Name:	**C1 Esterase Inhibitor (C1-INH)**
Catalog Number:	**A140C**
Sizes Available:	1000 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>90 % active protein
Purity:	>95 % by SDS PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.3
Extinction Coeff.	$A_{280\ nm}$ = 0.45 at 1.0 mg/mL
Molecular Weight:	110,000 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, HTLV-I/II, STS, 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

The protease inhibitor C1-INH prevents the spontaneous activation of complement and limits consumption of C2 and C4 by rapidly inactivating C1r, C1s and MASP2. It is the only plasma serine protease inhibitor (Serpin) capable of interacting with and inhibiting activated C1. C1-INH interacts with the catalytic sites of both C1r and C1s. The interaction with activated C1r and C1s is covalent resulting in complexes which are stable to SDS. The binding of C1-INH to activated C1 releases both C1r and C1s from the complex leaving C1q bound to the immune complex. The released complexes contain four molecules: C1-INH-C1r-C1s-C1-INH. The reaction of C1 esterase inhibitor with activated C1 is very fast with the estimated half-life of C1r and C1s being approximately 15 seconds in serum. In fact, at serum concentrations of C1-INH little or no additional C4 or C2 activation occurs 3 min after immune complexes are added because all the C1r and C1s molecules have been inactivated and removed from the C1q which remains bound to the immune complex (Ross, G.D. (1986); Morley, B.J. and Walport, M.J. (2000); Rother, K., et al. (1998); Ziccardi, R.J. (1982a and 1982b); Morgan, B.P. (1990)). C1-INH is thought to bind to and stabilize unactivated C1r and C1s in the C1 complex thus retarding their spontaneous activation (Ziccardi, RJ. (1982b)).

C1-INH plays an important role in suppression of inflammation and control of vascular permeability. Through its ability to inhibit complement proteases (C1r, C1s and MASP2) and to express a variety of other biological functions (Davis III, A.E. et al. (2008)) C1-INH is able to regulate inflammatory reactions in sepsis, endotoxic shock, ischemia-reperfusion injury, transplantation, and other bacterial and parasitic infections. Through its ability to control contact system activation it inhibits bradykinin generation and this controls vascular permeability. This function is most apparent in patients with a physical or functional deficiency of C1-INH. Hereditary angioedema (HAE) patients suffer from enhanced blood vessel permeability and tissue swelling or edema. Most

patients are heterozygous and have 15% to 30 % of the normal level of functional C1-INH in blood.

Physical Characteristics & Structure

C1-INH has an apparent molecular weight of 110,000 Da. It is a single chain protein that is highly glycosylated with approximately 30 to 40% carbohydrate. It is synthesized as a 500 amino acid protein. Removal if the signal peptide results in a plasma protein that contains 478 amino acids. The calculated molecular weight based on amino acids is 53,000 g/mole, however due to extensive glycosylation its mass is closer to 75,000. It runs abnormally on many gel systems giving apparent molecular weights from 90,000 to 115,000 MW. The glycosylation sites include six N-linked and six or seven O-linked sites. C1-INH is an extremely acidic protein with a pI of less than 3.0.

Assays

Assays that measure the functional level of C1-INH in serum are the most useful for diagnostic purposes because many HAE patients possess normal levels of antigen, but low functional levels of C1-INH (Dodds, A.W. and Sim, R.B. editors (1997)). There are a number of commercial assays on the market. In the laboratory a spectrophotometric assay based on the ability of C1-INH to inhibit the proteolytic activity of C1s is the most specific and convenient (Dodds, A.W. and Sim, R.B. editors (1997); Morgan, B.P. ed. (2000)). Purified C1s enzyme (CompTech #A104) is preincubated for 15 min with the diluted serum sample. The remaining activity of C1s is then measured by following the cleavage of a C1s spectrophotometric substrate (for example, N-alpha-carbobenzoxy-L-lysine-p-nitrophenyl ester).

An ELISA assay can be used to detect C1-INH-C1r-C1s-C1-INH complexes in blood (Dodds, A.W. and Sim, R.B. editors (1997)). In one format plates are coated with anti-C1s which captures the complexes and anti-C1-INH is used as the detecting antibody (Morgan, B.P. ed. (2000); Dodds, A.W. and Sim, R.B. editors (1997)).

In vivo

The normal plasma concentration of C1-INH is approximately 200 µg/mL (range 137-240 µg/mL). C1-INH is an acute phase protein and its concentration often doubles during infections and during inflammatory reactions such as infections and rheumatoid arthritis. INF-gamma, IL-6 and TNF-alpha stimulate synthesis of C1-INH. C1-INH normally has a rapid turnover rate *in vivo* with almost half of the C1-INH in blood being lost and replaced every 24 to 48 hours. This is at least partially due to consumption of C1-INH into protease-C1-INH complexes which are cleared.

Applications

The genetic disorder HAE is caused by a partial deficiency of C1-INH. Replacement therapy with a C1-INH concentrate produced by a number of drug companies has been approved for use in both Europe and the USA. These concentrates are administered intravenously and increase blood levels of C1-INH 2- to 3-fold. A typical treatment includes administration of 1000 Units (approximately 200 mg) and the blood level may remain elevated for 2-4 days. Note: C1-INH from Complement Technology, Inc. is for research only and is not for human or drug use.

Regulation

See ***In vivo*** section above.

Genetics

The gene for C1-INH is located on chromosome 11p11.2-13. The gene is composed of 8 exons and spans 18 kb. The EMBL/Genbank cDNA accession numbers for C1-INH are M13656 and X54486 (human) and Y10386 (mouse).

Deficiencies

One of the first diseases connected to the deficiency of a complement component was hereditary angioedema (HAE). Although it proved many years later to be incorrect, it was thought that the absence of C1-INH lead to spontaneous activation of C1, which it does, which lead to activation of C2, which it does, which led to release of a kinin from C2, which does not appear to be the case. The mechanism behind HAE is the loss of regulation of the proteases (plasma kallikrein and factor XIIa) that leads to the release of bradykinin. This kinin, not one from C2, appears to be the causative agent of the major symptoms of HAE attacks.

HAE is an autosomal dominant disease. This means that loss of only half of the normal plasma concentration of C1-INH can, in many individuals, lead to disease. This loss can be caused by the loss of a gene or mutations that lead to dysfunction. More than 50 mutations that cause the loss of function have been described. Acquired C1-INH deficiency has also been described and is generally due to the presence of antibodies that inhibit the biological function of the protein.

Diseases

The genetic disorder HAE is caused by a partial deficiency of C1-INH. See **General Description**, **Applications,** ***In vivo,*** and **Deficiencies** above for more details. Patients with HAE have low functional C1-INH levels in blood and have recurrent episodes of systemic or localized edema.

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.

References

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