Name:	**C1s Enzyme**
Catalog Number:	**A104**
Sizes Available:	250 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>90 % C1s will bind with excess C1-INH
Purity:	>90 % by SDS PAGE (Note: C1s enzyme is 86,000 unreduced, but upon reduction runs as 58,000 and 28,000 chains on SDS PAGE)
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Extinction Coeff.	$A_{280\,nm}$ = 0.96 at 1.0 mg/mL for pure C1s
Molecular Weight:	86,000 Da (2 chains)
Preservative:	None, 0.22 µm filtered.
Storage:	-70^{o}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

C1s enzyme is the activated form of C1s proenzyme. C1s is a subunit of the C1 complex which is the first complement component in the classical pathway of complement. C1s proenzyme is an inactive zymogen until C1 is activated. C1s proenzyme is activated when C1 binds to and is activated by antibodies bound to antigens (immune complexes) yielding C1r enzyme, the first protease that initiates the cascade. C1r enzyme in the C1 complex activates C1s proenzyme generating C1s enzyme. C1 complex is a non-covalent calcium-dependent complex of one C1q, two C1r and two C1s molecules. C1q binds by two or more of its six arms to the Fc domains of IgG or IgM. The binding of multiple arms to immune complexes causes the two C1r proteins in the complex (protease zymogens) to activate producing two proteases that cleave and activate the two C1s proenzymes in the complex. This activation of C1s proenzyme results in its cleavage into the two chain C1s enzyme with 58,000 and 28,000 dalton fragments.

Activated C1s enzyme cleaves complement component C4 releasing C4a and initiating covalent attachment of C4b to the activating surface. Activated C1s also cleaves C2 and the larger fragment of C2 binds to the surface-attached C4b forming C4b,C2a, the C3/C5 convertase of the classical pathway.

Physical Characteristics & Structure

C1s enzyme is a high molecular weight, two chain, trypsin-like protease composed of disulfide-linked chains of 58,000 and 28,000 daltons. C1s is present in plasma at 31 µg/mL. C1s proenzyme is a high molecular weight, single chain, trypsin-like zymogen (86,000 daltons). C1s proenzyme is activated by C1r enzyme (Dodds, A.W. and Sim, R.B. (1997); Morikis, D. and Lambris, J.D. (2005)). Two C1r form a C1r-C1r complex in the presence of calcium which in turn forms a stable complex with

two C1s molecules in the presence of calcium. This tetramer can exist in solution, but in the presence of C1q it binds to C1q forming the C1 complex which is stable in the presence of calcium. C1r self-activation, and subsequent C1s activation, is controlled by a weak association of C1r with C1esterase inhibitor (C1-INH) when it is in the C1 complex and similar stabilization occurs with purified C1r. C1s and C1r enzymes, however, are irreversibly inactivated by binding to C1-INH.

Function

C1s enzyme alone can be used to activate C4 or C2 but normallt it would do this while in complex with C1q. In the presence of Mg++ activated C4b and C2a can form fluid phase C4b,C2a the C3/C5 convertase of the classical pathway. In the presence of calcium C1s enzyme is still capable of binding to C1r and the complex is capable of binding to C1q. The resulting C1q-C1r-C1s complex is a fully active C1 molecule which will activate C4 and C2 in the fluid phase or on a cell bearing antibodies such as EA (Dodds, A.W. and Sim, R.B. (1997); Morgan, B.P. (2000)). EA are sheep erythrocytes with rabbit IgM anti-sheep erythrocyte antibodies bound to their surface (CompTech #B200) (Morgan, B.P. (2000)).

Assays

The activity of C1s is checked by measuring its ability to bind to the protease inhibitor C1 inhibitor (C1-INH, CompTech #A140). It may also be assayed by measuring its ability to cleave C4 or C2. It has the ability to form the activated C1 complex composed of one C1q, two C1r and two C1s molecules and this product C1 complex may be assayed. Finally, C1s is a trypsin-like serine protease and as such it cleaves many of the same synthetic substrates used for other protease assays (for example, Pefachrome from Centerchem, Inc.).

Applications

See section titled Function above.

Regulation

Activated C1s is controlled by C1-INH. C1s enzyme and C1-INH form a covalent complex that is resistant to separation on SDS gels. During complement activation C1 complex is rapidly activated by binding to immune complexes. The resulting activated C1s and C1r are rapidly inactivated by interaction with C1-INH (Ziccardi, R.J. (1982). Binding to immune complexes is fast (10-20 sec) and activation of the bound C1 complex takes several minutes, but C1-INH has also been shown to be fast and no active C1r or C1s remain 4 min after addition of immune complexes to plasma (Ross , G.D. (1986); Ziccardi, R.J. (1981)). The interaction of purified C1s enzyme and C1-INH is slower.

Genetics

The EMBL/Genbank cDNA accession number for C1s is J04080. The genes for C1r and C1s are closely linked and located on chromosome 12p13.

Deficiencies

Deficiencies of each of the three components of C1 have been found (Ross, G.D. (1986)). C1r and C1s deficient patients are prone to systemic lupus erythematosus (SLE) and recurrent pyogenic infections (Rother, K., et al. (1998)). They lack classical pathway function and may or may not exhibit antigen in blood.

Diseases

See section titled Deficiencies above.

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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Morgan, B.P. ed. (2000) Complement Methods and Protocols. (ISBN 0-89603-654-5) Humana Press, Inc., Totowa, New Jersey.

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Rother, K., Till, G.O., and Hänsch, G.M. (1998) The Complement System. (ISBN 3-540-61894-5) Springer-Verlag, Heidelberg.

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