Name:	**Factor Bb (fragment of factor B)**
Catalog Number:	**A155**
Sizes Available:	250 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Purity:	>95% by SDS-PAGE
Buffer:	Phosphate-buffered saline, pH 7.3
Molecular weight:	60,000 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

Factor Bb is the fragment of complement factor B that results from activation of the alternative pathway. The Bb fragment is made from factor B which was 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 comes from the C-terminal of factor B and it contains the proteolytic active site of the serine protease C3b,Bb (Morley, B.J. and Walport, M.J. (2000)). 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)). C3b,Bb is an unstable trypsin-like serine protease with a half-life of approximately 90 seconds. In the presence of factors that accelerate decay (factor H, DAF, and CR1) it is dissociated is seconds. This releases the fragment Bb into solution. Once released from C3b the Bb fragment is no longer active in complement and lacks a typical serine protease active site.

Physical Characteristics & Structure

Molecular weight: 60,000 daltons, single chain protein containing carbohydrate (Rother (1998); Morley & Walport). The protein is negatively charged at serum pH. 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. 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 angstrom resolution have been published.

Function

The fragments of factor B (Ba and Bb) have been proposed to elicit numerous biological responses; however, many of these activities have proved to be controversial with an inconsistent record of reproducibility. It is not yet clear whether these failures are due to different experimental conditions, more highly purified Ba and Bb or the need to test fresh, in situ-prepared fragments, as has been suggested. Both fragments have been reported to bind to B lymphocyte receptors and modulate proliferation (Kolb, W.P., et al. (1989)). Fragment Bb has been reported to induce activation and spreading of human and murine macrophages and monocytes (Sundsmo, J.S. and Gotze, O. (1981)), although the activity was inhibited by anti-C5 presumably by preventing secondary activation of C5 and release of C5a (Sundsmo & Gotze (1981)). Both factor B and the Bb fragment have been reported to stimulate intracellular killing of *Staph. aureus* by human monocytes (Leijh, P.C. et al. (1982)). Fragment Bb has also been reported to induce secretion of lysosomal hydrolases from peritoneal mononuclear phagocytes in an interleukin-like manner (Hirani, S. et al. (1985)). Bb has been reported to act as a B cell growth factor (Peters, M.G. (1988)) and it has also been reported to be a specific inhibitor of C5a desArg chemotactic activity (Perez, H.D. (1987)). Activation of bovine monocytes and neutrophils by Bb was found to be comparable to the activation by PMA and FMLP (Sethi, M.S. et al. (1990)). Evidence for protein receptors for Bb on human monocytes grown in culture for 24 hrs and on guinea pig macrophages has been reported (Saeki T. and Nagasawa, S. (1989)). Other studies have reported that Bb stimulates the microbicidal activity of bovine monocytes (Seith, M.S. and Tabel, H. (1990a)) and that it stimulates the oxidative burst (Seith, M.S. and Tabel, H. (1990b)) of bovine monocytes. More recently, Bb was demonstrated to induce apoptosis in leukemic cells through a suicide mechanism of myeloid lineage cells (Uwai, M. et al. (2000)). A factor isolated from fetal bovine serum that improved the long term survival of human endothelial cells in culture turned out to be the bovine Bb fragment (Cai, G. et al. (1997)).

The fragment Bb possesses the proteolytic site of C3b,Bb, 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 although highly sensitive fluorescent esterolytic assays may be able to detect residual Bb activity.

Assays

There are no convenient assays for the proteolytic activity of Bb primarily because its activity is so poor compared to a typical trypsin-like protease. It is usually referred to as inactive after its release from C3b. Several companies produce ELISA kits for measuring Bb levels in blood samples (Dodds, A.W. and Sim, R.B. (1997)).

In vivo

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. These have been used in numerous human and animal studies (Lynch, A.M., et al. (2008))

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. Mice deficient in factor B ($B^{-/-}$ mice), compared to wild-type, exhibit much lower or no pathology in a wide variety of diseases where alternative pathway activation is the cause of or exacerbates the pathology of these diseases. See Diseases section 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 in the absence of factor I and C3b 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 production of fragment Bb.

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 (Holers, V.M. (2000); Kolb, W.P. et al. (1989); Thurman and Holers, (2006); Morgan, B.P. (1990)).

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

MSDS available upon request.

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