ISOLATION AND PARTIAL CHARACTERIZATION OF A HUMAN PROTHROMBIN VARIANT : PROTHROMBIN BARCELONA

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SUMMARY. An abnormal prothrombin variant, Prothrombin Barcelona, has been isolated by chromatography on DEAE Sephadex, from several members of the same family. In the absence of any normal component, it was eluted in two unequal peaks. The second peak was homogeneous. This component had the same molecular weight as normal prothrombin but migrated slightly faster on disc gel electrophoresis. The first peak, the smaller one, was heterogeneous : in addition to a minor band similar to that of the second peak, a major one with less anodic mobility and with a molecular weight of 32,000 was found. A possible chromatographic artefact has been eliminated. The family study gave good arguments for an heterozygote state of both parents, the siblings being homozygote.

In 1971, Josso *et al.* (1) described in four siblings of a Spanish family an inborn prothrombin deficiency with a very low functional activity (5 % in one stage assay), but normal levels for immunochemical and staphylocoagulase assays. In the parents' plasmas, the prothrombin activity was about 50 % with normal levels of antigenic material. The variant was called Prothrombin Barcelona.

In the present work, we describe the isolation and partial characterization of this variant resulting from the patients'plasmas, as well as the isolation of prothrombin from the two parents' plasmas.

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MATERIAL AND METHODS. The ACD plasma of siblings or parents was collected by plasmapheresis, frozen at - 70° immediately after centrifugation, and stored at - 20°.

Starting from small quantities of plasma (30 to 50 ml), the purification procedure used was the method described in its main features by Morrison and Esnouf (2) and perfected by Shapiro (3). All the experiments were carried out by processing in parallel the patient's material and normal plasma, using two identical columns. Similar results have also been obtained with another purification procedure (4). The elution of the abnormal prothrombin material during chromatography was checked by staphylocoagulase (5) and immunochemical assays (6). Kinetics of the activation of prothrombin into thrombin were performed by a two stage method using Russell viper venom, phospholipids, human factors X, V and calcium. Acrylamide gel electrophoreses were carried out according to Davis (7) and to Weber and Osborn (8) for SDS gel electrophoreses. Protein estimation was done spectrophotometrically at 280 nm according to Shapiro (9).

RESULTS AND DISCUSSION. During the fractionation of the siblings' plasmas, no normal prothrombin was found. The abnormal protein, unlike the normal one, was eluted in two unequal peaks after DEAE Sephadex A_{50} chromatography (Fig. 1). The second peak (B_2) - the major one - was eluted approximately at the same ionic strength as the normal prothrombin and corresponded to a protein content of 1.7 mg, starting from 30 ml of plasma. The peak B_1 was eluted at lower ionic strength, without any corresponding material in the elution pattern of normal plasma. It represented about 60 % of peak B_2.

By disc gel electrophoresis, the peak B_2 was homogeneous, revealing a single band migrating slightly faster than normal

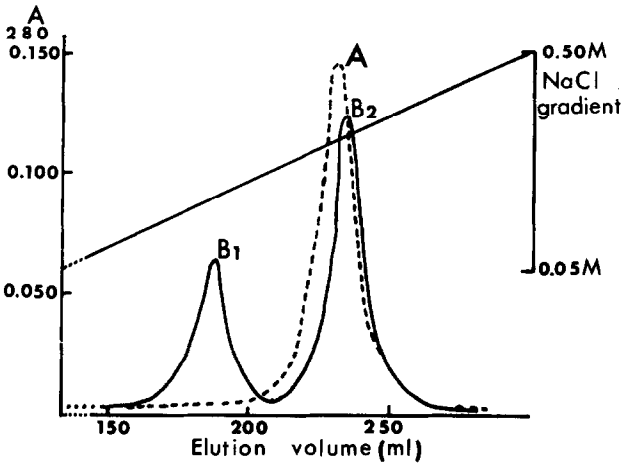

Figure 1. Chromatography on a DEAE Sephadex column (1.5 x 30 cm) of barium citrate eluate of sibling (———) and normal plasmas (-----) (30 ml of each). Elution was performed by a linear gradient of NaCl (0.05 M to 0.50 M) in Tris citrate buffer 0.05 M pH 6.5. Peak A represents normal prothrombin.

prothrombin (Fig. 2 a). This different migration could only be proved by continuing the run 1 hr 30 at 2 mA per gel after the marker reached the end of the gel. This difference was clearly demonstrated by the coelectrophoresis of an artificial mixture of the abnormal B_2 component with normal prothrombin : two distinct bands could be seen.

The first peak B_1 was electrophoretically resolved into two components : one minor band migrating like B_2 and a major one less anodic (Fig. 3 a). By disc gel immunoelectrophoresis, these two bands reacted against antiprothrombin antibodies.

