Name:	**Factor I**
Catalog Number:	**A138**
Sizes Available:	100 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	Cleaves C3b with factor H as a cofactor.
Purity:	>95% by SDS-PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Extinction Coeff.:	$A_{280\ nm}$ = 1.43 at 1.0 mg/mL
Molecular weight:	88,000 Da (2 chains)
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

Human factor I is purified from normal human serum (Fearon, D.T. (1977)). Factor I is a naturally glycosylated serine protease which cleaves and inactivates C3b and C4b. Factor I is highly specific but inactive without a cofactor such as the soluble factor H and C4b binding protein (C4BP) (Pangburn, M.K. et al (1977)). Membrane-bound cofactors include CR1 (complement receptor 1 (CD35) which is found on human red cells and other lymphoid cells) and MCP (membrane cofactor protein (CD46) which is found on most human nucleated cells). Factor I cleaves the alpha-peptide chain of C3b and C4b when these are bound to one of the cofactors. This cleavage inactivates all of the complement activating functions of these proteins producing iC3b and iC4b. Factor I can cleave the alpha chain of C3b twice and this releases a small fragment called C3f (17 amino acids, ~3 kDa). In the presence of CR1 factor can cleave iC3b releasing C3c from C3dg. C4b is cleaved rapidly at two sites separating C4c from C4d. Nomenclature for this protein has changed over time and it has been called C3b inactivator (C3b-INA), C3b/C4b inactivator, and conglutinin-activating factor (KAF).

Physical Characteristics & Structure

Molecular weight: 88,000 daltons composed of 2 disulfide-linked chains of 50,000 and 38,000 daltons. It is synthesized as a single chain molecule. The protein is heavily glycosylated with at least 6 glycosylation sites (3 on each chain) (Morley, B.J. et al. (2000)). The total carbohydrate content is approximately 10.7 %. The protein is negatively charged at serum pH and exhibits a heterogeneous pI = 4.5 to 6.0 due primarily to differences in sialic acid content.

CAS Number: 80295-66-5
MDL Number: MFCD00166552
EC Number: EC 3.4.21.45

Function

See general description above. Even though it is a serine protease with trypsin-like specificity for Lys/Arg, it exhibits virtually no activity against peptide or ester

substrates and its activity is only very slowly inactivated by DFP (diisopropyl fluorophosphate) or other serine protease class inhibitors. It is thought to have an inactive conformation except when bound in complex with its substrates and cofactors.

Assays

The most convenient assay measures the cleavage of substrates C3b or C4b by SDS gels. This must be done in the presence of the appropriate cofactor: factor H (for C3b) and C4BP (for C4b) (Morgan, B.P. (2000)). A typical C3b cleavage assay should contain approximately 4 µg C3b, 1 µg factor H and various amounts of factor I from 0 to 1 µg in a total volume of 15 µL. A typical C4b cleavage assay should contain approximately 4 µg C4b, 1 µg C4BP and various amounts of factor I from 0 to 0.1 µg in a total volume of 15 µL. The assays should be set up on wet ice, then incubated for 10 min at 37^{o}C at which time SDS sample buffer containing a reducing agent should be added to the tubes and the samples heated for 5 min. SDS PAGE gels run under reducing conditions should reveal cleavage of the alpha chains of the respective protein. A continuously monitored fluorescent assay has been reported (Pangburn, M.K. et al. (1983)) which takes advantage of the approximately 10-fold drop in fluorescence of ANS (8-anilino-1-naphthalenesulfonic acid) in the presence of C3b when that C3b is converted to iC3b.

Applications

Deficiencies have been treated for acute crises with fresh frozen plasma transfusions as well as fractions of plasma enriched in factor I.

In vivo

The average concentration is 34 µg/mL (0.39 µM) in human plasma. The protein is produced primarily in the liver although mRNA as well as protein expression has been identified in monocytes, endothelial cells, fibroblasts, muscle and nerve cells.

Regulation

Factor I plasma concentration increases with infections and it is one of the acute phase proteins. Synthesis in tissue cells is increased by bacterial lipopolysaccharide (LPS) and by gamma interferon.

Genetics

The gene for factor I is located on human chromosome 4q25. The gene spans 63 kb with 13 exons (CFI(3426)). In the mouse it is on chromosome 3 at 66.6 cM. Accession numbers: Human (cDNA: Y00318, J02770; Genomic X78594), Mouse (U47810).

Deficiencies

Deficiencies are autosomal recessive and are characterized by low levels of functional C3 due to uncontrolled complement activation (Ziegler , J.B. et al. (1975)). Complement activation occurs due to the inability to inactivate C3(H_2O) from tickover or the C3b produced by C3 convertases. Both C3 and factor B levels are severely depressed in complete deficiencies.

Diseases

Patients with factor I deficiencies often present with repeated pyrogenic infections, glomerulonephritis and/or SLE-like symptoms. Treatments include transfusions with normal plasma and partially purified factor I (Ziegler , J.B. et al. (1975)), but long term application of these elicit a neutralizing immune response.

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, HTLV-I/II, STS, and for antibodies to HCV, HIV-1 and HIV-II.

Hazard Code: B

MSDS available upon request.

References

Fearon, D.T. (1977) Purification of C3b inactivator and demonstration of its two polypeptide chain structure. J. Immunol. 119, 1248-1252.

Morgan, B.P. ed. (2000) Complement Methods and Protocols. Humana Press.

Morley, B.J. and Walport, M.J. (2000) The Complement Facts Book (ISBN 0127333606) Academic Press, London.

Pangburn, M.K., Schreiber, R.D. and Müller-Eberhard H.J. (1977) Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein beta1H for cleavage of C3b and C4b in solution. J Exp Med. 146,257-70.

Pangburn, M.K. and Müller-Eberhard H.J. (1983) Kinetic and thermodynamic analysis of the control of C3b by the complement regulatory proteins factors H and I. Biochemistry 22:178-185.

Ziegler , J.B. et al. (1975) Restoration by purified C3b inactivator of complement-mediated function *in vivo* in a patient with C3b inactivator deficiency. J. Clin. Invest. 55,668-672.

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