Name:	**C3a Anaphylatoxin (Not Recombinant)**
Catalog Number:	**A118**
Sizes Available:	50 µg
Concentration:	0.5 mg/mL (see Certificate of Analysis for the actual concentration)
Extinction Coeff.	$A_{276\,nm}$ = 0.41 at 1.0 mg/mL
Molecular weight:	9089 Da (single chain)
Form:	Frozen liquid
Purity:	>97% by SDS-PAGE
Buffer:	Phosphate buffered saline, pH 7.3 (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 C3a is prepared by cleavage of human C3 protein by a human C3 convertase. C3a is a member of the anaphylatoxin family of three proteins (C3a, C4a and C5a) produced by the activation of complement. It is an unglycosylated polypeptide containing 77 amino acids with a molecular mass of 9,089 daltons. Many of the biological functions of C3a are similar to those of C5a, but C3a is approximately 10- to 20-fold less active per microgram than C5a. C3a mediates many inflammatory responses including smooth muscle contraction, vasodilation, increased vascular permeability, and release of histamine from mast cells and basophils (Law, S.K.A. and Reid, K.B.M. (1995)). In contrast to C5a, C3a does not exhibit significant neutrophil stimulating activities and does not induce chemotaxis, granule release or superoxide production. C3a acts 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)). These activities of C3a are inactivated by removal of the C-terminal arginine and this occurs rapidly in plasma due to the action of carboxypeptidase N (Meuller-Ortiz, S.L., et al. (2009)).

Physical Characteristics & Structure

Molecular weight: 9,089 calculated molecular mass. Observed mass (MALDI-TOF) is 9092 $\pm$ 9 mass units.

Amino acid sequence (77 amino acids): SVQLTEKRMD KVGKYPKELR KCCEDGMREN PMRFSCQRRT RFISLGEACK KVFLDCCNYI TELRRQHARA SHLGLAR

X-ray-derived crystal structure: Huber, R. et al. (1980)

NMRderived structure: Nettesheim, D.G. et al. (1988); Murray, I. et al. (1999).

Function

See **General Description** above. Although C3a has some measurable chemotactic activity it is hundreds of times less active than C5a in this regard. Due to these differences

the role of C3a in this response is thought to be negilible *in vivo*. However, considering that hundreds of times more C3a than C5a is usually produced during complement activation, especially by alternative pathway activation, this belief may not be entirely correct (see sections below titled ***In vivo*** and **Regulation**). Both C3a and C5a, which are generally released together, can cause anaphylatic shock which is a generalized circulatory collapse similar to that caused by a strong allergic reaction.

Assays

Two well established assays for C3a (and C5a) 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.

More practical assays include ATP release from guinea pig platelets, serotonin relaease from guinea pig platelets, N-acetyl-beta-D-glucosamidase release from differentiated U937 cells and calcium release from differentiated U937 cells. These assays have been described in detail (Dodds, A.W. and Sim, R.B. (1997)).

ELISA kits for the assay of C3a levels (or more correctly C3a desArg levels) in blood and other fluids are sold by many companies. A radioimmunoassay for C3a/C3a desArg is also available. 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 approximately 17 nM C3a (corresponding to activation of about 0.3 % of the total C3). Although this may represent the resting concentration *in vivo* it is difficult to draw or store blood without some C3 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 C3 in blood (1200 µg/mL) would result in ~6,600 nM C3a (~60 µg/mL). Due to the sensitivity of many C3a responses and the fact that there is 20-fold more C3 than C5 in blood, a biological response can theoretically be initiated by activation of approximately 1/10,000 of the C3 in a local area.

Regulation

C3a levels are regulated by three processes: formation, inactivation and clearance. The enzymes that cleave C3 and release C3a (collectively called C3/C5 convertases) do so at a rate approximately 300-times the rate that these enzymes cleave C5 (Pangburn, M.K. and Müller-Eberhard, H.J. (1986); Rawal, N. and Pangburn, M.K. (2001)). Thus, although C3a is 10 to 20 times less active than C5a for most of the biological activities they share (see **General Description** above), C3a is produced at a much faster rate. C3a is "inactivated" by removal of its C-terminal arginine amino acid. The product C3a desArg (or C3a 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 C3a is converted to C3a desArg within minutes of its formation. "Inactivated" C3a still possesses some biological activities, but it is considered inactive for most C3a-specific functions. C3a desArg does, however, possess numerous activities of its own including its identification as the acylation-

stimulaing protein (ASP) released by adipose tissue. This function attributed to C3a desArg controls lipid metabolism through the receptor C5L2 (see C3a desArg product description). Because of the large number of cells bearing C3a receptors (endothelial, immune, smooth muscle, neuronal, etc.) the capture, internalization and digestion of C3a and C3a desArg results in its removal from circulation.

Deficiencies

A deficiency of C3 or a deficiency of the enzymes that cleave C3 to generate C3a result in the absence of C3a. There are no known complete deficiencies of all of the C3 convertases. Examples of C3 deficient humans (Ghannam A, et al. (2008)) and mice (Wessels, M.R. et al. (1995)) exist, but the degree to which pathologies associated with C3 deficiency are due to the lack of C3 or the absence of C3a is unclear. Information on this has been acquired from C3aR knock-out animals (Singer, L. et al. (1994)). C3 deficiencies result in increased susceptibility to pyogenic infections, severely reduced adaptive immune responses by both the B-cell and T-cell systems, membranoproliferative glomerulonephritis and SLE. C3aR KO mice demonstrate that part of these effects are due to the loss of C3a in C3 deficiency.

Diseases

See sections above for many biological effects of C3a connected with inflammatory reactions in many diseases and anaphylactic shock. In addition, a role for C3a in asthma has been well documented (Zhang, X. and Kohl, J. (2010); Wills-Karp, M. (2007)).

Precautions/Toxicity/Hazards

The source of C3a 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.

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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@complementtech.com
Web: www.ComplementTech.com