SDS gel electrophoresis of the B_2 fraction revealed essentially one band with the same mobility as normal prothrombin, corresponding to a M.W. of 75,000. As for B_1, in addition to this band, another one was found at 32,000 daltons (Fig. 3 b), corresponding most likely to the less anodic band described above.

The immunochemical reactivity of the B_2 component was normal, its clotting activity deficiency was similar to that of the whole plasma.

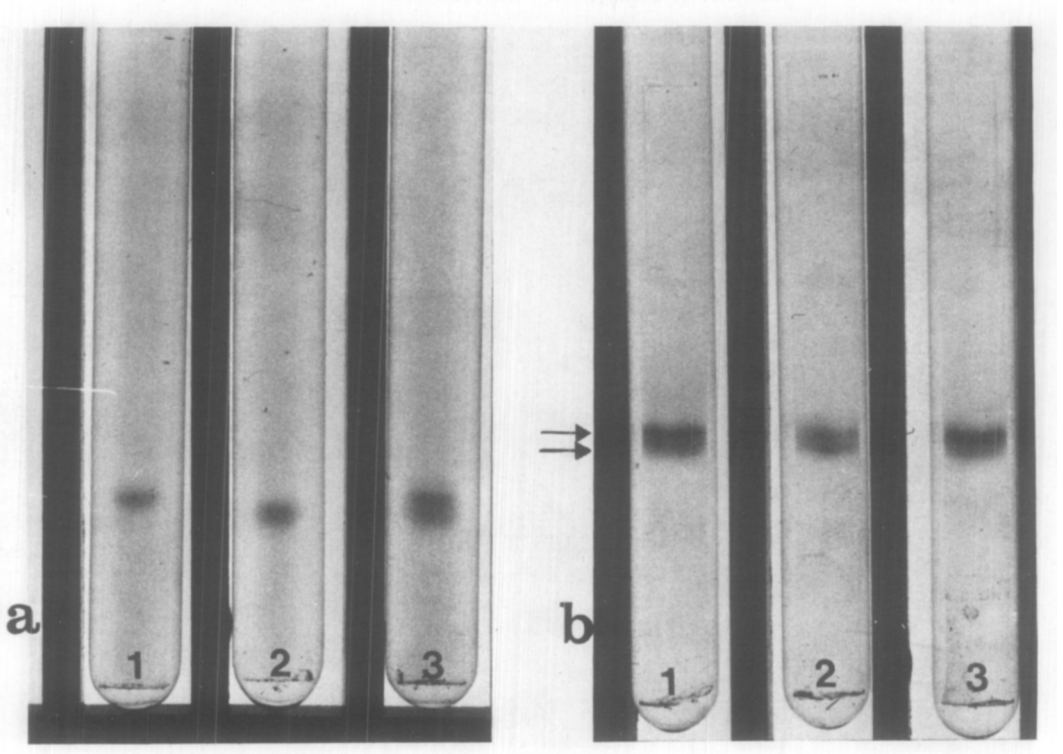

Figure 2. Polyacrylamide gel electrophoreses (7,5 % polyacrylamide, height 10 cm) performed during prolonged time (see text).
a. 1 = normal prothrombin
2 = B_2 sibling component
3 = mixture of normal prothrombin + B_2 sibling component.
b. 1 = peak B_2 from mother's plasma
2 = mixture of normal prothrombin + B_2 sibling component
3 = peak B_2 from father's plasma.

The peaks B_2 of both parents show similarly to the mixture in gel 2, two bands with slight different electrophoretic mobility. The two arrows indicate the positions of the two bands.

The activation studies of prothrombin into thrombin of the B_1 and B_2 products by two stage assay showed the following results (Fig. 4) : the B_2 product could be converted into thrombin much more slowly than normal prothrombin and at a decreased rate. Conversely, the B_1 product could not be activated at all into thrombin.

In order to see whether B_1 was due to an artificial conversion of B_2 during chromatography, B_2 was rechromatographed

