peptide bonds A and B of prothrombin in a random fashion and produce both thrombin and meizothrombin. Despite the fact that meizothrombin was not detected in the original studies on the pathways of factor X_a-catalyzed prothrombin activation later observations reopened the possibility of meizothrombin formation. Primary cleavage of bond B (i.e. meizothrombin formation) was reported for the mutant prothrombin molecule, prothrombin Barcelona (21). However, this molecule is different from native prothrombin since the arginine at site A has been substituted for by cystein (22) and consequently site A cannot be cleaved. One year later, Novoa and Seegers (23) observed that during the activation of prethrombin 1 by factor X_a

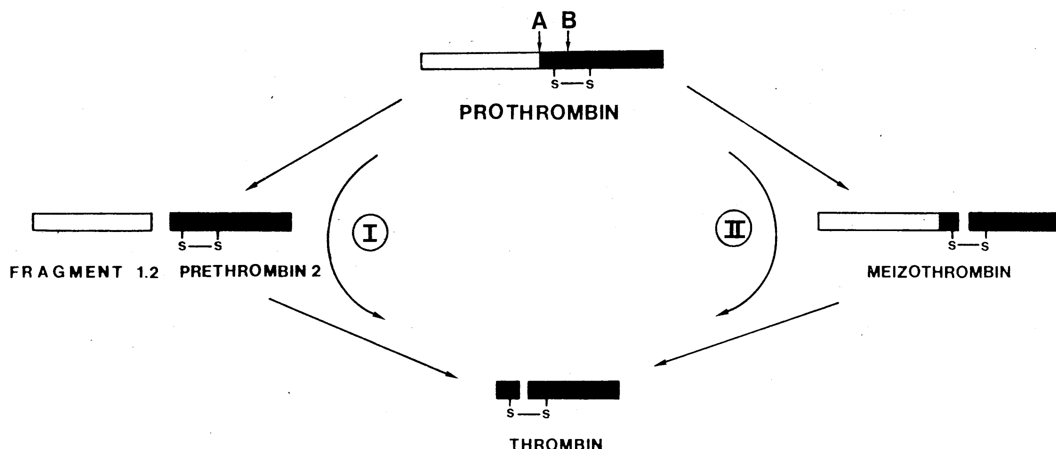

Fig. 2 Pathways of prothrombin activation

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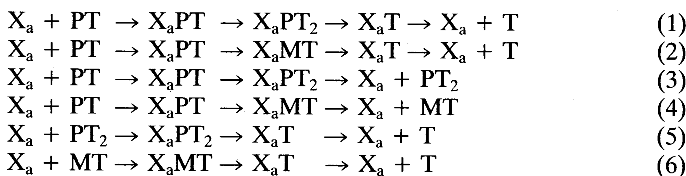
the generation of esterase activity preceeds clotting activity. This indicates that besides thrombin other catalytically active species (e.g. meizothrombin) may have been formed.

Although the studies mentioned above show that the order of bond cleavage in prothrombin need in principle not to be compulsory, the actual physical proof of meizothrombin formation during factor X_a-catalyzed prothrombin activation remained, for a long time, elusive. This has most likely been caused by the fact that meizothrombin and meizothrombin-desF1 are hard to detect by conventional gel electrophoretic techniques since they comigrate with prothrombin or other activation products on both non-reduced and reduced gels. It was not until 1986, that conclusive evidence for the occurrence of meizothrombin during factor X_a-catalyzed prothrombin activation was put forward by Rosing et al. (4), followed shortly thereafter by a report on meizothrombin formation by Krishnaswamy et al. (5). In both studies fluorescent active-site directed probes were used to detect meizothrombin. In addition, Rosing et al. (4) developed an amidolytic assay that enables quantitation of thrombin and meizothrombin in prothrombin activation mixtures. This procedure is based on the observation that the amidolytic activity of thrombin can be readily inhibited by antithrombin III plus heparin, while meizothrombin is rather insensitive to this combination of inhibitors.

It appears now well established that both pathways I and II can occur during factor X_a-catalyzed prothrombin activation and that considerable amounts of meizothrombin can be produced. This immediately raises questions concerning the precise reaction conditions under which meizothrombin will be formed and regarding the functions and the physiological significance of this activation product.

