Name:	**C1r Proenzyme**
Catalog Number:	**A101C**
Sizes Available:	100 µg/vial
Concentration:	0.5 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>70% versus normal human serum standard (see Cert of Analysis).
Purity:	>90 % by SDS PAGE
Buffer:	10 mM Imidazole, 400 mM NaCl, 1 mM $CaCl_2$, pH 6.0
Extinction Coeff.	$A_{280\ nm}$ = 1.15 at 1.0 mg/mL for pure C1s
Molecular Weight:	92,000 Da (1 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

C1r proenzyme is a single chain 92,000 dalton protein that is the native form of C1r enzyme. C1r is a subunit of the C1 complex which is the first complement component in the cascade referred to as the classical pathway of complement. C1r proenzyme is an inactive zymogen until C1 is activated. C1r 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. C1 is a non-covalent calcium-dependent complex of one C1q, two C1r and two C1s molecules. Each C1q binds through 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 protease zymogens in the complex (Morikis, D. and Lambris, J.D. (2005)). The activation of C1r results from cleavage of C1r into two fragments of 57,000 and 35,000 daltons. Activation of the bound C1s molecule is the only known function of C1r enzyme. Activated C1s 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

C1r proenzyme is a 92,000 dalton, single chain peptide that exhibits trypsin-like proteolytic specificity for arginyl bonds present in its two natural substrates, C1s and itself. C1r is present in plasma at 34 ug/mL (0.2µM) (Cooper, 1985). C1r proenzyme is an unstable zymogen and it spontaneously activates (Dodds, A.W. and Sim, R.B. editors (1997); Morikis, D. and Lambris, J.D. editors. (2005)) by cleaving a peptide bond in C1r producing a 57,000 dalton heavy chain and a 35,000 dalton light chain. This is the form sold as C1r enzyme (Cat# A102). This self-activation occurs rapidly in the C1 complex

upon binding to an immune complex and it occurs slowly with pure C1r. 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 is controlled in part by a weak association with C1esterase inhibitor (C1-INH) when it is in the C1 complex and similar stabilization occurs with purified C1r (Ziccardi, R.J. (1982)). C1r enzyme, however, is irreversibly inactivated by binding to C1-INH.

Function

The biological functions of C1r proenzyme are described above in the General Description and Physical Characteristics sections. C1r proenzyme can be used in the presence of calcium to form the C1 complex with C1q and C1s proenzyme. The C1r proenzyme will rapidly self-activate to C1r enzyme when the C1q in the C1 complex binds to immune complexes such as EA cells bearing antibodies. EA are sheep erythrocytes with rabbit IgM anti-sheep erythrocytes antibodies bound to their surface (CompTech #B200) (Morgan, B.P. ed. (2000)). The activated C1r enzyme will rapidly activate the two C1s proenzymes to form C1s enzymes and the resulting C1q-$C1r_2$-$C1s_2$ complex is a fully active C1 molecule which will activate C4 and C2 (Dodds, A.W. and Sim, R.B. editors (1997); Morgan, B.P. ed. (2000)).

Assays

The unit of classical pathway activity is the CH50. A similar unit, the C1rH50, can be used to quantitate the activity of C1r proenzyme for which we have developed a hemolytic assay. The C1rH50 assay uses our newly developed C1r-Dpl (Cat# A302) and measures the lysis of EA (classical pathway) as a function of the concentration of added test sample or standard purified C1r proenzyme (Cat# A 101). A C1rH50 unit is the amount of functional C1r proenzyme needed to lyse 50% of 3 x 107 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when that amount of C1r proenzyme is incubated with the recommended volume of C1r-Dpl in GVB++ (CompTech #B100) in a total volume of 500 µL for 30 min at 37 ºC. This amount of C1r proenzyme indicates the sensitivity of the assay for C1r proenzyme which is typically less than 10 ng C1r proenzyme with 10 µL C1r-Dpl. See the Certificate of Analysis for lot specific values.

Applications

See sections titled Function and Assays above.

Regulation

Activated C1r is rapidly inactivated by C1-INH. The spontaneous activation of C1r observed with pure C1 and pure C1r proenzyme is minimized by the presence of C1-INH which rapidly inactivates spontaneously activated C1r enzyme. Stabilization of the proenzyme is also due to existence of a weak complex between C1-INH and C1r proenzyme. This association apparently stabilizes C1 thus preventing spontaneous activation in serum (Ziccardi, R.J. (1982)). Separation of C1-INH from C1 during purification is one of the reasons that isolated C1 and C1r proenzyme are unstable and prone to spontaneous activation.

Genetics

The EMBL/Genbank cDNA accession number for C1r is M14058. 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 C1r 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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