Name:	**Factor B**
Catalog Number:	**A135C**
Sizes Available:	250 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>90% versus normal human serum standard.
Purity:	>95% by SDS-PAGE
Buffer:	10 mM Sodium phosphate, 145 mM NaCl, pH 7.2
Molecular weight:	93,000 Da (single chain)
Extinction Coeff.:	$A_{280\ nm}$ = 1.27 at 1.0 mg/mL
Preservative:	None, 0.22 µm filtered
Storage:	-70°C or below. Avoid repeated 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

Complement factor B (fB) is purified from normal human serum. Complement factor B is a glycosylated protein composed of a single 93,000 Da polypeptide chain. It is an essential component of the alternative pathway of complement activation and is found in plasma at approximately 200 µg/mL. In the presence of Mg^{++} factor B binds to C3b and the C3b,B complex can be activated by factor D, a serine protease that circulates as an active trypsin-like serine protease. Cleavage of factor B by factor D causes the release of the Ba fragment (33,000 Da) and leaves the 60,000 Bb fragment bound to C3b. This Bb subunit is a serine protease. C3b,Bb is called a C3 and a C5 convertase because it converts both of these proteins to their active forms by cleaving off the small peptides C3a and C5a, respectively (Morikis, D. and Lambris, J.D. (2005); Morley, B.J. and Walport, M.J. (2000)).

Another role for factor B is in the initiation of the alternative pathway. Continuous conversion of native C3 to a C3b-like form is a result of spontaneous hydrolysis of the thioester in C3. This C3(H_2O) binds factor B in a Mg++ stabilized complex. Factor B in the C3(H_2O),B complex can be activated by factor D releasing Ba. During alternative pathway initiation, fluid phase C3(H_2O),Bb cleaves C3 producing metastable C3b which can attach to carbohydrates on cell surfaces and on plasma proteins. If this C3b attaches to a host cell or protein it is rapidly inactivated by a variety of mechanisms due to the actions of factor H, CR1, MCP, and factor I. C3b that attaches to a foreign target lacking these regulators remains active long enough to bind factor B and form C3b,Bb as described above. This is the cell surface-bound C3/C5 convertase of the alternative pathway of complement. C3b,Bb is an unstable trypsin-like serine protease with a half-life of approximately 90 seconds in the absence of factors that accelerate decay (factor H, DAF, and CR1). The proteolytic site is in the C-terminal domain of the Bb subunit (Morley, B.J. and Walport, M.J. (2000)).

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 and form another C3/C5 convertase and deposit more C3b in

an expanding ring of attached proteins. C3b,Bb with a second C3b nearby becomes a more efficient C5 convertase and it cleaves C5 releasing C5a and depositing C5b-9 complexes in the bilipid layer of the target cell. This amplification mechanism of the alternative pathway can deposit 2,000,000 C3b molecules on a yeast cell or 30,000 C3b on a bacterium 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.

The major protein of cobra venom is able the bind factor B from many species of animals. The protein, known as cobra venom factor (CVF), once combined with factor B and activated by factor D forms the C3 convertase CVF,Bb which circulates as a stable complex (half-life ~7 hrs) and is able to cleave C3 and C5. This complex is not decayed by any of the decay accelerating factors that shorten the half-life of C3b,Bb to a few seconds in blood. Thus, the cobra can generate an enzyme that releases C3a and tahe advantage of its vasodilatating effect to speed dissemination of the other toxins in the venom. CVF from some species of cobras (*naja naja Kaouthia*) form enzymes that can also bind C5 with high affinity. The resulting CVF,Bb enzyme can cleave C5 thus releasing the anaphylatoxin C5a (Rawal, N. and Pangburn, M.K. (2001)).

The fragments of factor B (Ba and Bb) have been proposed to elicit several biological responses. The smaller fragment Ba has been reported to have chemotactic activity with neutrophils and macrophages, but this effect is so much lower than that of C5a or even C5adesArg that its effect *in vivo* may be negligible (Morgan BP, (1990)). Fragment Bb has been reported to stimulate macrophage spreading, to enhance monocyte-mediated cytotoxicity and to promote B lymphocyte proliferation. The larger fragment Bb possesses the proteolytic site, but once Bb is released from C3b it no longer expresses proteolytic activity toward C3 or C5. Reports of low level proteolytic activity towards synthetic substrates have been shown to be due to contaminating thrombin in some Bb preparations. Reported activities toward clotting factors probably have a similar explanation.

Physical Characteristics & Structure

Molecular weight: 93,000 daltons, single chain protein containing 7.3-8.6% carbohydrate (Rother (1998)). The protein is negatively charged at serum pH and exhibits a heterogeneous pI = 5.6-6.1. Factor B is one of the heat labile proteins of the complement system. It binds divalent metal ions such as Mg++ or Ni++, which stabilize its interaction with C3b and C3b-like molecules. In the electron microscope factor B exhibits a three domain structure one domain of which is Ba. Bb contains two domains one of which is a von Willebrand factor-like A domain that binds to Mg++ and to C3b and the other, the C-terminal domain, contains the active serine protease site. The Ba domain actually contains of three SCR or CCP domains which interact with C3b. Crystal structures for the serine protease domain at 2.1 angstrom resolution (Jing, H. (2000)), the A domain (Milder, F.J. (2007)) and the whole protein (Bhattacharya, A.A. (2004)) at 2.3 A resolution have been published.

