Name:	**C5a desArg Anaphylatoxin (Not Recombinant)**
Catalog Number:	**A119**
Sizes Available:	50 µg
Concentration:	0.5 mg/ml (see Certificate of Analysis for actual concentration)
Extinction Coeff.	$A_{280\ nm}$ = 0.41 at 1.0 mg/ml
Molecular weight:	10,250 Da (single chain)
Form:	Liquid
Purity:	> 97% by SDS-PAGE
Buffer:	20 mM HEPES, 100 mM NaCl, pH 7.2 (No carrier proteins added)
Preservative	None
Endotoxin:	Typically < 0.1 EU/µg
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 C5a is prepared from human C5 protein cleaved into C5a and C5b by human C5 convertase. The C5a is converted to C5a desArg by proteolytic removal of the C-terminal arginine. The primary carboxypeptidase responsible for Arg removal is serum carboxypeptidase N, but there are several different carboxypepticases in serum. C5a desArg is a naturally glycosylated polypeptide containing 73 amino acids with a molecular weight of approx. 10,250 daltons. It contains 25% carbohydrate attached to a single Asn residue at position 64. This carbohydrate is of variable structure leading to a broad distribution of MW upon analysis by mass spectroscopy. C5a is the most potent anaplylatoxin (compared to C3a and C4a). C5a desArg is produced when C5a is "inactivated" by removal of its C-terminal arginine amino acid. This cleavage occurs by the action of the plasma enzyme carboxypeptidase N. This inactivation is rapid and most C5a is converted to C5a desArg within minutes of its formation. "Inactivated" C5a still possesses approx. 1% of its anaphylatoxic and chemotatic activities, but its stimulatory activity is only reduced 10-fold. Thus, C5a desArg retains considerable biological activity even though it is frequently called inactivated C5a. Its biological properties include being weakly chemotactic for neutrophils (PMN), causing smooth muscle contraction, increasing vascular permeability, causing histamine and TNF-alpha release, and causing lysosomal degranulation of immune cells. C5a and C5a desArg act through the C5a Receptor (C5aR, CD88, a G-protein coupled receptor) on PMN, monocytes, alveolar macrophages, and mast cells. A second receptor of unknown function (C5L2, gpr77) has been identified. Due to the widespread expression of C5a receptors and the results from C5aR KO mice it is believed that C5a and its receptors have many non-immunolgical functions in organ development, CNS development, neurodegeneration, tissue regeneration and hematopoiesis (Monk, P.N. et al. (2007)).

Native versus Recombinant C5a desArg

Numerous recombinant forms of C5a and C5a desArg are sold by many companies. In side-by-side biological testing, we have found that our native native

proteins are 10- to 100-fold more active per µg than all but one of these recombinant proteins. Structurally not a single one of the recombinant proteins on the market has the correct amino acid sequence or structure. They have extra amino acids at the N-terminal (such as 6 His tags), different amino acids in the sequence itself (some were produced from the original, but incorrect amino acid sequence), and none possess the 25% carbohydrate at Asn 64. In fact, one recombinant C5a on the market has approximately 30 additional amino acids at the N-terminal end due to the cloning vector used. This is a 40% addition of nonsense structure to the C5a molecule. Both our C5a and our C5adesArg are native proteins produced by the native human C5 convertase.

Physical Characteristics & Structure

Molecular weight: 10,250 (± 1,000 due to variable glycosylation)
Deglycosylated MW: Calculated monoisotopic mass 8112; Calculated average mass 8117.
Isoelectric point: pI = 8.8
Carbohydrate content: ~25% carbohydrate (heterogeneous)
Amino acid sequence: TLQKKIEEIA AKYKHSVVKK CCYDGACVNN DETCEQRAAR ISLGPRCIKA FTECCVVASQ LRANISHKDM QLG
CAS Number: 80295-54-1
MDL Number: MFCD00130842
NMRderived structure: FEBS Lett. 238:289-294, 1988; Biochemistry 28:172-185, 1989; Biochemistry 29:2895-2905, 1990; Proteins 28:261-267, 1997.

