Name:	**C2 Protein**
Catalog Number:	**A112**
Sizes Available:	50 µg/vial
Concentration:	0.5 mg/ml (see Certificate of Analysis for exact conc.)
Form:	Frozen liquid
Activity:	>90% versus normal human serum standard (see Cert of Analysis)
Purity:	>95% by SDS-PAGE
Buffer:	25 mM sodium phosphate, 100 mM NaCl, pH 6.0
Extinction Coeff.	$A_{280\ nm}$ = 8.9 at 1.0 mg/ml
Molecular Weight:	93,000 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 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

C2 is central to the activation of both the classical and the lectin pathways of complement. It 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 fragment (C2a) binds to C4b and forms the C3/C5 convertase enzyme complex C4b,C2a. This enzyme activates C3, deposits C3b on or near the C4b,C2a site and thus is converted from a weak C5 convertase to a highly efficient C5 convertase (C4b,C2a,C3b) with a K_m for C5 3000-fold lower than that of the C4b,C2a enzyme alone (Rawal N. and Pangburn M.K. (2003)).

Physical Characteristics & Structure

Native human C2 is a naturally glycosylated polypeptide expressed as a 752 amino acid protein containing a 20 amino acid signal sequence. The mature protein contains 732 amino acids and is glycosylated at 8 potential sites all of which are N-linked sites (Morley, B.J. and Walport, M.J. (2000)). The calculated molecular weight of C2 is 81,000 daltons, but due to the high carbohydrate content (approximately 16%) it has been reported to run on reduced SDS-PAGE gels at a wide variety of apparent molecular weights. Depending on the SDS-PAGE system used C2 has been reported to appear as if it is 93,000, 102,000, 110,000, and 117,000 daltons. Using the Novex NuPAGE system with MOPS buffer on a 4-12% Tris-Glycine gel at CompTech C2 appears to be 93,000 daltons.

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.

Function

C2 is activated by proteolytic cleavage by the C1s enzyme in the activated C1q-C1r-C1s complex of the classical pathway or by MASP in the MBL- or Ficolin-MASP complexes of the lectin pathway. Release of C2b leaves C2a which binds to C4b on the activating surface forming the C3 convertase (C4b,C2a). C4b is the regulatory subunit allowing C2a to function as a protease to cleave C3 and C5. C2 is active in extremely small amounts. It takes less than a nanogram of C2 to produce 50% lysis of antibody-sensitized erythrocytes (EA) in a standard C2H50 assay.

C2 and factor B are the two most heat sensitive complement components. Both may be largely inactivated by a 6 minute incubation at 56ºC (Morgan, B.P. 2000). Care must be taken not to inactivate the many other heat sensitive complement components and it should be noted that heat inactivation only reduces the concentration of active C2, it does not completely inactivate it or remove the C2 protein.

Assays

C2 may be assayed using C2-depleted human and antibody-sensitized sheep erythrocytes (EA). Hemolytic titers using EA and C2-dpl serum are extremely sensitive to C2 with 50% lysis requiring less than 1 ng C2. One note of caution, however, there is a C2 bypass which allows human C2-deficient and C2-depleted serums to lyse EA under certain conditions (Knutzen Steuer, K.L. et al. (1989); May, J.E. and Frank, M.M. (1973)). One must titer the reagents in each assay to find the best balance between low background lysis and maximum sensitivity for C2.

The unit of classical pathway activity is the CH50. A similar unit, the C2H50, is used to quantitate the activity of C2 and C2-Dpl. A C2H50 unit is the amount of functional C2 needed to lyse 50% of 3×10^7 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when that amount of C2 (CompTech #A112) is incubated with the recommended volume of C2-Dpl (CompTech #A312) in GVB^{++} (CompTech #B100) in a total volume of 500 µL for 30 min at 37ºC. This amount of C2 indicates the sensitivity of the assay for C2 which is typically about 1 ng C2 with 5 µL C2-Dpl. See the Certificate of Analysis for lot specific values.

In vivo

Plasma contains approximately 20 ug/mL of C2 with considerable variation between individuals (normal range 11-35 ug/mL). The primary site of biosynthesis of C2 is the liver although it is also produced locally by monocytes, macrophages, lung cells, brain cells and epithelial cells.

Regulation

C2 synthesis is accelerated by IFNgamma in macrophages, lung epithelial cells, glioma cells, fibroblasts and liver cells.

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 37ºC. 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.

Genetics

The gene for C2 resides on the sixth chromosome at 6p21.3 in the same region as C4 (6p21.3). The gene for C2 has 18 exons which span approximately 18 kb of chromosome 6. Gene accession numbers: Human (X04481,K1236, M26301, L09706, L09707, L09708), Mouse (M57891,J05661,M60563,J05661).

Deficiencies

C2 deficiency is the most common complement deficiency. The null allele occurs in almost 1% of people of European origin.

Diseases

Deficiency of C2 in humans is correlated with an increased incidence of systemic lupus erythematosus (SLE), glomerulonephritis, vasculitis and recurrent bacterial infections.

References

Knutzen Steuer KL, Sloan LB, Oglesby TJ, Farries TC, Nickells MW, Densen P, Harley JB, Atkinson JP. (1989) Lysis of sensitized sheep erythrocytes in human sera deficient in the second component of complement. J Immunol. 143:2256-61.

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Morley, B.J. and Walport, M.J. (2000) The Complement Facts Book (ISBN 0127333606) Academic Press, London.

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