Function

Factor B is a zymogen of a serine protease. It must be bound to C3b, C3(H_2O) or CVF to be activated by the serine protease factor D which cleaves the Arg233-Lys234 bond in factor B. Even in its active form while bound to C3b, the Bb enzyme is a very inefficient protease. It cleaves C3 at about 1/1000 the rate of trypsin. But unlike trypsin it is highly specific for the single bond in C3 or C5. The turnover number for C3 is 107 C3/min/enzyme and cleavage of C5 has a turnover number of 0.3 C5/min/enzyme. The activity toward C5 is slower than some RNA-based enzymes (ribozymes), but it is apparently sufficient to support the biological effects of complement including immune system stimulation by C5a and target killing by C5b-9.

Assays

The most convenient assays measure the cleavage of the substrate C3 or reconstitution of alternative pathway function in factor B-depleted serum (B-Dpl). Assays using purified components are complicated by the short half-life of the active enzyme complex between C3b and Bb (90 sec at 37^{o}C in the absence of decay accelerating factors such as factor H). Also, these assays are extremely sensitive to contaminating factor H (1 ng factor H can greatly alter the measured activity) which is frequently present in purified C3 preparations (Complement technology, Inc. specifically removes trace amounts of factor H). Nevertheless an accurate relatively easy to perform fluorescence-based assay has been described (Dodds and Sim (1997)). The most convenient assays are alternative pathway reconstitution assays using lysis of rabbit erythrocytes and factor B-depleted human serum (B-Dpl) (Morgan (2000)). ELISAs for Ba or Bb (Dodds and Sim (1997)) can also be used for measuring factor B split products and ELISA kits for this are sold commercially by several companies.

Applications

Split products of factor B in plasma are indicative of activation of the alternative pathway in vivo. ELISA kits for measurement of Ba and Bb are commercially available.

In vivo

The average concentration is 200 µg/mL (range 170-258 µg/mL) in human plasma. The protein is produced primarily in the liver although mRNA as well as protein expression has been identified in PMN, macrophages, endothelial cells, fibroblasts, and alveolar type II epithelial cells. Factor B is an acute phase protein whose plasma levels increases during inflammation.

Regulation

Factor P (properdin) stabilizes the complex of factor B with C3b as well as the preformed C3/C5 convertase, C3b,Bb. These complexes are destabilized by factor H and by the membrane-bound regulators DAF and CR1. The term nephritic factor describes a group of autoantibodies that bind to and stabilize the C3b,Bb complex. These occur in type II membranoproliferative glomerulonephritis (MPGN). The antibody-stabilized enzyme cannot be regulated, is deposited in the kidneys and leads to kidney damage.

The plasma concentration of factor B increases with infections and inflammation and it is thus considered one of the acute phase proteins. Increased synthesis has been shown to be stimulated by LPS and cytokines (IL1, TNFα, IL6, IFNγ and IFNβ). PDGF, FGF, and EGF down regulate synthesis of factor B.

Genetics

The gene for factor B is located on human chromosome 6p21.3 within the MHC class III region between the class I and class II regions. The factor B gene lies between the larger gene for C2 (to which it is highly homologous) and genes for C4A and C4B. The gene is composed of 18 exons and spans 6 kb.

Deficiencies

No natural deficiencies of factor B have been identified in humans or animals. However, factor B-deficient mice have been generated by disruption of the factor B gene. In pathogen free environments $B^{-/-}$ mice exhibit no overt phenotype. However, animals lacking factor B are more susceptible to infectious diseases compared to wild type mice (Holers V.M. (2000), Thurman and Holers, (2006)). . In contrast, $B^{-/-}$ mice exhibit much lower or no pathology in a wide variety of diseases where alternative pathway activation is the cause of or exacerbates the pathology. These diseases are listed below. Acquired and secondary deficiencies do occur in humans. Human factor I deficiencies exhibit very low factor B levels due to the fact that C3b is not inactivated and accumulates in blood. This results in binding of factor B, cleavage by factor D and rapid release of Bb by factor H. Transfusions with normal plasma or reconstitution with factor I temporarily stop or slow consumption of factor B.

Diseases

While mice with complete deficiencies of factor B exhibit increased susceptibility to infections, they also show reduced or the complete absence of pathology in many inflammatory diseases including SLE (systemic lupus erythematosus), rheumatoid arthritis, intestinal and renal ischemia/reperfusion injury, immune-mediated spontaneous fetal loss and asthma (Thurman and Holers, (2006)).

Precautions/Toxicity/Hazards

This protein is purified from human serum and 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, HTLV-I/II, STS, and for antibodies to HCV, HIV-1 and HIV-II.

Hazard Code: B WGK Germany 3

MSDS available upon request.

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