Name:	**Factor H**
Catalog Number:	**A137C**
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 7.3
Extinction Coeff.:	$A_{280\ nm}$ = 1.24 at 1.0 mg/mL
Molecular weight:	155,000 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

Complement factor H (fH) is purified from normal human serum. It is an essential regulatory component of the alternative pathway of complement. It is critical for prevention of complement activation on host cells and tissues, especially the kidney. It has two functional activities: 1) it controls the formation and decay of the alternative pathway C3/C5 convertase (decay accelerating activity) and 2) it acts as a cofactor for factor I which proteolytically inactivates C3b when C3b is bound to factor H (cofactor activity).

Factor H is a 155,000 Da protein composed of 20 homologous domains arranged like beads on a semi-flexible string. The N-terminal 5 domains bind to C3b and inhibit binding of factor B thus reducing the formation of C3/C5 convertase. Factor H also binds to preformed C3/C5 convertases (C3b,Bb and C3b,Bb,C3b) and causes rapid release of the catalytic subunit Bb (decay acceleration). These activities are essential for controlling the spontaneous activation of the alternative pathway amplification process in plasma. In addition, factor H controls the formation and decay of these enzymes when C3b is attached to the surface of particles. It is most effective on host cells and less effective on foreign particles for reasons described below.

The alternative pathway of complement is constantly activating by "tickover" producing fluid phase C3b-like C3(H_2O) and C3b. Factor H can bind to these proteins and act as a cofactor so that factor I (a serine protease that circulates in active form) can cleave their alpha chains producing inactive proteins (iC3b or iC3(H_2O)). If C3b is not inactivated in this way it continues to form C3 convertases and consumes factor B and C3. If C3b is attached to surfaces it is converted to iC3b by factors H and I in a similar manner. Factor H is more effective when C3b resides on a host cell due to the presence of host markers recognized by factor H. Complement-mediated damage to the host is minimized due to host specific recognition by factor H.

Factor H appears to regulate discrimination between potential pathogens and host cells and tissues by recognizing host markers. C3b attached to a surface can initiate the amplification cascade of the alternative pathway. Factor H prevents this on host cells and allows it to occur on surfaces that do not bear host-like markers. These host-specific

structures are thought to be polyanionic clusters such as sialic acids and sulfated glycosoaminoglycans. Recognition of host markers occurs through multiple polyanion binding sites located in domains 6-20 of factor H. One site is located in domain 7 and a mutation in this domain (Y402H) is strongly associated with complement activation and tissue destruction in age-related macular degeneration (Zipfel, P.F. et al. (2006)). A tentative site is located in the domain 12-14 region and a very important site is located at the C-terminal in domains 19-20. This C-terminal site appears to be the main site that aids binding to host surfaces. Mutations affecting or located in these domains lead to activation of the alternative pathway of complement in inherited hemolytic uremic syndrome (Zipfel, P.F. et al. (2006)). This site appears to be the site involved in polyanion-dependent dimer and tetramer formation of factor H (Pangburn, M.K. et al. (2009)).

Physical Characteristics & Structure

Molecular weight: 155,000 Da. Factor H is a heavily glycosylated (16%) protein composed of a single polypeptide chain. The pI = 5.4 – 6.0. The structure consists of 20 homologous domains of approximately 60 amino acids each connected by short spacers of 3 to 8 amino acids. Some interdomain linkages appear to be highly flexible and some appear to be rigid. Each domain has two disulfides and numerous invariant amino acids. These domains are referred to as CCP (Complement Control Protein domains) or SCR (Short Consensus Repeat domains) although CCP has recently gained wider acceptance. Both NMR- and crystal-derived structures have been published for various domains. The domains are ellipsoid and the overall length of factor H has been estimated to be between 40 and 80 nm.

Function

See General Description above.

Assays

Functional assays of factor H measure either its decay accelerating activity or its factor I cofactor activity. The most convenient cofactor assay measures the cleavage of purified C3b by SDS gels. This must be done in the presence of factor I (Morgan, B.P. (2000)). A typical C3b cleavage assay should contain approximately 4 µg C3b, various amounts of factor H from 0.1 to 1 µg and 1 µg factor I in a total volume of 15 µL. The assays should be set up on wet ice, then incubated for 10 min at 37ºC at which time SDS sample buffer containing 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 chain of C3b. A continuously monitored fluorescent assay has been reported (Pangburn, M.K. et al. (1983)) which takes advantage of the approximately 8-fold drop in fluorescence of ANS (8-anilino-1-naphthalenesulfonic acid) in the presence of C3b when that C3b is converted to iC3b.

