Fibrin cross-linking in congenital factor XIII deficiency

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SUMMARY Homozygous patients with factor XIII deficiency are devoid of immunologically identifiable A protein, the active enzymatic component. Quantitative studies of transamidase activity of the factor are available in only a few cases, and the fibrin cross-linking pattern is not well known. The present paper deals with the quantitative estimation of factor XIII transamidase activity (dansylcadaverine system), factor XIII molecular subunits, and the corresponding fibrin cross-linking pattern in seven homozygous patients with factor XIII deficiency. The results indicate that transamidase activity was present in all patients, and the range was 0.5-1.7%. The pattern of fibrin stabilisation showed an absence of cross-linking in two patients, the presence of γ - γ -dimers (traces) in four, and γ - γ -dimers plus incomplete α -polymers (traces) in one patient. In conclusion, the homozygous patients reported here were not completely devoid of functioning factor XIII.

Plasma factor XIII, the fibrin stabilising factor, is a zymogen which, after activation by thrombin,¹ catalyses the formation of $\epsilon(\gamma$ -glutamyl) lysine cross-links between fibrin monomers.² The zymogen contains two types of subunits and, according to Bohn *et al.*,³ the subunit plasma structure is A₂S. Subunit A is the enzymatically active component whereas subunit S plays a role in regulating the rate of calcium-dependent activation of the zymogen.⁴

Patients with severe factor XIII congenital deficiency are devoid of immunologically identifiable A protein but retain, albeit in reduced amount, subunit S.⁵⁻⁸

The minimum level of factor XIII activity requested for normal haemostasis has been evaluated at between 1 and 2%.^{9,10} Moreover, the clinical expression of this haemorrhagic disorder is variable, and one could admit a relationship between the residual enzymatic activity and the severity of cross-linking impairment. Since this relation has been studied in only isolated cases,¹¹⁻¹³ we report here the values of the specific enzymatic activity, the molecular subunit components, and the fibrin cross-linking pattern in seven homozygotes for factor XIII deficiency.

Material and methods

The patients have been reported previously.⁸ They

had not received blood components during the last three months. Case 1 had not been transfused for one year before the present study. Blood was collected into 0.1 vol 3.8% sodium citrate anticoagulant and centrifuged at 1500 g for 20 minutes and deep frozen (-30°C). The transamidase activity was measured, as previously reported¹⁴ using the fluorimetric method described by Lorand *et al.*¹⁵ employing Dansylcadaverine (Sigma) and Hammarsten-casein, respectively, as donor and recipient for the amide group in the transamidase reaction. In order to render the method more sensitive, amine incorporation was stopped 60 minutes later, instead of 30 minutes as described by Lorand. The enzymatic activity of each plasma was calculated as the difference between the fluorescence emitted by the test plasma and that of the blank (control mixture including the plasma of the same subject with only the monodansylcadaverine missing but otherwise similarly treated). The calibration curve was constructed by diluting a pool of 10 normal plasmas at 1:20, 1:40, 1:80, 1:160, 1:320 in saline, and a linear relationship between the rate of amine incorporation and plasma concentration was obtained. In order to exclude the presence of some transamidating activity in the thrombin, the test was repeated omitting plasma; dansylcadaverine failed completely to be incorporated into casein. All plasmas from homozygous patients were examined for transamidase activity with or without the enzyme activation induced by thrombin.

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Subunits A and S were determined immunologically in agarose gel containing antibodies according to the Laurell technique^{16,17} as previously reported.^{7,8}

Dodecylsulphate-polyacrilamide gel electrophoresis of solubilised reduced fibrin was performed according to Weber and Osborn¹⁸ principles, as previously reported.¹⁹ Fibrin was prepared from plasma according to Finlayson and Morton.²⁰ 0.1 ml of plasma was placed in a glass test tube (5 × 75 mm) and mixed with 0.1 ml of Michelis buffer 0.05 M pH 7.3 containing 30 mmol/l cysteine. To this mixture, brought to 37°C in a water bath, 0.01 ml of thrombin (test-thrombin Behringwerke) diluted at 60 NIH U/ml in 0.25 M CaCl₂ (or 0.03 M EDTA) was added. Clots formed in all test tubes and were wound, as they formed, on glass rods of 2.5 mm diameter. After 2 hours at 37°C, the clots, still attached to the rods, were removed from the formed clot liquor for washing. Each clot was washed by immersing the rod to which it was attached in 200 ml of refrigerated saline stirred at 4°C. After 3 minutes the clot was carefully dried with filter paper; this procedure was repeated three times. After the final washing step each clot was placed in a test tube (5 × 75 mm) containing 0.3 ml of urea 9 M, sodium-dodecylsulphate (SDS) 3% and beta-mercaptoethanol 3% in sodium phosphate 0.04 M pH 7.1. After incubation, with occasional stirring in a water bath at 37°C for 16 hours (during which time all clots were completely dissolved), 0.1 ml aliquot was removed and mixed with 0.01 ml blue bromophenol (0.22%) and 0.02 ml glycerin; 20 µl of this mixture were submitted to electrophoresis.