Thrombin and Meizothrombin Formation During Factor X_a-Catalyzed Prothrombin Activation

The fact that factor X_a can apparently cleave two peptide bonds in prothrombin in a random order may result in a rather complex pattern of product generation during a time course of prothrombin activation. Considering the prothrombin activation pathways presented in Fig. 2 there are six possible reactions that may contribute to products formation:



$$X_a + PT \rightarrow X_aPT \rightarrow X_aPT_2 \rightarrow X_aT \rightarrow X_a + T \quad (1)$$
$$X_a + PT \rightarrow X_aPT \rightarrow X_aMT \rightarrow X_aT \rightarrow X_a + T \quad (2)$$
$$X_a + PT \rightarrow X_aPT \rightarrow X_aPT_2 \rightarrow X_a + PT_2 \quad (3)$$
$$X_a + PT \rightarrow X_aPT \rightarrow X_aMT \rightarrow X_a + MT \quad (4)$$
$$X_a + PT_2 \rightarrow X_aPT_2 \rightarrow X_aT \rightarrow X_a + T \quad (5)$$
$$X_a + MT \rightarrow X_aMT \rightarrow X_aT \rightarrow X_a + T \quad (6)$$

In these reaction sequences X_aPT, X_aPT_2, X_aMT and X_aT represent enzymebound reaction intermediates of factor X_a with prothrombin, prethrombin 2, meizothrombin and thrombin, respectively. The contribution of each of the above reactions depends on the extend of prothrombin activation. Therefore, it will be helpful to distinguish three phases in a time course of prothrombin activation. The *pre-steady state* starts immediately after onset of the reaction and covers the period in which the enzyme-bound intermediates (X_aPT, X_aPT_2, X_aMT and X_aT) build up to their so-called steady-state level. In the *initial steady state* that follows, the concentrations of enzyme-bound intermediates do not further change and the reaction rate is virtually constant, since this period is restricted to the time interval over which the concentration of prothrombin does not greatly change. The steady state is followed by the *post-steady state* during which the prothrombin concentration begins to decrease with concomittant decrease of the prothrombin activation rate.

The pre-steady state is usually very short (10^{-7}–1s) and involves formation of levels of product that are too low to be detected by the conventional techniques used to analyze prothrombin activation. The majority of the kinetic studies of prothrombin activation have been carried out in the initial steady state period, a condition that involves reaction 1 to 4. In the initial steady state reactions 5 and 6 hardly contribute to thrombin formation since the concentration of prothrombin greatly exeeds that of meizothrombin and prethrombin 2, i.e. conditions that favor reactions 1 to 4. This is especially true if one considers that the catalytic efficiencies of the factor X_a-catalyzed conversion of prothrombin, meizothrombin and prethrombin 2 are approximately the same (14, 24). In the post-steady state reaction 1–4 slow down because of the decrease of the prothrombin concentration. Under these conditions reaction 5 and/or 6 progressively contribute to thrombin formation since the reaction conditions favor meizothrombin and/or prethrombin 2 conversion because the concentrations of these intermediates approach and finally exceed the prothrombin concentration.

Effect of the Composition of the Prothrombin Activating Complex

Although factor X_a is the coagulation factor that is responsible for prothrombin activation, the enzyme by itself is actually a very poor activator of prothrombin. In order to obtain reaction rates sufficiently high to account for thrombin formation in vivo, additional components are required. These so-called accessory components are: calcium ions, negatively charged phospholipids and the protein cofactor, factor V_a. Calcium ions appear to participate in the binding of factor X_a and prothrombin to the phospholipid bilayer, an interaction that also involves the γ-carboxyglutamic residues of these proteins and polar head groups of the procoagulant membrane. Phospholipids are thought to stimulate the reaction by bringing the proteins in close proximity so that they can efficiently interact with one another (25). The major effect of factor V_a is related to a tremendous increase of the V_{max} by which factor X_a can activate prothrombin (25, 26).

Table 1 shows the effect of the accessory components on the rates at which the various reaction products are formed during prothrombin activation under initial steady-state conditions. With all possible combinations of the prothrombinase components substantial meizothrombin formation occurs. In most cases the initial rate of meizothrombin formation even exceeds that of thrombin formation. The only reaction condition at which meizothrombin is formed slower than thrombin is prothrombin activation by factor X_a in the presence of Ca^{2+}-ions and factor V_a (ref. 4, Table 1). Another striking feature of the data in Table 1 is that

Table 1 The effects of accessory components on product formation during steady-state prothrombin activation

Activator	Product formation Prethrombin 2 (%)	Meizothrombin (%)	Thrombin (%)
X_a, Ca^{2+}	95	3	2
X_a, Ca^{2+}, PL	82	9	9
X_a, Ca^{2+}, V_a	not detectable	18	82
X_a, Ca^{2+}, V_a, PL	not detectable	65	35

Prethrombin 2 formation was determined by quantitation of the reaction products on SDS-polyacrylamide gels as described in ref. 27. Initial rates of meizothrombin and thrombin formation were determined by measurement of the amidolytic activity towards the chromogenic substrate S 2238 as described in ref. 4. Reaction conditions were: 50 mM Tris (pH 7.9), 175 mM NaCl, 0.5 mg/ml ovalbumin, 5 mM $CaCl_2$, 2 μM prothrombin, varying amounts of factor X_a with or without 50 μM phospholipid (PS/PC; 25/75; mole/mole) and 5 nM factor V_a.

