BOVINE FACTOR IX (CHRISTMAS FACTOR). FURTHER EVIDENCE OF HOMOLOGY WITH FACTOR X (STUART FACTOR) AND PROTHROMBIN

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1. Introduction

Factor IX is a single-chain glycoprotein involved in the intrinsic pathway of blood coagulation. The molecular characteristics of factor IX and the mechanism of its activation by factor XI_a (activated factor XI) have recently been described [1,2]. In the presence of factor VIII, calcium, and phospholipid, factor IX_a catalyzes the conversion of factor X to X_a. Factor X_a in the presence of factor V, calcium, and phospholipid in turn converts prothrombin to thrombin. Factor X_a and thrombin are both inactivated by diisopropylphosphorofluoridate (DFP), but the same has not been established for factor IX_a. Factor X_a and thrombin are homologous with other mammalian serine proteases [3]. The amino terminal regions of factor IX, prothrombin, and the light chain of factor X are also homologous, but factor IX is immunologically distinct [4]. Nevertheless, evidence is presented here showing that considerable homology exists between bovine factors IX and X and prothrombin, suggesting that factor IX_a has evolved from the same precursor and therefore is probably also a serine protease.

2. Materials and methods

Bovine factor IX was prepared and activated according to the method of Fujikawa et al. [1,2]. The protein was reduced and pyridylethylated as described by Friedman et al. [5]. The light and heavy chains and the activation peptide of factor IX_a were fractionated by gel filtration [2]. Digestion with cyanogen bromide was carried out as described [3] and the fragments separated by gel filtration on Sephadex G-75. The smaller fragments were further purified by recycling on Sephadex G-50 'superfine'. Both columns were eluted with 9% formic acid. Automatic sequence determination was carried out with the Beckman Sequencer (model 890B) according to the method of Edman and Begg [6], as modified by Hermodson et al. [7]. 4-Vinylpyridine monomer (practical grade) was a product of J. T. Baker Chemical Co. and was further purified by vacuum distillation and stored at −20°C in the dark.

3. Results and discussion

The sequence of the first 15 residues of the heavy chain of factor IX_a is shown in fig. 1 together with the sequences of the corresponding regions of thrombin [8] and the heavy chain of factor X_a [3]. Eight of these 15 residues are identical in all three proteins, and many of the remaining residues represent conservative substitutions.

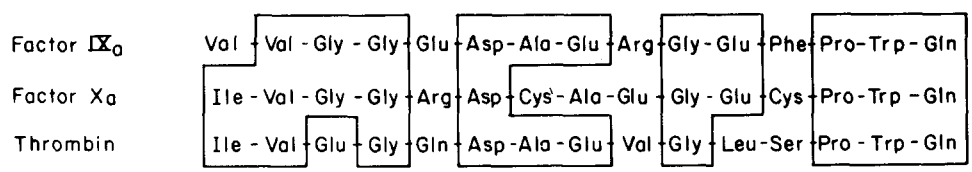

Fig. 1. Amino-terminal sequences of the heavy chains of bovine factors IX_a and X_a and the B-chain of thrombin. Residues enclosed by solid lines are identical.

