Name:	C5a Anaphylatoxin (Not Recombinant)
Catalog Number:	A144C and A144(300)C
Sizes Available:	30 µg and 300 µg
Concentration:	0.5 mg/mL (see Certificate of Analysis for the actual concentration)
Extinction Coeff.	$A_{280 \, nm} = 0.41$ at 1.0 mg/ml
Molecular weight:	10,400 Da (single chain)
Form:	Frozen liquid
Activity:	>70% by myeloperoxidase release assay
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:	-70ºC 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 by cleavage of the peptide bond between C5a and C5b by the human C5 convertase. C5a is a naturally glycosylated polypeptide containing 74 amino acids with a molecular weight of approx. 10,400 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). Its biological properties include being strongly 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 acts 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

Numerous recombinant forms of C5a are sold by many companies. In side-by-side biological testing, we have found that our native C5a is 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: 10,400 ($\pm$ 1,000 due to variable glycosylation)
> Deglycosylated MW: 8,271 (observed). Calculated monoisotopic mass 8268; Calculated average mass 8273.
> Isoelectric point: pI = 8.9
> Carbohydrate content: ~25% carbohydrate (heterogeneous)
> Amino acid sequence: TLQKKIEEIA AKYKHSVVKK CCYDGACVNN DETCEQRAAR ISLGPRCIKA FTECCVVASQ LRANISHKDM QLGR
> 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 causes neutrophils to become adherent to endothelium and to migrate to the site of complement activation by chemotaxis where it stimulates release of PMN granule contents and reactive oxygen species. The biological properties of C5a include being strongly 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 acts 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 (Dodds, A.W. and Sim, R.B. (1997)). 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. In addition, assays have been described that measure 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)). Functional responses have been detected in the sub-picomolar concentration range for purified human C5a (Gerard, C. et al. (1981); Hugli, T.E. et al. (1981)).

ELISA kits for the assay of C5a levels (or more correctly C5a desArg levels) in blood and other fluids are sold by many companies. A radioimmunoassay for C5a/C5a 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

The resting serum concentration has been reported to be approximately 4 nM although it is difficult to draw or store 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.

Regulation

C5a levels are regulated by three processes: formation, inactivation 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 is "inactivated" by removal of its C-terminal arginine amino acid. The product C5a desArg (or C5a without the C-terminal arginine) is produced by the action of the plasma enzyme carboxypeptidase N (Mueller-Ortiz S.L. et al. (2009)). 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 results in its rapid removal from circulation.

Deficiencies

A deficiency of C5 or a deficiency of the enzymes that cleave C5 to generate C5a result in the absence of C5a. 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. 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 the absence of C5a 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   WGK Germany 3

MSDS available upon request.

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