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prethrombin 2 is actually the major reaction product that is formed during steady-state prothrombin activation by factor X_a in the presence of calcium ions. Its initial rate of formation is much higher than that of thrombin and meizothrombin. Although phospholipids strongly stimulate the overall rate of prothrombin activation by factor X_a (25) they do not have much effect on the ratios at which the different reaction products are formed (Table 1). However, factor V_a drastically affects the pattern of product formation. As was originally shown for bovine prothrombin by Rosing et al. (25, 27) and later for human prothrombin by Krishnaswamy et al. (28), large amounts of prethrombin 2 accumulate during prothrombin activation in the absence of factor V_a while prethrombin 2 is not detectable in the presence of factor V_a. Thus, factor V_a causes a shift in prothrombin activation from a process in which mainly prethrombin 2 (reaction 3) and small amounts of thrombin and meizothrombin are produced into one in which only thrombin (reaction 1 and/or 2) and meizothrombin (reaction 4) are formed. Whether the fact that meizothrombin is formed signifies the conclusion (28) that the conversion of prothrombin into thrombin in the presence of factor V_a exclusively occurs via meizothrombin as intermediate (reaction 2 and/ or reaction 4 followed by reaction 6) remains in our opinion to be established[2].

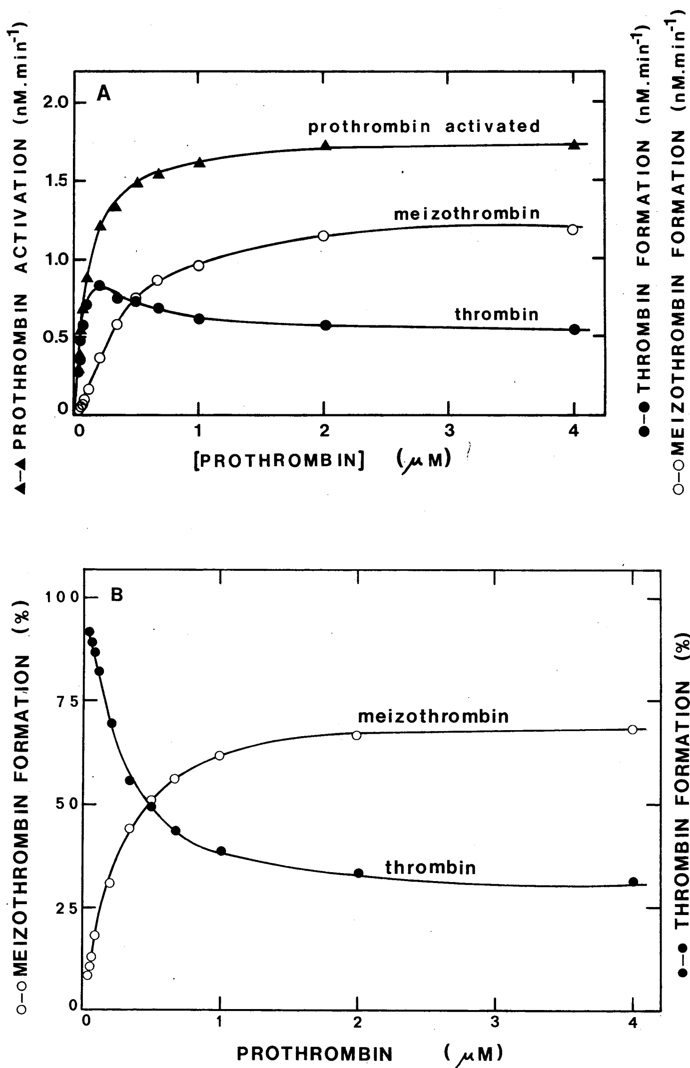

Fig. 3 Initial rates of thrombin and meizothrombin formation as a function of the prothrombin concentration. A. Bovine prothrombin was activated in a reaction mixture containing 50 mM Tris (pH 7.9), 175 mM NaCl, 0.5 mg/ml ovalbumin, 5 mM $CaCl_2$, 50 μM phospholipid (PS/PC; 25/75; mole/mole), 5 nM bovine factor V_a and 5 pM bovine factor X_a. Rates of thrombin- and meizothrombin formation were determined as described in ref. 4. The rate of prothrombin activation was taken to equal the sum of the rates of thrombin- and meizothrombin formation. B. The points in panel B were obtained from the data presented in panel A. In panel B is plotted, the percentage of product that can be attributed to thrombin and meizothrombin when varying concentrations of prothrombin are activated by the complete prothrombinase complex under initial steady state conditions. 100% equals the sum of the amounts of thrombin and meizothrombin formed

It should be pointed out that the data in Table 1 concern initial rates of product formation (i.e. initial steady-state conditions). The meizothrombin that is formed during prothrombin activation is, of course, a transient reaction product that during the time course of the reaction will be further processed to thrombin by factor X_a. This explains that on a quantitative basis the amount of meizothrombin that accumulates only reaches some 10–20% of the total prothrombin available (4) and that in a later stage of the reaction the amount of thrombin will exceed the amount of meizothrombin present. However, when prothrombin activation is carried out in the presence of phospholipids, meizothrombin-desF1 can accumulate to a much larger extent since this molecule lacks the ability to bind to phospholipid and can only be processed to thrombin at much lower velocities by factor X_a in a phospholipid-independent manner.

Effect of Prothrombin Concentrations

