Name:	**C4b**
Catalog Number:	**A108C**
Sizes Available:	250 µg/vial
Concentration:	1.0 mg/ml (see Certificate of Analysis for exact conc.)
Form:	Liquid
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
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

C4b is derived from native C4 upon proteolytic cleavage by C1s and release of C4a. C4b is central to the function of both the classical and lectin pathways of complement (Morley, B.J. and Walport, M.J. (2000); Law, S.K.A. and Reid, K.B.M. (1995)). Upon proteolytic activation of C4 the thioester in C4 becomes reactive and couples C4b covalently to amino groups (preferred by the C4A isotype) or hydroxyl groups (preferred by the C4B isotype) on the target surface (Tack B.J., et al. (1980); Law, S.K., et al. (1980); Isenman, D.E. and Young, J.R. (1986)). C1s also activates C2 and the C2a fragment binds to the C4b on the surface forming a C3 convertase (C4b,C2a). This enzyme cleaves a peptide bond in C3 releasing the anaphylatoxin C3a and activating C3b. In the lectin pathway MASP-2 activates C4 and C2 to form the same C3 convertase.

At CompTech C4b is prepared by cleavage of purified C4 (CompTech #A105) with purified active C1s enzyme (CompTech #A104). C1s rapidly cleaves only a single bond in C4 while cleavage by other proteases, such as trypsin, results in multiple cleavages at many other sites in the protein. C4b sold by CompTech is made in solution and the reactive thioester has reacted with water because no surface was available. This protein will not spontaneously attach to surfaces.

Physical Characteristics & Structure

Native human C4b is a glycosylated (~7%) polypeptide containing three disulfide-linked chains. The molecular weight predicted from the amino acid sequence is 180,500 and that observed is 195,000 Daltons. On reduced SDS-PAGE gels C4b shows a three chain structure with an alpha prime chain (α'-chain) of 87,000 Da, a beta chain of 75,000 Da and a gamma chain of 33,000 Da. Alpha prime and beta chains are linked through a single disulfide bond. The pI of C4b is approx. 7.5. 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).

Upon cleavage of C4 (205,000 Da) by C1s, C4a (77 amino acid fragment, 8,759 Da) is released from the N-terminal of the alpha chain and C4b (195,000 Da) becomes attached covalently to the surface of the activator.

In humans there are two common isotypes of C4 designated C4A and C4B. They differ in sequence at amino acids #1120-1125 with C4A = PCPVLD and C4B = LSPVIH. This

difference and the fact that this protein covalently attaches to and remains on erythrocytes produces the serological antigenic determinants known as Chido and Rogers on human blood cells (Atkinson, J.P. et al. (1988)). Because of the hydrolysis of the thioester of C4, C4b has a free sulfhydryl group. Because of the Chido/Rogers difference, C4A has an additional free sulfhydryl group and both of these –SH groups are located in the C4d domain.

Function

The generation of C4b is essential for effective complement activation by the classical and lectin pathways of complement. Following recognition of a target by immunoglobulins or by lectins complement C4 is cleaved forming C4b. Nascent C4b can attach to the surface of the target and there it forms a C3 convertase after binding C2a. This enzyme (C4b,C2a) cleaves C3 and deposits C3b on the target surface. This tri-molecular complex of C4b,C2a,C3b can now cleave C5 efficiently and produces cell lysis by C5b-9 formation. C4b,C2a also possesses a weak C5 convertase activity that can activate C5 without C3b at ~1/2000 the rate of C4b,C2a,C3b at the C5 concentration found in blood. The difference is in the Km for C5, not in the catalytic rate (kcat) of the enzyme (Rawal, N and Pangburn, M.K. (2003)). C3b is generally considered to be necessary for effective C5 activation.

The complement receptor CR1 found on erythrocytes of primates binds both C3b and C4b and acts as a cofactor for the inactivation of both proteins by factor I forming iC3b and iC4b.

Assays

C4b alone has no enzymatic activity. It is a structural component of the classical and lectin pathway C3 convertase (C4b,C2a) as well as a structural component of the C5 convertase of these pathways (C4b,C2a,C3b). Assays of function include measurement of binding to C2a and formation of the C3 convertase, binding to C4b binding protein (CompTech #A109) and cleavage by the protease factor I in the presence of the cofactor C4b binding protein (C4BP) forming iC4b and subsequently C4c and C4d.

In vivo

The normal serum concentration of C4 is 400 µg/mL with the normal range from 200 to 600µg/mL. The primary site of synthesis is the liver, but C4 has also found to be synthesized in macrophages and other cells.

Regulation

C4b is regulated by both fluid phase and membrane-bound proteins. C4b is inactivated by factor I, a serine protease that cleaves C4b at two locations when C4b is bound to a cofactor. Cleavage causes a structural rearrangement in C4b forming iC4b (inactive C4b) and subsequently forming C4c and C4d (after the second cleavage). Both products (iC4b and C4c/C4d) lack the functional binding sites that C4b possessed for C2a, CR1 and C4BP. Cleavage and inactivation of C4b by factor I requires that a cofactor be bound to C4b. The primary fluid phase cofactor is C4b binding protein (C4BP, CompTech #A109). Some cell membranes, such as human erythrocytes, possess CR1 which can act as a cofactor for factor I. Most human cells and tissues have MCP (membrane cofactor protein) which also acts as a cofactor for factor I. CR1 only acts on C4b bound to adjacent cells or immune complexes. It does not function as a cofactor or receptor for C4b attached to the same cell as the CR1. In

contrast, MCP only acts on C4b attached to the cell membrane bearing the MCP. In the absence of factor I the interactions of C4b with C4BP and CR1 inhibit C4b complement functions through competition with binding of C2 and C2a and through decay acceleration of the C3 convertase C4b,C2a. DAF (decay accelerating factor) is another membrane-bound protein that is present on most human cells and it interacts with C4b. It is not a cofactor for factor I and only promotes the dissociation of C3 and C5 convertases containing C4b. The interactions of CR1, MCP, C4BP, and DAF with C4b do not inactive the C4b itself and so C4b is capable of continuing all of its complement functions once dissociated from them so long as the C4b has not been cleaved by factor I.

Genetics

Human chromosome location of C4 gene 6p21.3. The genes for C4A and C4B are located in tandem on chromosome 6 within the class III region of the MHC. The mouse chromosome location is on chromosome 17. The human C4 genes each span 14 to 21 kb with 41 exons.

Deficiencies

Complete human C4 deficiencies are rare due to the fact that there are two C4A and two C4B genes. Nevertheless, 35% of the population fails to express from one of the four genes, while 8% lacks expression from two and ~1% lack expression from three genes. The extremely rare individuals with complete deficiencies often suffer from systemic lupus erythematosus, immune complex disease and increased susceptibility to infections. Even partial deficiencies of C4A expression predispose individuals to immune complex disease.

Diseases

See **Deficiencies** section above.

Precautions/Toxicity/Hazards

The source is human serum, 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. MSDS available upon request.

References

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