Name:	**C4a Anaphylatoxin (Not Recombinant)**
Catalog Number:	**A106**
Sizes Available:	50 µg
Concentration:	0.5 mg/mL (see Certificate of Analysis for the actual concentration)
Extinction Coeff.	$A_{276\,nm}$ = 0.456 at 1.0 mg/mL
Molecular weight:	8,759 Da (single chain)
Form:	Frozen liquid
Purity:	>95% by SDS-PAGE
Buffer:	HEPES buffered saline, pH 7.2 (No carrier proteins added)
Preservative	None
Presence of desArg:	< 3 %
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

Natural human C4a is prepared by cleavage of human C4 protein by human C1s. It is produced during activation of both the classical and lectin pathways of complement. C4a is a member of the anaphylatoxin family of three proteins (C3a, C4a and C5a) produced by the activation of complement (Hugli, T.E. et al. (1981)). It is an unglycosylated polypeptide containing 77 amino acids with a molecular mass of 8,759 daltons. Many of the biological functions of C4a are similar to those of C3a, but the specific activities are far below those of C3a. C4a activity is so low, in fact, that it was initially thought to be inactive. These measured activities include inducing muscle contraction in the guinea pig ileum test (spasmogenic activity), desensitization of muscle to C3a stimulation suggesting that the same receptor for both C3a and C4a is involved (tachyphylactic activity) and inducing vascular permeability in human skin (Gorski J.P. et al. (1979)). C4a does not show tachyphylactic activity against C5a or chemotactic activity. Removal of the C-terminal arginine by serum carboxypeptidase N destroys all these activities (Meuller-Ortiz, S.L., et al. (2009)). C4a appears to act through the C3a receptor (C3aR) which is a G-protein coupled receptor found widely distributed on peripheral tissues, lymphoid cells (neutrohphils, monocyes, and eosinophils) and in the central nervous system (astrocytes, neurons and glial cells) (Law, S.K.A. and Reid, K.B.M. (1995)).

Physical Characteristics & Structure

Molecular weight: 8,759 calculated molecular mass. Observed mass (MALDI-TOF) is 8,762 $\pm$ 9 mass units. pI = 9.0 to 9.5 (Gorski, J.P. et al. (1981))

Amino acid sequence (77 amino acids): NVNFQKAINE KLGQYASPTA KRCCQDGVTR LPMMRSCEQR AARVQQPDCR EPFLSCCQFA ESLRKKSRDK GQAGLQR

C4a is thought to be structurally very similar to C3a and C5a to which it is homologous. Thus its 3D structure is probably similar to the X-ray-derived crystal structure of C3a (Huber, R. et al. (1980)) and the NMR derived structure of C3a: Nettesheim, D.G. et al. (1988); Murray, I. et al. (1999).

Function

See **General Description** above. C4a exhibits much weaker biological activities than C3a and C5a. Its activity in inducing erythema and edema in human skin is 25,000-fold weaker than that of C5a and 100-fold weaker than C3a per nanomole. The spasmogenic activity of C4a is 2000-fold weaker than C5a and 100-fold weaker than that of C3a. Due to these differences the role of C4a in these responses *in vivo* is thought to be negligible.

Assays

Two well established assays for C4a and C3a functional activities include induction of contraction in the guinea pig ileum and the permeation of a dye such as trypan blue from the vasculature into skin. The anaphylatoxins also induce mast cell degranulation, (measured as histamine release), platelet aggregation, IL-1 release from monocytes and the release of prostaglandins and leukotrienes from many cells and tissues. The other assays used for C3a (Dodds, A.W. and Sim, R.B. (1997)) should also respond to C4a, but few reports have described utilizing these assays with C4a.

ELISA kits for the assay of C4a levels (or more correctly C4a desArg levels) in blood and other fluids are sold by several companies. These measurements are useful for detecting complement activation *in vivo*, but the interpretation of their meaning is complicated by the fact that clearance of the anaphylatoxins is rapid.

In vivo

Freshly drawn normal human serum contains significant levels of all three anaphylatoxins. Although these may represent the resting concentration *in vivo* it is difficult to draw or store blood without some complement activation so a true *in vivo* concentration is difficult to determine. The presence of EDTA and Futhan in the collection tubes can minimize this background (Pfeifer, P.H. et al. (1999)). Full activation of all C4 in blood (600 µg/mL) would result in ~3,400 nM C4a (~30 µg/mL). Due to the low biological activity of C4a it could require activation of most of the C4 in a small region to achieve the micromolar C4a concentrations necessary to elicit a response.

Regulation

C4a levels are regulated by three processes: formation, inactivation and clearance. There are two enzymes that cleave C4 and release C4a: C1s and MASP-2. C4a is "inactivated" by removal of its C-terminal arginine amino acid. The product C4a desArg (or C4a without the C-terminal arginine) is produced by the action of the plasma enzyme carboxypeptidase N (Mueller-Ortiz S.L. et al. (2009)). The inactivation is rapid and most C4a is converted to C4a desArg within minutes of its formation. Inactivated C4a lack measurable biological activity. Because of the large number of cells bearing C3a/C4a receptors (endothelial, immune, smooth muscle, neuronal, etc.) the capture, internalization and digestion of C4a and C4a desArg probably results in its removal from circulation.

Deficiencies

A deficiency of C4 or a deficiency of all of the enzymes that cleave C4 to generate C4a could result in the absence of C4a. There are no known complete deficiencies of all of the C4 cleaving enzymes. Examples of C4 deficient humans and mice exist (Wessels, M.R.

et al. (1995)), but the degree to which pathologies associated with C4 deficiency are due to the lack of C4 or the absence of C4a is unclear.

Diseases

There are no known diseases connected to C4a or C4a desArg.

Precautions/Toxicity/Hazards

The source of C4a is human serum, therefore appropriate precautions must be observed 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.

Injection can cause anaphylatic shock which is a generalized circulatory collapse similar to that caused by an allergic reaction.

Hazard Code: B WGK Germany 3

MSDS available upon request.

References

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Complement Technology, Inc.
4801 Troup Hwy, Suite 701
Tyler, Texas 75703 USA
Phone: 903-581-8284
FAX: 903-581-0491
Email: contactCTI@aol.com
Web: www.ComplementTech.com