A parameter that profoundly influences the ratio of the initial steady-state rates of meizothrombin over thrombin formation is the prothrombin concentrations. This is illustrated in an experiment in which initial rates of thrombin and meizothrombin formation by the complete prothrombinase complex (i.e. X_a, V_a, Ca^{2+} and phospholipids) were determined at different prothrombin concentrations (Fig. 3A). At low concentrations of prothrombin virtually all product that is formed is thrombin. Higher prothrombin concentrations lead to increased rates of meizothrombin formation as compared to thrombin formation, until at concentrations of prothrombin well over 1 μM about 70% of the initially generated product is meizothrombin (Fig. 3B). Thus, a change of the prothrombin concentration leads to a shift in the ratio of thrombin over meizothrombin formed. As yet, there is no mechanistic explanation for this phenomenon and further kinetic studies will be required to understand this prothrombin-depen-

[2] It has been suggested (28) that in the absence of factor V_a thrombin is formed via prethrombin 2 (pathway I, Fig. 2) whereas with factor V_a it is formed via meizothrombin (pathway II, Fig. 2). Although this will be true for the major part of the thrombin that is finally generated, the kinetic pathway of thrombin formation under initial steady state conditions remains to be established. For that one has to differentiate between an intermediate (i.e. transient product) that has dissociated from the enzyme and which at a later stage may be converted to thrombin and, a true kinetic intermediate that remains bound to the enzyme and which is directly processed to thrombin. The meizothrombin and prethrombin 2 that are observed during prothrombin activation are transient products which have dissociated from the enzyme. As such, they are temporarily lost for further conversion by factor X_a since in the initial steady-state phase they have to compete for the enzyme with the excess of prothrombin present. This makes it unlikely that the initially formed thrombin results from cleavage of accumulated transient products. These thrombin molecules will be generated from enzyme-bound reaction intermediates i.e. enzyme-prethrombin 2 or enzyme-meizothrombin (reaction 1 or 2, p. 3). To establish the kinetic pathway of initial steady-state thrombin formation it will be necessary to obtain information about the occurrence of enzyme-bound intermediates.

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dent change of the pattern of product formation. It will be clear, however, that this observation may have important implications for in vivo prothrombin activation. Prothrombin is present in plasma at a concentration of approximately 2 μM and thus meizothrombin will likely be the major product of prothrombin activation in the early stage of coagulation. When during coagulation the prothrombin concentration drops, the activation pathway will shift to thrombin formation.

Properties of Meizothrombin

One of the most striking characteristics of the enzymatically active prothrombin derivatives (meizothrombin, meizothrombin-desF1 and thrombin) is the fact that these enzymes very efficiently remove the fragment 1 region of prothrombin and/or meizothrombin. Therefore, it will hardly be surprising that as yet there are no reports on the purification of fully intact and active meizothrombin. Bovine meizothrombin-desF1 has been purified (15, 23, 30, 31) since this is a relatively stable end product. In contrast, human meizothrombin-desF1 much more readily autocatalyzes to thrombin (18) and for this molecule there is no reported purification procedure. As a consequence, the knowledge of the properties of meizothrombin is either based on studies with bovine meizothrombin-desF1 (15, 23, 30, 31) or on studies of the enzymatic activities of meizothrombin and meizothrombin-desF1 that are transiently formed during prothrombin activation (16–18, 32).

