PROTEOLYSIS OF BLOOD COAGULATION FACTOR X BY ACTIVATED FACTOR X

Difference between the bovine and human proteins

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1. Introduction

Factor X is a plasma glycoprotein that is involved in both the intrinsic and extrinsic pathway of blood coagulation. During the coagulation process, factor X is converted to factor Xa (EC 3.4.21.6), a serine protease catalysing the activation of prothrombin and the enzymatic conversion of several other coagulation factors [1,2]. One of the substrates of factor Xa is its zymogen, factor X. In the bovine system, the cleavage of factor X by factor Xa can result in the activation of factor X [3–5]. In the human system however, factor Xa cleaves a peptide from factor X that contains the active-site serine residue [6]. Thus, the bovine and human system differ with respect to the effect of factor Xa on factor X (activation *vs* inactivation).

Here, the proteolysis of factor X by factor Xa in various combinations of human and bovine proteins is described. We conclude that the enzymes of both species have similar substrate specificities and that structural differences between the human and bovine zymogen account for the different autocatalytic pathways.

2. Materials and methods

Bovine factor X and factor Xa were prepared as in [7,8]. Human factor X was purified as in [6]. Human factor Xa was prepared by the complete activation of purified factor X using the purified factor X-activator from Russell's viper venom [9], as detailed elsewhere (K. M., R. M. B., submitted). Phospholipid vesicles were prepared by dispersing a phospholipid extract from human brain [6] in 100 mM NaCl, 50 mM Tris–HCl (pH 7.5) using a Branson (model B12) sonifier.

Lipid phosphorus was quantitated after $HClO_4$ combustion according to [10]. SDS–Polyacrylamide gel electrophoresis was performed as in [6]. Factor Xa activity was measured spectrophotometrically by adding samples to cuvettes containing 5 mM EDTA, 100 μg/ml ovalbumin and 0.22 mM of the substrate Benzoyl-Ile–Glu(piperidyl)–Glu–Arg–*p*-nitroanilide (S 2337, Kabi Diagnostica, Sweden) in 200 mM NaCl, 50 mM Tris–HCl, pH 8.0 (final vol. 1 ml), and by recording A_{405} at 37°C. The factor Xa activity in the samples was expressed in nkat/ml. Factor X concentrations were derived from the factor Xa activities measured after the complete conversion of the factor X in the samples to factor Xa using Russell's viper venom; under the assay conditions the factor X concentration in normal human plasma is equivalent to a factor Xa activity of 6.8 nkat/ml.

3. Results and discussion

Fig.1a shows the development of factor Xa activity during the incubation of bovine factor Xa with bovine and human factor X in the presence of Ca^{2+} and phospholipid. When bovine factor X is the substrate, the factor Xa activity increases after an initial lag period; this confirms the activation of bovine factor X by bovine factor Xa [3–5]. However, when human factor X is the substrate, the factor Xa activity remains constant, indicating that bovine factor Xa does not activate human factor X. Similar results are obtained when human factor Xa is used in stead of bovine factor Xa (fig.1b).

Fig.2 shows the SDS–polyacrylamide gels of samples taken after different incubation times from the reaction mixtures described in fig.1. Bovine and

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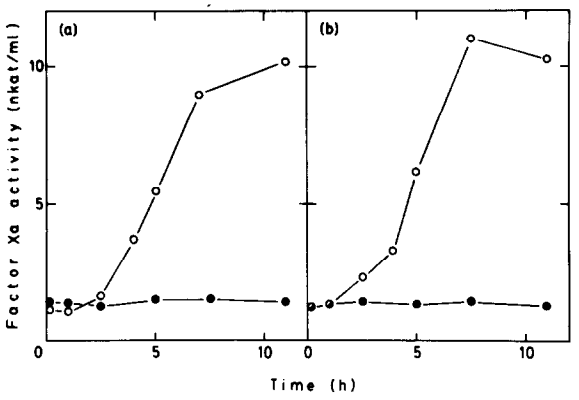

Fig.1. Activation of factor X by factor Xa. Bovine (○) and human (●) factor X (equivalent to 34 nkat/ml factor Xa after complete activation by Russell's viper venom) were incubated with bovine (a) and human (b) factor Xa (1.1 nkat/ml) in the presence of phospholipid (100 μM) and $CaCl_2$ (10 mM) in 100 mM NaCl, 50 mM Tris–HCl (pH 7.5) at 37°C. At the times indicated, samples from the incubation mixtures were assayed for factor Xa activity.

human factor Xa catalyse essentially the same conversions when incubated with either bovine or human factor X.