Electrophoresis was performed using 125 × 6 mm glass tubes filled up to 81 mm with polyacrilamide 10% in sodium phosphate 0.15 M pH 7.1-7.2 containing 0.1% SDS. Electrode buffer was sodium phosphate/0.075 M pH 7.1-7.2 containing 0.1% SDS. Electrophoresis at room temperature was carried out using 300B Bio Rad apparatus, for 1 hour at

4 mA/tube and an additional 5 hours at 7 mA/tube (bromophenol blue at the bottom of the tube). The gels were stained in Coomassie brilliant blue (Serva). Normal fibrin obtained in the presence of Ca⁺⁺ showed α-polymer and γ-γ-dimer bands, whereas normal fibrin obtained in the presence of EDTA (not cross-linked) showed α, β, and γ-monomer bands. No plasma protein contaminants were present except for a minimal trace of albumin migrating with the α chains. Furthermore, the thrombin used for testing fibrin cross-linking was found to be devoid of contaminating factor XIIIa, as shown by the appearance of the same pattern of cross-linking when recalcified plasma, instead of thrombin-clotted plasma, was used.

Results

The Table gives the factor XIII transamidase activity measured with and without thrombin activation and the molecular subunits of the factor and describes the fibrin stabilisation pattern observed in each patient's plasma.

Transamidase activity dependent on thrombin activation ranging from 0.50 to 1.70% was detected in all homozygous patients. No fibrin cross-link was observed in two cases (3 and 5) whereas γ-γ-dimers and γ-γ-dimers plus incomplete α-polymers were detected in four (1, 2, 6, 7) and one (4) cases respectively. It should be noted that case 4 had the highest transamidase plasma activity level. No enzymatic activity was measurable if thrombin was omitted. Subunit A was immunologically undetectable whereas subunit S was found to be low in all patients.

The Figure shows the fibrin stabilising pattern detected in the seven homozygous patients with factor XIII deficiency.

Discussion

The seven homozygous patients with congenital

Factor XIII molecular subunit plasma content, transamidase, and fibrin stabilising activity

Case	H subunits (%)		Transamidase activity (%)		Fibrin stabilisation pattern
	'A'	'S'	With thrombin	Without thrombin	
1	Absent	30	0.65	Absent	γ-γ-dimer (traces)
2	Absent	30	0.60	Absent	γ-γ-dimer (traces)
3	Absent	50	0.50	Absent	No stabilisation
4	Absent	34	1.70	Absent	γ-γ-dimer and α-polymer (traces)
5	Absent	33	0.80	Absent	No stabilisation
6	Absent	30	1.00	Absent	γ-γ-dimer
7	Absent	20	0.80	Absent	γ-γ-dimer (traces)

Polyacrylamide gel electrophoresis of solubilised reduced fibrin. Tube A: normal fibrin not cross-linked (EDTA plasma content 1.5 mmol/l); Tube B: normal fibrin completely cross-linked (CaCl₂ plasma content 12 mmol/l); Tubes 1 to 7 are from cases 1 to 7.

factor XIII deficiency here presented had plasma transamidating activity ranging from 0.5 to 1.7%. In none of them was subunit A measurable since the Laurell technique is not sensitive enough to measure levels below 5%. Subunit S has also been found to be reduced, and this finding could be due to a genetic mechanism which involves the two components.⁸

The enzymatic activity found in these patients could be ascribed to a transamidating enzyme different from factor XIII. But the transamidase factor XIII is unique in requiring thrombin for its activation, and, when activated, there is an initial formation of γ - γ -dimer followed by α -polymer formation. The pattern of polymerisation of fibrin monomer is quite different with other transamidases.²¹

In our study, the enzyme required thrombin for its transamidating activity and induced γ - γ -dimers or γ - γ -dimers plus α -polymers, showing that the sequence of the specific factor XIII action was respected. Thus this activity must be ascribed to factor XIII and not to other transamidating enzymes.

The activity found in our patients could be due to previous blood transfusion or could be an expression of a residual genetic synthesis. Considering that the half-life of plasma factor XIII is estimated at four to seven days^{10 22} we think it is unlikely that the activity we measured can represent that of transfusions which were stopped at least three months before the present study was begun.

In conclusion, these patients with congenital factor XIII deficiency are not completely devoid of functioning enzyme, which in different degrees induces a partial fibrin cross-linking. No clinical correlation could be drawn from these few cases. We hope that further studies, including sensitive and reliable methods for detecting factor XIII levels ranging from 0 to 2%, will be able to demonstrate that, in this genetic disorder, the variable picture of haemorrhagic manifestations may be due to different degrees of genetic factor deficiency.

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