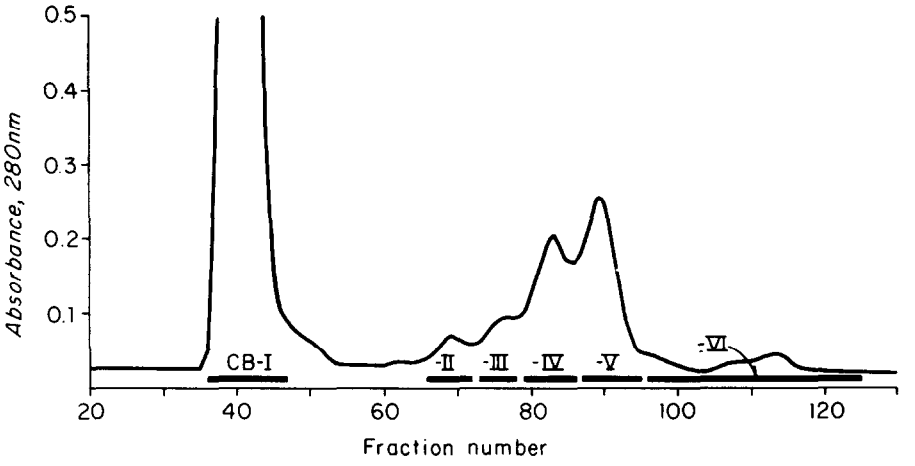

Fig. 2. Gel filtration of the cyanogen bromide digest of S-pyridylethyl bovine factor IX. The lyophilized digest (100 mg) was applied to a 2.5 X 115 cm column of Sephadex G-75 and eluted with 9% formic acid. The flow rate was 35 ml/hr; fractions were collected every 8.7 min. Fractions were combined as indicated by the black bars and peptides were recovered by lyophilization. The column was monitored at 280 nm with an LKB Uvicord-II UV Absorptiometer (model 8300).

To further compare the sequence of factor IX with those of factor X and prothrombin, factor IX was fragmented with cyanogen bromide, Amino acid analysis yields three methionines [1], and thus four major fragments would be expected. The fractionation of the digest by gel filtration is shown in fig. 2. Peaks CB-I through CB-V were analyzed with the Sequencer. Preliminary evidence indicates that CB-II is a fragment produced in low amounts resulting from the presence of a poorly-cleaved methionyl peptide bond. No amino acids were found in CB-VI after acid hydrolysis. Fragment CB-IV was found to correspond to the regions of factor X_a and thrombin containing the DFP-reactive serine. The sequence of the first 24 residues of CB-IV is shown in fig. 3 together with the homologous sequences of factor X_a [3] and thrombin [8]. Of these 24 residues, 14 are identical to those in the other two proteins.

The functional roles of the light chains of factors IX_a and X are not yet known. However, factor X binds calcium [9] and the amino acid composition of a calcium-binding peptide isolated from factor X [10]

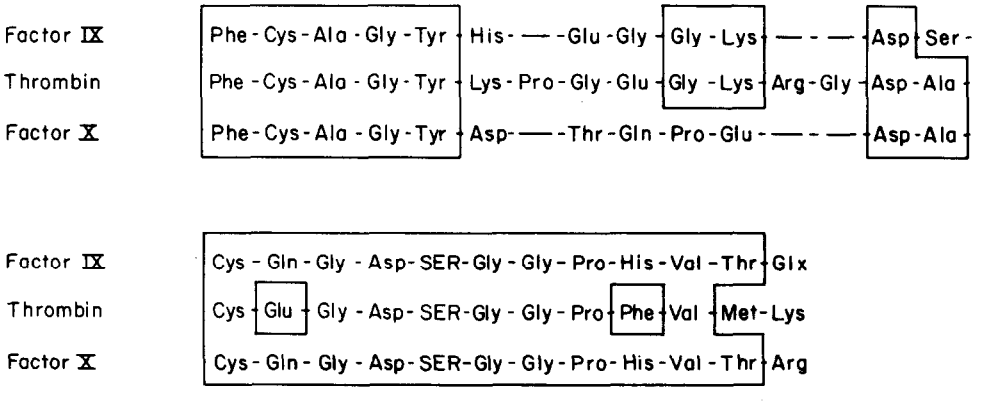

Fig. 3. Amino acid sequence of factor IX compared to homologous sequences surrounding the DFP-reactive serine in thrombin and factor X. The serine is shown in capital letters. Dashes indicate gaps inserted to optimize homology among the sequences. Residues enclosed by solid lines are identical.

