Name:	**C4 Protein**
Catalog Number:	**A105**
Sizes Available:	250 µg
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Liquid
Activity:	>80% versus normal human serum standard (see Cert. of Analysis)
Purity:	>90% by SDS-PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.3
Extinction Coeff.	$A_{280\ nm}$ = 1.03 at 1.0 mg/mL
Molecular Weight:	205,000 Da (3 chains)
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

Native human C4 is a naturally glycosylated (6.9%) polypeptide containing three disulfide-linked chains. C4 is central to the activation of both the classical and the lectin pathways of complement activation. Initiation of each pathway generates proteolytic enzyme complexes (C1q/C1r/C1s in the classical pathway and MBL/MASP1/MASP2 in the lectin pathway) which are bound to the target surface. These enzymes cleave a peptide bond in C4 releasing the anaphylatoxin C4a and activating C4b. Like C3, the thioester of metastable C4b is highly reactive and is capable of reacting with and covalently coupling C4b to amino or hydroxyl groups on the target surface. There are two variants of C4 that are common in man (C4A and C4B). Animals with only one type generally have C4B. The favored sites of attachment for variants C4A and C4B differ. Metastable C4b produced from C4A attaches primarily to amino groups while C4b produced from the C4B variant binds well to hydroxyl groups as well as amino groups (Law, S.K.A. and Dodds, A.W. (1997)). Surface-bound C4b forms the basis for formation of the C3/C5 convertase enzyme complex C4b,C2a. This enzyme activates C3, deposits C3b and thus converts itself from a weak C5 convertase to a highly efficient C5 convertase with a K_m for C5 3000-fold lower than that of the C4b,C2a enzyme alone (Rawal N. and Pangburn M.K. (2003)). Surface-bound C4b is a weak opsonin and is recognized by receptors (CR1) on erythrocytes, lymphoid, and phagocytic cells. All of the complement activating functions of C4b are lost upon cleavage of the alpha chain generating C4c and C4d. The protease factor I cleaves C4b only when C4b is bound with one of the factor I cofactors: C4b binding protein (C4bBP), membrane cofactor protein (MCP) or complement receptor 1 (CR1).

Physical Characteristics & Structure

C4 is synthesized as a single chain protein, but circulates as a 3 chain molecule of 205,000 Da in plasma. During excretion the protein is glycosylated (6.9 %), forms an intramolecular thioester in the alpha chain and undergoes limited tyrosine sulfation. The

three disulfide-linked chains have molecular weights of 97,000 (alpha), 75,000 (beta), and 33,000 (gamma). During complement activation C4a (8,757 MW) is cleaved off the N-terminal of the alpha chain producing C4b (192,000 Da). Cleavage of surface-bound C4b by factor I yields the soluble C4c fragment (147,000 Da) and the cell-bound C4d fragment (45,000 Da).

Function

C4 is activated by proteolytic cleavage by C1s enzyme and the activated C1q-C1r-C1s complex of the classical pathway or M1 (MBL/MASP1/MASP2 complex) of the lectin pathway. Release of C4a leaves the metastable C4b in which the thioester is briefly able to covalently attach C4b to the activating surface. These same proteases (C1 and M1) cleave and activate C2 and the C2a subunit binds to C4b forming a C3 convertase (C4b,C2a). C4b is the regulatory subunit allowing C2a to function as a protease to cleave C3 and C5. Upon cleavage of C3, C3b is depositied at the enzyme site forming the C5 convertase C3b,C4b,C2a. The C3b subunit binds C5 allowing the C4b,C2a enzyme to proteolytically cleave C5. Although C4b,C2a is capable of cleaving C5, the K_m for C5 is 3000-fold higher and thus it is proportionally less efficient at activating C5 than the C3b,C4b,C2a enzyme.

The C4B variant binds better to cells due to its ability to covalently attach through both carbohydrates and amino groups on proteins. This is also the apparent source of its higher hemolytic activity in complement activation. C4A prefers amino groups making it better at attaching to proteins such as immune complexes.

Native C3 and C4 circulate in plasma with intramolecular thioester bonds linking a glycine and a glutamine residue in their C3d or C4d domains. These thioester bonds are susceptible to nucleophilic attack by amines such as ammonia, methylamine, hydroxylamine and hydrazine, all of which have been used to inactivate complement in serum.

Purified C4 is extremely sensitive to freeze/thaw losing 5-10% of its activity with each freeze/thaw cycle. It is also sensitive to intermediate temperatures such as -20^{o}C. The longer it remains at intermediate temperatures the more activity is lost. A few hours at -20^{o}C can completely inactivate it, even though it remains completely frozen.

Assays

C4 may be assayed using C4-depleted human or C4-deficient guinea pig serum due to the fact that antibody-sensitized sheep erythrocytes (EA) are very poorly lysed in the absence of C4. Hemolytic titers using EA and C4-D guinea pig serum are extremely sensitive to C4 with 50% lysis requiring less than 1 ng C4. One note of caution, however, there is a C4 bypass which allows C4-deficient and C4-depleted serums to lyse EA efficiently under certain conditions (May, J.E. and Frank, M.M. (1973); Knutzen Steuer, K.L. et al. (1989)).

In vivo

Plasma contains approximately 400 ug/mL of C4 with wide variations between individuals (normal range 250-550 µg/mL). The primary site of biosynthesis of C4 is the liver although it is also produced locally in tissues by monocytes, macrophages, the mammary gland, lung, spleen, kidney, brain and intestinal epithelial cells.

The red cell serological antigenic markers Chido and Rogers are the result of inadvertent attachment of C4b to blood cells. These antigenic determinants generally segregate with C4A (Rogers) and C4B (Chido).

Regulation

C4 is an acute phase protein meaning that its concentration in blood is elevated during inflammation. Biosynthesis of C4 is upregulated by interferon gamma and IL-6 in blood cells and in intestinal epithelium. Bacterial lipopolysaccharide (LPS) down regulates expression and can counteract INF-gamma upregulation.

Genetics

The gene for C4 resides on the sixth chromosome at 6p21.3. The genes for C4A and C4B are normally 10 kb apart and 21 kb long although C4B can be 21 kb or 14.6 kb long depending on the absence of a retroposon in intron 9. Both genes have 41 exons. Gene accession numbers: Human (M59815, M59816, AF019413, K02403, and U24578), Mouse (J05095 and M11729).

Deficiencies

Complete deficiencies of C4 are rare due to the presence of four genes in most individuals . Deficient individuals have been found to lack classical pathway function and to suffer from severe immune complex disease similar to individuals with complete lack of the C4A isotype, but who possess the C4B isotype (see Diseases, below).

Diseases

Deficiency of the C4A variant in humans is correlated with an increased incidence of autoimmune disorders, primarily systemic lupus erythematosus (SLE). It is thought that the increased reactivity of C4A with amino groups found on proteins is important for effective activation of complement on immune complexes. Low concentrations of C4A due to heterozygous or homozygous states (most humans carry four C4 genes) is associated with less efficient clearance of immune complexes.

References

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