BRIEF REVIEW

Protein-C: Biochemistry, Physiology, and Clinical Implications

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PROPERTIES OF THE PROTEIN-C ANTICOAGULANT PATHWAY

Protein-C is a vitamin-K-dependent plasma zymogen. After activation, activated protein-C differs from the vitamin-K-dependent plasma clotting factors in that it potently inhibits coagulation by inactivating factors V and VIII, and it facilitates fibrinolysis in vivo by elevating circulating plasminogen activator levels. Clinical evidence of protein-C involvement in the regulation of coagulation comes from the observation that low levels of protein-C are associated with recurrent familial thrombosis. To understand the role of protein-C in hemostasis, it is useful to discuss the protein-C anticoagulant pathway. This pathway can be conveniently divided into three parts: (1) protein-C activation; (2) the inhibition of coagulation through the inactivation of factors V/Va and VIII/VIIIa by activated protein-C; and (3) the inhibition of activated protein-C by a plasma protease inhibitor specific for this enzyme. Each part of this pathway involves unique proteins and/or receptor surfaces. A schematic diagram of the role of protein-C in the regulation of blood coagulation is shown in Fig. 1.

PROTEIN-C STRUCTURE

Human protein-C, like the bovine zymogen, is isolated as a two-chain, disulfide-linked protein. Both the active site and the peptide released during activation reside on the heavy chain (mol wt = 41,000). Thrombin, the only currently known protein-C activator of physiologic relevance, activates human protein-C by a single cleavage that appears to be between Arg 12 and Leu 13 of the heavy chain. The light chain (mol wt = 21,000) contains γ-carboxyglutamic acid residues that are involved in Ca²⁺-dependent membrane association. The light chain of bovine protein-C also contains one residue of β-hydroxyaspartic acid at position 71. The functional importance of this residue remains to be elucidated.

THE ROLE OF COFACTORS AND THE ENDOTHELIAL CELL SURFACE IN PROTEIN-C ACTIVATION

Although thrombin can activate protein-C, the rate of activation is rather slow and is inhibited further by physiologic Ca²⁺ concentrations. As a result, little protein-C is activated when blood clots in vitro. This observation led to the hypothesis that a cofactor for thrombin-catalyzed protein-C activation might exist on the surface of the vascular endothelium. Both organ perfusion and tissue culture experiments confirmed the existence of a high-affinity thrombin receptor (Kₐ = 0.5) on the surface of the vascular endothelium that potently stimulates protein-C activation. Smooth muscle cells, fibroblasts, and glial cells lack detectable cofactor activity. This cofactor, now referred to as thrombomodulin, has been isolated from extracts of rabbit lung. Thrombomodulin is a protein (mol wt = 74,000) that forms a 1:1 complex with thrombin, and this complex then rapidly activates protein-C in a Ca²⁺-dependent reaction. When bound to thrombomodulin, thrombin no longer clots fibrinogen, activates factor V, or triggers platelet activation. Thus, thrombomodulin appears to possess two distinct anticoagulant functions: (1) the ability to stimulate thrombin-dependent protein-C activation, and (2) the ability to directly inhibit thrombin’s procoagulant activities. Since these functions are catalyzed on the surface of the vascular endothelium, it is important to note that the endothelial cell surface area exposed to blood (and hence the presumptive receptor density) rises to approximately 5,000 sq cm/ml in the microcirculation. By comparison, in major vessels or in tissue culture experiments, the surface-to-volume ratio is approximately 1–10 sq cm/ml. Thus, thrombomodulin-mediated protein-C activation and thrombin clearance will occur primarily in the microcirculation. Consistent with this hypothesis, Lollar and Owen demonstrated rapid binding of thrombin to the microcirculation in vitro. Factors such as stasis, which delays the access of thrombin to the microcirculation, will inhibit protein-C activation as well as endothelial cell-mediated thrombin clearance.

An alternative pathway for protein-C activation has recently been proposed. Salem et al. noted that small
amounts of protein-C were activated when human blood clotted in vitro. They demonstrated that factor Va could also enhance the rate of thrombin-catalyzed protein-C activation. Factor Va differs from thrombomodulin in many respects: factor-Va-dependent activation of protein-C is not Ca2+-dependent; if a complex between thrombin and factor Va does form, the affinity is at least 100-fold lower than the affinity of thrombomodulin for thrombin; factor Va does not alter the macromolecular substrate specificity of thrombin; and the rate of protein activation is no more than 10% that obtained with thrombomodulin.15,16 How these two activation systems interact remains to be determined.

