To the Editor:

Earlier this year, we reported on the molecular cloning, expression, and partial characterization of a novel human Kunitz-type inhibitor with striking overall domain organization similarity and considerable primary sequence homology to human tissue factor pathway inhibitor. Inasmuch as the purified recombinant protein readily inhibited the amidolytic activity of a complex of factor VIIa and tissue factor, we provisionally referred to this inhibitor as TFPI-2. Purified recombinant TFPI-2 exhibited a molecular mass of 32 kD by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the presence and absence of reducing agent. In addition to inhibiting factor VIIa-tissue factor, the purified TFPI-2 readily inhibited trypsin, but, in contrast to TFPI, inhibited factor Xa only weakly. In addition, TFPI-2 had no detectable inhibitory activity towards the amidolytic activity of human thrombin. Furthermore, recombinant TFPI-2 bound avidly to heparin-agarose columns, and its inhibitory activity against factor VIIa-tissue factor was markedly enhanced in the presence of heparin.

Northern analysis indicated that TFPI-2 is transcribed in umbilical vein endothelial cells, liver, and placenta. A retrospective examination of the literature concerning placental-specific proteins showed a striking similarity between TFPI-2 and placental protein 5 (PP5). PP5 is a glycoprotein (30 to 36 kD) that was originally isolated from the human placenta using heparin-Sepharose chromatography as the initial chromatographic step. Subsequent studies showed that this protein was also produced by endothelial cells and inhibited the activity of human thrombin and plasmin. Using a time-resolved immunofluorometric assay, Butzow et al first demonstrated the presence of PP5 in sera from men (0.43 μg/L) and women (0.49 μg/L), providing evidence that PP5 was not a pregnancy-specific protein, although PP5 concentrations were dramatically higher in pregnancy plasma samples (18 to 30 μg/L). Further evidence that PP5 and TFPI-2 are equivalent was obtained from amino-terminal amino acid sequence analyses of purified PP5. PP5 isolated from placenta in the absence of protease inhibitors yielded a 30-kD band by SDS-PAGE under nonreducing conditions that migrated as three bands at 16.8 kD, 18.3 kD, and 19.0 kD under reducing conditions. Amino-terminal amino acid sequence analysis of each of the latter polypeptides showed the following sequences: 19 kD, Ala-Ala-Ala-GluGlu-Pro-Thr-Gly-Asn-Asn-Ala-X-Ile; 18.3 kD, Asn-X-Ile-Glu-Asn-X-Phe-Pro-X-Glu-Ala-Thr-X-Met; and 16.8 kD, Ala-Leu-Leu-Leu-X-Tyr-Tyr. With the exception of the amino-terminal Ala residue of the 19.0-kD fragment, these sequences correspond exactly to residues 1-13, 111-124, and 25-31, respectively, in human TFPI-2. It is interesting to note that the three polypeptide chains of PP5 obtained in the above study in the absence of protease inhibitors presumably resulted from cleavages C-terminal to Arg residues in TFPI-2/PP5 catalyzed by an unidentified serine protease(s) in the crude placental homogenate. Consistent with this possibility, addition of benzamidine to the purification procedure resulted in the purification of PP5 that exhibited a single band (36 kD) in the presence and absence of reducing agent and a blocked N-terminus.

The ability of PP5 to inhibit thrombin-induced coagulation of fibrinogen appears to be inconsistent with our finding that TFPI-2 failed to inhibit the amidolytic activity of human thrombin. However, a previous report failed to show inhibition of thrombin activity by PP5 using chromogenic substrates. Moreover, we have recently found that TFPI-2, in addition to its ability to inhibit trypsin and factor VIIa-tissue factor, is a potent inhibitor of plasmin (Petersen, Foster, and Kisiel, manuscript in preparation), consistent with an earlier report demonstrating plasmin inhibition by PP5. Taken collectively, these data provide compelling evidence for the molecular equivalence of human TFPI-2 and PP5.

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REFERENCES


Evidence that a second human tissue factor pathway inhibitor (TFPI-2) and human placental protein 5 are equivalent [letter]

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