

Name:	Factor D
Catalog Number:	A136
Sizes Available:	25 µg/vial
Concentration:	0.1 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>95% versus normal human serum standard
Purity:	>95% by SDS-PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.3
Extinction Coeff.:	$A_{280\text{ nm}} = 1.1$ at 1.0 mg/mL
Molecular weight:	24,000 Da (single chain)
Preservative:	None, 0.22 µm filtered
Storage:	-70°C or below. Avoid freeze/thaw.
Source:	Normal human serum (shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II).
Precautions:	Use normal precautions for handling human blood products.
Origin:	Manufactured in the USA.

General Description

Factor D is a glycosylated protein composed of a single 24,000 Da polypeptide chain. It is an essential component of the alternative pathway of complement activation. Its only known function is to cleave and activate factor B when factor B is bound to C3b or a C3b-like protein such as C3(H₂O) or C3bB30. Factor D is a serine protease that circulates as a mature protease, but it exhibits a highly restricted specificity and it appears to be substrate activated. Factor D cleaves factor B bound to C3b between Arg233 and Lys234 causing the release of the Ba fragment (33,000 Da) and leaving the 60,000 Bb fragment bound to C3b. The C3b,Bb complex is called a C3 or C5 convertase because it converts these proteins to their active forms by cleaving off the small peptides C3a and C5a, respectively (Law, S.K.A. and Reid, K.B.M. (1995); Morikis, D. and Lambris, J.D. (2005)).

A unique feature of the alternative pathway is the ability of C3b,Bb to amplify itself on the surface of a complement-activating target particle. This enzyme cleaves C3 producing metastable C3b which can attach to the cell near the initial C3b. Each C3b deposited can bind factor B which is activated by factor D forming another C3/C5 convertase. Thus, factor D is a required component for alternative pathway amplification and the concentration of factor D is rate limiting. This amplification mechanism of the alternative pathway can deposit 2,000,000 C3b molecules on a yeast cell or 30,000 C3b on a single bacterial cell 10-15 min after they come in contact with blood. These numbers represent a monolayer of covalently attached opsonins (C3b, iC3b and C3d) which are ligands for phagocytic immune cells. The numbers of C3b and C5b-9 deposited far exceed those produced by the classical or lectin pathway due to the factor B-containing convertase and its ability to amplify itself and spread across the surface of a target.

Physical Characteristics & Structure

Molecular weight: 24,000 daltons, single chain protein with no N-linked glycosylation. Factor D is synthesized as a 246 amino acid proteins with a 13 amino acid signal peptide and a five amino acid activation peptide. Both peptides have been remove

from circulating factor D. The protein exhibits a pI = 7.4. The 3D structure of factor D was solved at 2 angstrom resolution (Narayana, S.V.L. (1994)). The structure resembles chymotrypsin closely except for a disrupted catalytic triad. Activation of the triad structure is believed to be the result of substrate-induced conformational changes that result upon interaction with the C3b,B complex. As a result of this unique structure, factor D exhibits minimal activity on synthetic substrates and minimal inhibition by serine protease inhibitors.

CAS Number: 37213-56-2

Function

Factor D is a trypsin-like serine protease that cleaves only one substrate, namely, complement factor B. Furthermore, it only cleaves factor B when that protein is bound to a cofactor such as C3b, C3(H₂O), or cobra venom factor. For further details about its function see the General Description above and Assays below.

Assays

There are biological and synthetic substrate assays for factor D. Biological assays measure the function of factor D in complement activation – the cleavage of factor B when it is bound to C3b. Mixtures of C3b, factor B, 0.5 mM MgCl₂ and factor D result in the cleavage of factor B (93,000 Da) into Bb (60,000 Da) and Ba (33,000 Da) which can be detected by SDS PAGE. Alternatively, the inactivation of factor B can be followed by assaying the factor B remaining. Radiolabeled factor B has also been used, but the labeling process produces two forms of factor B which differ significantly in the rates of cleavage by factor D.

Synthetic substrate assays for factor D take advantage of its weak proteolytic activity toward Arg- and Lys-containing ester and thioester peptides. One such assay used CBZ-Lys-thiobenzyl ester (Volanakis, J.E. et al. (1993)). The split products are detected at 405 nm by reacting the freed thiol with DTNB. All of the synthetic substrate assays suffer from interference from the much more active proteases such as thrombin and plasmin which if present in the factor D preparation even at low ppm levels can be detected in these assays. Fortunately there is a simple way to test for these and similar proteases: control assays containing the serine protease inhibitor benzamidine at 15 mM must be run. Factor D is only minimally affected by benzamidine. If the factor D in the presence of benzamidine is only slightly less active than without this inhibitor then the activity measured is that of factor D and not from contaminating proteases.

Applications

The alternative pathway cannot activate without factor D and much pathological damage is done by primary or secondary activation of the alternative pathway of complement. Therefore, pharmaceutical companies have investigated various drugs to inhibit it. Due to the distorted active site, except when bound to its substrate, effective small molecule inhibitors have not yet been found. However, humanized anti-factor D is under investigation and has the advantage that very low plasma concentration of factor D requires little antibody. On the other hand, the high biosynthetic rate may need to be overcome with excess drug (see *In vivo* section below).

In vivo

Serum concentration of factor D has been reported to be between 1 and 2 $\mu\text{g/mL}$ and CompTech and others have determined 1.4 $\mu\text{g/mL}$ to be closest to the normal concentration in human serum. Factor D is a trypsin-like serine protease that circulates in its activated form without its activation peptide, however, as mentioned above its proteolytic activity is substrate-induced. It is synthesized in the expressed in the kidney, adipocytes, and macrophages. Its primary site of synthesis appears to be adipose tissue and it is also known as adipsin. Adipsin is thought to also be involved in fat metabolism. Factor D is made as a zymogen that is apparently activated only by MASP-1 (Takahashi, M. et al. (2010)). MASP-1 deficient mice lack a functional alternative pathway and factor D was found to be circulating in zymogen form with its activation peptide still attached. Restoration of alternative pathway function in these mice was achieved with addition of MASP-1.

Regulation

Due to the unique structure of factor D, it is only an active protease when bound to its substrate C3b,B and thus its regulation is built-in. No known regulators of factor D exist. None of the protease inhibitors in plasma affect factor D function. Factor D has been reported to have a high rate of synthesis as well as a high rate of catabolism by the kidney (Volanakis J.E. et al. (1985)).

Genetics

Early reports placed the gene on the X chromosome, but it is located on chromosome 19 in humans (Location: 19p13.3, GeneID 1675).

Deficiencies

Numerous cases of factor D deficiency have been reported. Homozygous individuals suffer from recurrent and sometimes fatal infections especially meningococcal infections. The inheritance is autosomal recessive. Other presentations include pneumococcal neonatal sepsis, and recurrent neisserial infections.

Precautions/Toxicity/Hazards

This protein is purified from human serum, therefore precautions appropriate for handling any blood-derived product must be used even though the source was shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II.

Hazard Code: B WGK Germany 3

MSDS available upon request.

References

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