Name:	Fragment C2a (larger fragment of complement protein C2) *
Catalog Number:	A172
Sizes Available:	30 µg/vial
Concentration:	1.0 mg/ml (see Certificate of Analysis for actual concentration)
Molecular weight:	73,000 Da (single chain)
Extinction Coeff.	$A_{280\ nm}$ = 1.081 at 1.0 mg/ml
Form:	Frozen Liquid
Purity:	>95% by SDS-PAGE (see Certificate of Analysis).
Buffer:	10 mM Sodium phosphate, 145 mM NaCl, pH 7.3
Preservative:	None, 0.22 µm filtered.
Storage:	-70ºC 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.

* Complement scientists have decided to designate the smaller of all C fragments with an 'a', and the larger with a 'b' and hence more recent literature may refer the larger C2 fragment as C2b. Complement Technology, Inc. uses the current Uniprot names which follow the original naming practice.

General Description

The larger fragment of complement protein C2 is C2a that results from the activation of the classical and lectin pathways. CompTech prepares the C2a fragment from complement protein C2 which was purified from normal human serum. C2a forms the proteolytic subunit of the C3 and C5 convertase of both pathways. Initiation of each pathway generates proteolytic enzyme complexes which are bound to the target surface (C1q/C1r/C1s in the classical pathway and MBL/ Ficolin/ MASPs in the lectin pathway). C1s and MASP in these complexes activate both C4 and C2. They cleave a peptide bond in C4 depositing C4b on the surface. They also cleave C2 into two fragments, the larger catalytic fragment C2a (73 kDa) and the smaller fragment C2b (34 kDa). The larger fragment binds to C4b and forms the C3/C5 convertase enzyme complex C4b,C2a (Nagasawa S. & Stroud, R. M. 1977), which activates C3, cleaving it to C3b and C3a. Deposition C3b on or near the C4b,C2a site converts the C3/C5 converatse, which is a weak C5 convertase, to a highly efficient C5 convertase (C4b,C2a,C3b) with a Km for C5 3000-fold lower than that of the C4b,C2a enzyme alone (Rawal N. and Pangburn M.K. 2003).

Upon cleavage of C2 by C1s or MASP two fragments are produced. The larger, C2a, with 509 amino acids forms the C3/C5 convertase of the classical and lectin pathways. C2a comes from the C-terminal of C2 while the smaller fragment, C2b, with 223 amino acids is from the N-terminal. Both contain carbohydrate. C2 has numerous allotypes (A, B, and C) and electrophoretic isoforms that can be separated by isoelectric focusing. The isoforms have pIs in the 6.0 to 6.3 pH range.

C4BP regulates the functional activity of C2 during complement activation. The C4b,C2a complex is inherently unstable with a natural half-life of several minutes at 37º C. In the presence of C4BP the decay of this complex is shortened to seconds due to the decay

accelerating activity of C4BP. This activity of C4BP on C4b,C2a is similar to the decay accelerating activity of factor H on C3b,Bb. Another similarity between factor H and C4BP is that once bound to their targets (C3b or C4b, respectively) they act as cofactors for cleavage by factor I. When C4BP binds to C4b,C2a the C2a is rapidly released and factor I cleaves the alpha chain of C4b producing iC4b. Thus, the site for C2a binding is destroyed and additional C3/C5 convertases cannot be formed.

Physical Characteristics & Structure

Human C2a (larger fragment) is composed of a single polypeptide chain. Nagasawa S. & Stroud, R. M. (1977) have reported C2a to have a molecular size of 73,000 Dalton as determined by SDS/polyacrylamide gel electrophoresis gels. At CompTech, using the Novex NuPAGE gel electrophoresis system with MOPS buffer on a 4-12% Tris-Glycine gel, purified human C2a migrates as a ~64,000 Dalton band under non-reduced and reduced conditions. C2a contains two domains one of which is a von Willebrand factor-like A (vWFA) domain that binds to Mg++ and to C4b and the other, the C-terminal domain, contains the active serine protease (SP) site. The crystal structure for the Mg2+-bound C2a at 1.9 angstrom resolution has been published (Krishnan et al., 2007).

The concentration of purified human C2a is determined by using the calculated extinction coefficient based on the amino acid sequence of C2a using ProtParam and assumes all pairs of Cys residues form cystines (i.e., a pair of cystine molecules are joined by a disulfide bond).

Assays

There are no convenient assays for the proteolytic activity of C2a. It is usually referred to as inactive after its release from C2.

References

Nagasawa S. & Stroud, R. M. (1977) Cleavage of C2 by Cis into the antigenically distinct fragments C2a and C2b. PNAS 74, 2998-3001.

Rawal N. and Pangburn M.K. (2003) Formation of high affinity C5 convertase of the classical pathway of complement. J Biol Chem. 278:38476-83.

Krishnan V., Xu Y., Macon K., Volanakis J. E. & Narayan V. L. (2009) The Crystal Structure of C2a, the Catalytic Fragment of Classical Pathway C3- and C5-convertase of Human Complement. J Mol Biol. 2007 367: 224–233.