Bovine factor X (M_r 63 000) is converted to a product with M_r 49 000 via an intermediate (M_r 58 000). In the same incubation mixture, factor Xa activity develops after an initial lag period, suggesting that only the final (M_r 49 000) product has factor Xa activity. Interpretation of these observations based on the data in [3,4,8,11,12] indicates that the M_r 58 000 component is formed by the cleavage of a small peptide from the C-terminal portion of the heavy chain, whereas the M_r 49 000 component is formed by the subsequent removal of a glycopeptide (the activation peptide) from the N-terminal portion of the heavy chain. The latter cleavage is accompanied by the appearance of factor Xa activity (see fig.1). It should be noted that the proteolysis of bovine factor X in the presence of human factor Xa (see fig.2b) may result from the action of both the human factor Xa added and of the bovine factor Xa formed. However, since no significant amounts of factor Xa (<1 nkat/ml) are formed in the absence of added factor Xa (data not shown), the conversions observed in fig.2b are at least initiated by human factor Xa.

Human factor X (M_r 68 000) has a molecular mass slightly higher than that of bovine factor X (M_r 63 000). It is converted to products with M_r 55 000, 34 000, 21 000 and 13 000 (see fig.2). These products

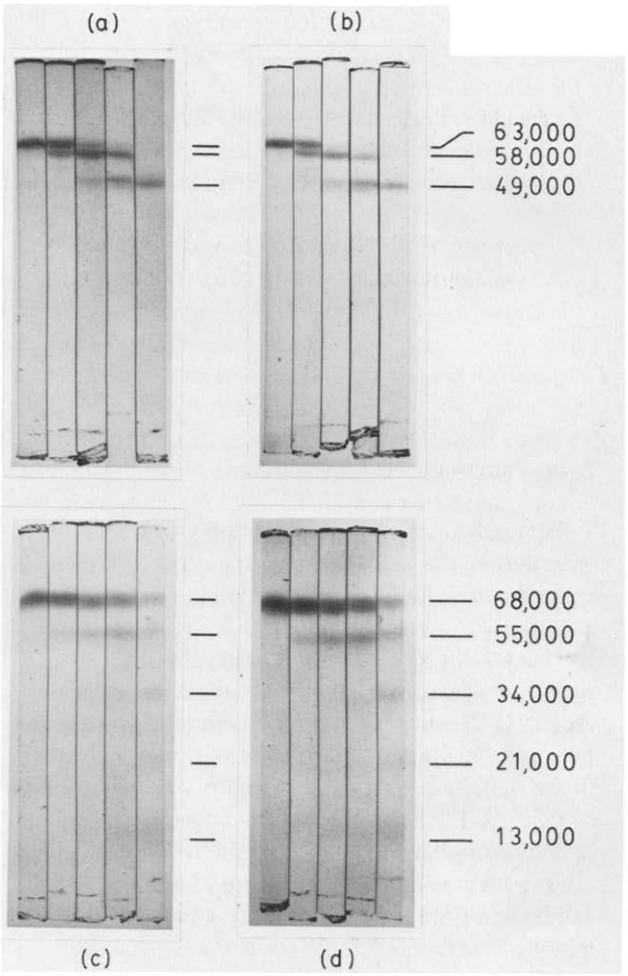

Fig.2. Proteolysis of factor X by factor Xa. The incubation mixtures were those in fig.1, containing bovine factor X and bovine factor Xa (a), bovine factor X and human factor Xa (b), human factor X and bovine factor Xa (c), and human factor X and human factor Xa (d). Each panel shows 10% SDS–polyacrylamide gels of samples withdrawn after (from left to right) 0, 1, 2.5, 4 and 11 h incubation.

correspond with factor γX, factor αγX, fragment-α and fragment-γ [6]. The first product, factor γX (M_r 55 000), is formed by the cleavage of fragment-γ (M_r 13 000) from the C-terminal region of the heavy chain. Since fragment-γ contains the active-site serine residue [6], the subsequent conversion of factor γX to factor αγX (M_r 34 000) by the removal of fragment-α (the activation peptide, M_r 21 000) from the N-terminal region of the heavy chain cannot result in the appearance of factor Xa activity (cf. fig.1).

In conclusion, both bovine and human factor X

are cleaved by factor Xa in two subsequent steps: in each case the cleavage of a C-terminal peptide from the heavy chain (step 1) is followed by the removal of the activation peptide from the N-terminal portion of the heavy chain (step 2). In the bovine system, the C-terminal peptide (M_r 5000) is not essential for factor Xa activity, whereas in the human system this fragment (M_r 13 000) contains the active-site serine residue. Since the substrate specificity of bovine factor Xa is similar to that of the human enzyme (cf. fig.1,2), this discrepancy must originate from structural differences between the C-terminal regions of the heavy chain of bovine and human factor X.

Human and bovine factor X also differ with respect to the N-terminal region of the heavy chain [13]: the human activation peptide has a higher carbohydrate content and contains 20–30 amino acids more than its bovine counterpart. This explains the difference in M_r value between human (68 000) and bovine (63 000) factor X.

At present it is not clear whether these structural differences contribute to differences in the mechanism of factor X-activation in the bovine and human species.

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