ANTICOAGULANT FUNCTIONS OF ACTIVATED PROTEIN-C

Activated protein-C functions as an anticoagulant by inactivating factor V7 and factor VIII8 since in human plasma, only these factors are inactivated.7 The thrombin-activated forms, factors Va and Villa, are inactivated by activated protein-C at least 30 times more rapidly than their precursors.2,7,25 The inactivation of factor Va has been studied in considerably more detail than that of factor Villa. The rate of inactivation is increased approximately 50-fold by phospholipid and Ca2+6,25. Factor Xa protects factor Va from inactivation both in the purified system26 and on the platelet surface.27,28

Activated protein-C appears to be the terminal enzyme in the pathway and the enzyme directly responsible for factor V and VIII inactivation. However, another vitamin-K-dependent protein in plasma, protein-S, is required for optimal activated protein-C anticoagulant activity.29 Walker29,30 has presented evidence that protein-S functions as a cofactor, at least in part, by forming a complex with activated protein-C on the surface of phospholipid vesicles30 and increasing the rate of factor Va inactivation.

CLEARANCE OF ACTIVATED PROTEIN-C

The question of how activated protein-C is inhibited gained considerable interest when Marlar and Griffin hypothesized that patients with simultaneous factor V/VIII deficiency lacked the ability to inhibit activated protein-C.31 They observed a slow time-dependent inhibition of activated protein-C in normal human plasma, but not in the plasma of factor-V/VIII-deficient patients. Although Giddings and Bloom32 also observed reduced activated protein-C inhibitory activity in another group of patients, Canfield and Kisiel33 failed to detect any differences. Initial attempts to isolate the inhibitor suggested that the inhibitor was a protein distinct from other known proteinase inhibitors.34 Recently, Suzuki et al.34 isolated the activated protein-C inhibitor in functional form. Two observations suggest that activated protein-C inhibitor deficiency may not cause simultaneous factor V/VIII deficiency. First, as cited above, the precursor proteins are relatively resistant to inactivation by activated protein-C. Second, there is no direct correlation between the degree of inhibitor deficiency and the severity of the factor V/VIII deficiency.12,33,36 Hopefully, with the availability of a functional inhibitor to activated protein-C and the appropriate exchange of patient plasmas, the role of this inhibitor in factor V/VIII deficiency can be more clearly elucidated.

PROTEIN-C DEFICIENCY AND THROMBOTIC DISEASE

Patients with decreased levels of protein-C appear to be at risk of thrombosis.11,12 In several families studied, decreases in the protein-C antigen level to approximately 40% of normal appear to be sufficient to cause recurrent thrombotic disease. All patients appear to respond favorably to oral anticoagulant therapy.11,12

The clinical use of oral anticoagulants complicates the diagnosis of protein-C deficiency. Both the antigen level15 and activity of vitamin-K-dependent clotting factors decline during therapy. To estimate protein-C levels in patients receiving oral anticoagulants, the antigen levels have been normalized by simultaneously
measuring the prothrombin level and/or the factor X level. This approach may not be adequate, however, since protein-C levels decrease in response to oral anticoagulants more rapidly than factor X and prothrombin. Thus, this approach may give erroneous results. Protein-C levels in suspected deficient patients should also be examined from at least four separate blood samples and under conditions where there is no evidence of coagulation occurring.

Functional assays for protein-C are currently being developed. One approach is to employ purified thrombomodulin to activate protein-C in plasma and subsequently measure activated protein-C by amidolytic assays.

Ultimately, the relationship between protein-C deficiency and thrombotic disease will require study of many more families in which the mode of inheritance is established by immunologic and functional assays. From these studies, a much clearer relationship between protein-C levels and thrombotic disease will emerge.

EVIDENCE FOR PROTEIN-C ACTIVATION DURING COAGULATION

Most patients with evidence of intravascular coagulation undergo significant reductions in their plasma protein-C antigen levels. Following surgery or certain infections, the levels of protein-C drop to 30%-60% of normal. What proportion of this antigen is complexed with the inhibitor remains uncertain. Hence, these represent minimum estimates of protein-C consumption.

Based on animal studies, it would appear likely that thrombin generation during coagulation is responsible for protein-C activation and subsequent consumption. In rabbits or dogs, thrombin infusion at subcoagulant levels leads to selective protein-C activation in a receptor-mediated process that is dependent on normal vitamin K status.

PERSPECTIVES

With our current knowledge of the protein-C system, it appears likely that patients deficient in either protein-S or thrombomodulin, as well as those deficient in protein-C, will exhibit recurrent thrombotic complications. The influence of degenerative vascular disease on endothelial cell-mediated protein-C activation may provide new insights into how vascular disease is linked to thrombosis. The in vitro and in vivo effectiveness of protein-C suggests that protein-C, activated protein-C, or perhaps, thrombomodulin may one day be useful in the management of thrombotic disease.

*Other reviews which deal with the various aspects of protein C structure, function, activation, and synthesis in greater detail can be found in references 42 and 43.

REFERENCES

5. Mammen E, Thomas W, Seegers W: Activation of purified prothrombin to autoprothrombin I or autoprothrombin II (platelet cofactor II) or autoprothrombin II-A. Thromb Diath Haemorrh 5:218, 1960


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