It is well established that a fully developed active site is present in all active prothrombin derivatives (thrombin, meizothrombin and meizothrombin-desF1) since the esterolytic and amidolytic activities are the same for each of these enzymes (4, 15, 16, 21, 23, 30). Meizothrombin and meizothrombin-desF1 appear to be inhibited effectively by small thrombin inhibitors such as DFP, benzamidine, DAPA and hirudin (5, 15, 16, 18, 19, 21, 23, 24). All three enzymes appear quite efficient in the proteolysis of site 1 (to cleave off fragment 1) and site 2 (to result in a shorter thrombin A-chain or to cleave off fragment 1.2.3). It is, however, with respect to other macromolecular substrates that differences in catalytic activities between thrombin and meizothrombin may be observed. Thus, meizothrombin and meizothrombin-desF1 are very poor enzymes when it comes to converting fibrinogen into fibrin (4, 15–17, 21, 23, 30). Both meizothrombins exhibit a clotting activity that is less than 10% of that of thrombin (4). Inhibition of meizothrombin by antithrombin-III occurs at rates that are comparable to those obtained with thrombin (15, 31, 32) but in the presence of heparin the inhibition of thrombin is much faster (4, 32). This appears to be caused by the fact that in the case of meizothrombin and meizothrombin-desF1 the interaction with antithrombin III is not stimulated by heparin (32), a phenomenon which can be explained by the observation that meizothrombin does not bind to heparin (32). As yet there is no information on the inhibition of meizothrombin by the other plasma protease inhibitors.

Further data concerning other macromolecular thrombin substrates (e.g. factor V, factor VIII, protein C, factor XIII and platelet glycoproteins) are still lacking and, therefore, many questions concerning the relative efficiency of meizothrombin as compared to thrombin remain unanswered. To appreciate the possible physiological function of meizothrombin it will be necessary to obtain such data.

Importance of Meizothrombin Formation During Prothrombin Activation

Although it is now well established that in model systems considerable amounts of meizothrombin can be formed during factor X_a-catalyzed prothrombin activation it is as yet not known whether the accumulation of this intermediate product is an event with an important physiological function. For this it will first of all be necessary to establish whether meizothrombin formation also occurs under physiological conditions (e.g. in plasma). This may actually turn out to be difficult to prove since the current gel electrophoretic techniques to detect meizothrombin are relatively insensitive (4) and the high protein content of plasma samples will interfere with gel electrophoretic analysis of prothrombin activation products. Also the possibility to quantitate meizothrombin by amidolytic assay (4) will be difficult because of the protease inhibitors present in plasma.

With respect to the functional properties of meizothrombin it will be necessary to establish the potency of meizothrombin in catalyzing reactions in which thrombin plays a role, for example the activation of factors V and VIII and protein C as well as the regulation of platelet function. Moreover, it will be necessary to determine the efficiency by which meizothrombin will be inhibited by plasma protease inhibitors other than antithrombin III (e.g. α_2-macroglobulin and α_1-antitrypsin).

It remains an intriguing finding that the relative amounts of meizothrombin that are formed depend upon the prothrombin concentration. This indicates that if meizothrombin is formed in the plasma milieu this will only occur in the early stages of the reaction when the concentration of prothrombin is still high. Thus, in the initial phase of prothrombin activation an activation product may be formed which differs significantly from thrombin and which, therefore, may exert different functions. In that respect it should be emphasized that meizothrombin retains the fragment 1 region and thus is capable to bind to phospholipid membranes. This makes it especially suitable to participate in reactions with substrates that can also bind to phospholipids. Meizothrombin also contains the so-called kringle structures in the fragment 1 and fragment 2 region (34). Although the precise function of the kringles of prothrombin is not known it has been suggested that such structures are important for the interactions with other proteins (35) and this may provide meizothrombin with a biological specificity different from thrombin. Since meizothrombin is poorly inhibited by antithrombin III in the presence of heparin it may perform its catalytic functions over a relatively long period. Thus, meizothrombin is potentially capable of expressing functions that may not be performed by thrombin since this latter molecule has different specificities and can be inhibited much faster. For the time being, however, such discussions remain rather speculative and it is necessary to await additional information on the function and the in vivo formation of meizothrombin in order to establish the physiological importance of this prothrombin activation product.

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