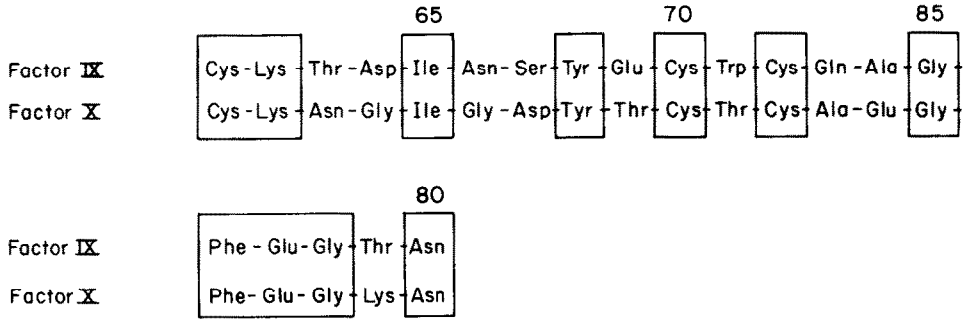

Fig. 4. Amino-terminal sequence of fragment CB-I from factor IX compared to the homologous sequence in the light chain of factor X. The numbers refer to the sequence of the light chain of factor X [11]. Residues enclosed by solid lines are identical.

closely corresponds to residues 5 through 43 of the light chain [11]. This region of factor X is known to be homologous to the amino terminal regions of factor IX and prothrombin [4], the latter also being a calcium-binding protein [12]. In addition, the light chains of factor IX_a and X are similar in size [2]. Further evidence of homology between these light chains is shown in fig. 4. The amino terminal sequence of CB-I of factor IX is aligned with residues 61 through 80 of the light chain of factor X [11]. Of these 20 residues, 11 are identical in both proteins. It is thus apparent that the light chain regions of factors IX_a and X are homologous.

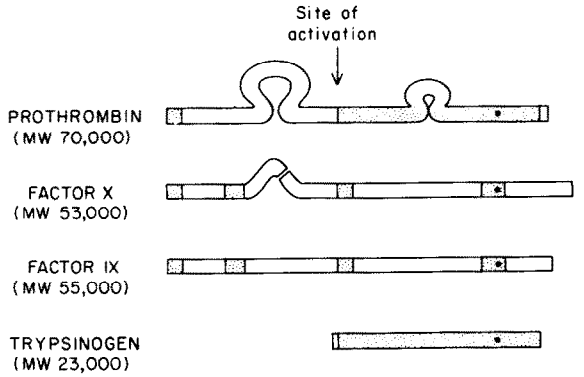

Fig. 5. Comparison of sequence homology and length of the polypeptide chains of bovine prothrombin, factor X, factor IX, and trypsinogen. The molecular weights are given for the intact proteins. The length of each bar is proportional to the molecular weight of the peptide portion of the protein, without carbohydrate (E. W. Davie and K. Titani, personal communication). Stippled areas indicate known homologous sequences. The large dots indicate the positions of the DFP-reactive serine (except in the case of factor IX where its reactivity has not been proven). Activation at the site indicated yields the B-chain of thrombin, the heavy chains of factors X_a and IX_a, and trypsin, respectively.

The relationships among the polypeptide chains of bovine prothrombin, factors X and IX, and trypsinogen are diagrammed in fig. 5. The amino terminal regions of prothrombin, factor IX, and the light chain of factor X are homologous, as are the four active enzymes themselves. Thrombin is somewhat larger than trypsin, the additional residues occurring as several short insertions [8,13,14]. Complete comparison of the regions preceding the sites of activation in the clotting factors is not yet possible. However, the correlation of segments preceding the active enzymes indicates differences in linear separation of the homologous regions. This is especially striking when prothrombin and factor IX are compared. Since both zymogens are single chains, there must have occurred either a massive single or several smaller additions or deletions during the divergent evolution of these two proteins. Since factor X is also smaller than prothrombin but contains two chains, it is possible either that factor X has evolved from a single chain shorter than prothrombin or that a peptide segment has been lost prior to isolation. Analogously, since factor IX is somewhat shorter than factor X, an insertion or deletion may have occurred also.

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