Function

C5a released from C5 by C5 convertases initiates a multitude of inflammatory reactions. C5a is rapidly converted to C5a desArg which, although it is less active than C5a, still causes neutrophils to become adherent to endothelium and to migrate to the site of complement activation where it stimulates release of PMN granule contents and reactive oxygen species. The biological properties of C5a desArg include being weakly chemotactic for neutrophils (PMN), causing smooth muscle contraction, increasing vascular permeability, causing histamine release, and initiating lysosomal degranulation of a variety of immune cells. C5a and C5a desArg act through the C5a Receptor (C5aR, a G-protein coupled receptor) on PMN, monocytes, alveolar macrophages, dendritic cells, mast cells, glial cells and smooth muscle cells. Rapid release of C5a and other anaphylatoxins can cause systemic effects as well as local changes. Anaphylatic shock is a generalized circulatory collapse similar to that caused by an allergic reaction and is caused by C3a and C5a which are generally released together.

Assays

The multitude of biological functions of C5a has resulted in the use of many different assay systems. The most typical biological assays being smooth muscle contraction assays using guinea pig ileum, chemotaxis assays using neutrophils or granule-release assays using human PMN or similar cell lines. Granule release is generally followed by measuring the release of myeloperoxidase. Functional responses have been detected in the picomolar concentration range (Gerard, C. et al. (1981); Hugli, T.E. et al. (1981)).

ELISA kits for the assay of C5a and C5a desArg in blood and other fluids are sold by many 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

The resting serum concentration of C5a desArg has been reported to be approximately 4 nM although it is difficult to draw, store and test blood without 1 to 10 % C5 activation (Watkins, J. (1987)). The presence of EDTA and Futhan in the collection tubes can minimize this background. Full activation of all C5 in blood (75 µg/mL) would result in ~380 nM C5a (~3.9 µg/mL). Due to the extreme sensitivity of many C5a responses, a response can theoretically be initiated by activation of approximately one millionth of the C5 in a local area (sub-picomolar C5a).

Regulation

C5a desArg levels are regulated by two processes: formation and clearance. The enzymes that cleave C5 and release C5a (collectively called C5 convertases) do so at very slow rates. Operating at Vmax the best enzymes only cleave one C5 every three minutes (Rawal, N. and Pangburn, M.K. (2001)). C5a desArg is created when C5a is "inactivated" by removal of its C-terminal arginine amino acid. The product C5a desArg is produced by the action of the plasma enzyme carboxypeptidase N. This inactivation is rapid and most C5a is converted to C5a desArg within minutes of its formation. "Inactivated" C5a still possesses approx. 1% of its anaphylatoxic and chemotatic activities, but its stimulatory activity is only reduced 10-fold. Thus, C5a desArg retains considerable biological activity even though it is frequently called inactivated C5a. Because of the large number of cells bearing C5a receptors (endothelial, immune, smooth muscle, neuronal, etc.) the capture, internalization and digestion of C5a and C5a desArg results in their rapid removal from circulation.

Deficiencies

A deficiency of C5 or a deficiency of the enzymes that cleave C5 to generate C5a would result in the absence of C5a and C5a desArg. A knock-out mouse deficient in carboxypeptidase N has been created and found to be hypersensitive to complement activation and CVF administration (Mueller-Ortiz S.L. et al. (2009)). Administration of human C5a was 100% lethal in these KO mice probably due to their inability to inactivate C5a to C5a desArg. There are no known complete deficiencies of C5 convertases. Examples of C5 deficient humans and mice exist. In fact, many laboratory mouse strains in common use were shown to have been bred with a deficiency of C5 (A/HeJ, AKR/J, DBA/2J, NZB/B1NJ, SWR/J, and B10.D2/nSnJ). The lack of C5 prevents formation of the membrane attack complex of complement and precludes formation of C5a and C5a desArg. Humans lacking C5 are susceptible to repeated infections from a wide variety of organisms, primarily gram-negative bacteria. Meningococcal and gonococcal neisserial infections are especially problematic. The degree to which pathologies associated with C5 deficiency are due to the lack of C5 or due to the absence of C5a and C5a desArg is unclear but information on this is being acquired from receptor knock-out animals.

Diseases

See Deficiencies above.

Precautions/Toxicity/Hazards

This protein is purified from human serum and 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.

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

Hazard Code: B

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@complementtech.com
Web: www.ComplementTech.com