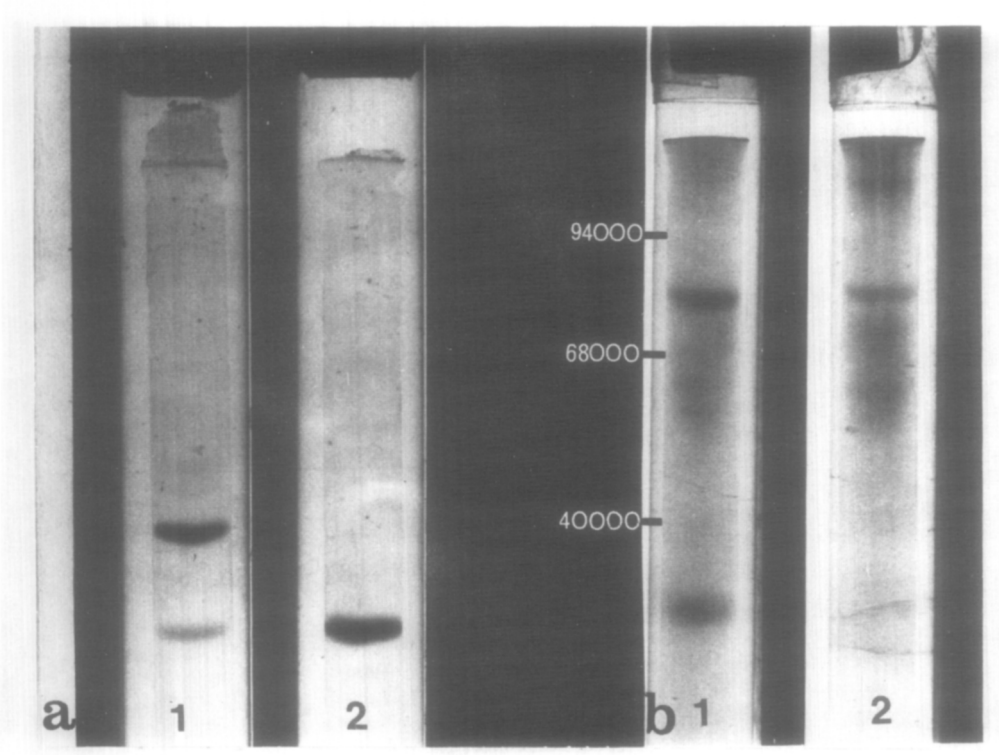

Figure 3. a. Acrylamide gel electrophoreses of siblings' B_1 component in gel 1 and siblings' B_2 component in gel 2.
b. SDS gel electrophoreses of the same samples (B_1 in gel 1 and B_2 in gel 2) : B_1 and B_2 show a band of electrophoretic mobility corresponding to the molecular weight of normal prothrombin : 75,000 daltons. In B_1, the major additional band had a mobility corresponding to 32,000 daltons. The markers used were Phosphorylase a (94,000), Albumin (68,000) and Aldolase (40,000).

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under the same conditions. As a result of this procedure, it was found quantitatively eluted in a single peak at the same ionic strength as the first time.

Identical results have been obtained reproducibly with all siblings'plasmas examined up to now (3 out of 4).

The purification procedure was also applied to the plasma of each of the two parents : in both cases, two peaks were obtained at the same ionic strength as the peaks B_1 and B_2 described above, with slight differences : for both parents, the amount of the first peak was smaller than in the siblings'B_1, and it was

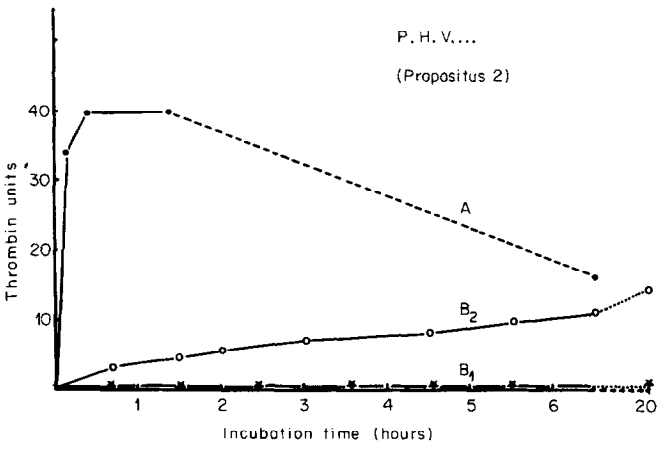

Figure 4. Kinetics of prothrombin conversion into thrombin using a two stage assay (see text). A = normal prothrombin. B_1 and B_2 represent respectively the peaks B_1 and B_2 of the siblings. The different samples were adjusted to the same prothrombin concentration according to immunochemical assay : 20 antigenic units per ml in the activation mixture (1 ml of undiluted normal control plasma being defined as containing 100 antigenic units).

slightly more important for the mother's plasma than for the father's. Electrophoreses of the products of these two peaks revealed complete identity between the two parents and showed the following characteristics : the first peak had the same components as those of siblings' B_1. The second peak was demonstrated to be heterogeneous (Fig. 2 b). By the same long electrophoresis as described above, it divided into two components, like the artificial mixture of siblings' B_2 and normal prothrombin. One of them migrated like normal prothrombin, the other one was slightly faster just as the B_2 component of siblings.

In conclusion, all these results are in good agreement with the following genetical hypothesis : the parents are heterozygote for the prothrombin Barcelone trait, the siblings being homozygote. This prothrombin Barcelona variant has two major characteristics : (1) the existence of a molecule migrating slightly faster than normal prothrombin ; (2) the abnormal occurrence of

an additional peak during DEAE Sephadex chromatography. This peak reveals a molecular entity of less anodic mobility with a molecular weight of 32,000, reacting against antiprothrombin antibodies. This peak does not seem to be due to an artificial production during chromatography as it does not appear during rechromatography. The meaning of its occurrence, not clear up to now, is presently under investigation.

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