Decay acceleration is more difficult to assay because it requires the measurement of the increased rate of decay of the C3 convertase C3b,Bb in the presence of factor H. C3b,Bb has a natural half life at 37ºC of 90 seconds, but by substituting Ni^{++} for Mg^{++} ions and working at room temperature, the half life can be increased to about 20 min. Two assays may be used: 1) lysis of sheep erythrocytes coated with C3b,Bb (Dodds, A.W. and Sim, R.B. (1997)) or 2) zymosan or erythrocytes coated with C3b,Bb made

with ^{125}I-B (Pangburn, M.K., et al. (2000)). In the EsC3b,Bb lysis assay, the higher the factor H concentration during the preincubation period the less lysis is seen in the second step where lysis occurs in the presence of EDTA and the terminal pathway proteins (Dodds, A.W. and Sim, R.B. (1997)). In the assays using ZymC3b,Bb with radiolabeled factor B, a ten minute incubation followed by a rapid wash of the cells results in less radioactivity on the cells incubated in the presence of factor H than on the cells in the absence of factor H.

Applications

Purified factor H as well as whole plasma from normal donors has been used as replacement therapy in inherited HUS. This treatment is effective but temporary.

In vivo

The serum concentration is generally accepted as 500 µg /mL, however, literature values for the extinction coefficient vary from 0.9 to 1.95 $A_{280\ nm}$ for a 1 mg/mL solution (Pangburn, M.K. et al. (2009)) and estimates of plasma concentrations range from 250 to 564 µg/ml. The primary site of synthesis is the liver. In addition, synthesis has been demonstrated in monocytes, endothelial cells, fibroblasts, platelets, glial cells and myoblasts. In these cells and in liver synthesis is increased by IFNgamma.

Regulation

IFNgamma has been shown to increase liver synthesis of factor H and a similar effect is seen on synthesis by monocytes, endothelial cells, fibroblasts and myoblasts.

Genetics

The human chromosome location is 1q32 while the mouse is 1,74.1 cM. It spans 22 exons. Accession numbers: human Y00716 and mouse M12660

Deficiencies

Human factor H deficiencies are rare and usually result in pathology. In the absence of factor H there is uncontrolled activation of the alternative pathway of complement. This results in low C3 and factor B levels. Patients suffer from renal disease, infections especially meningococcal infections, glomerulonephritis, systemic lupus erythematosus and are chronically hypocomplementemic. In factor H knock-out mice the primary cause of death is kidney failure due to massive uncontrolled complement activation.

Diseases

Surprisingly, the complement field was known for years as an immune system without a disease. By this it was meant that although complement seemed to be involved in most diseases where the immune system was activated, no diseases had been shown to be due to complement itself and there were questions about its importance to disease processes where it was involved, such a SLE. Then multiple examples of the failure of natural complement control systems began to reveal the destructive role complement played in many diseases. The first example was PNH (paroxsysmal nocturnal hemoglobinuria) where an acquired inability to attach lipid anchors to two membrane-bound regulatory proteins (DAF and CD59) resulted in human erythrocytes which were

attacked by complement and lysed in 3 to 6 days instead of circulating for 60 to 90 days (Nicholson-Weller , A., et al. (1983); Pangburn, M.K., et al. (1983); Brodsky, R.A. (2008)). Even though factor H protects these cells allowing extended time in circulation (Ferreira, V.P. and Pangburn, M.K. (2007)) it cannot overcome the absence of the two cell-bound regulators.

Atypical hemolytic uremic syndrome (aHUS) was shown to be associated with mutations affecting the C-terminal of factor H (Zipfel, 2006). As described above in the General Description, this region of factor H binds factor H to host surfaces and to deposited C3b. This two-site attachment at the C-terminal (domains 19-20) anchors factor H to the surface which allows the N-terminal regulatory domains to inhibit further activation at that site. Mutations that reduce the effectiveness of this anchor lead to reduced control of alternative pathway activation on host tissues. Because control of spontaneous fluid phase activation by the n-terminal domains is intact serum from affected individuals contains a fully functional alternative pathway that cannot be adequately controlled on surfaces. Thus, aHUS is characterized by spontaneous complement activation that destroys the kidney.

In 2005 four research groups using the first data from the human genome project simultaneously identified a strong link between age-related macular degeneration and a single amino acid change in domain 7 of factor H (de Córdoba, S.R. and de Jorge, E.G. (2008)). This region is known to contain a high affinity polyanion binding site and by a mechanism that is still unclear this appears to lead to complement activation primarily on retinal tissue late in life.

Precautions/Toxicity/Hazards

This protein is purified from pooled 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.
CAS Number: 80295-65-4

References

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