Name:	**C4a des Arg Anaphylatoxin (Not Recombinant)**
Catalog Number:	**A107**
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:	8,603 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)). Removal of the C-terminal arginine by serum carboxypeptidase N (Meuller-Ortiz, S.L., et al. (2009)) yields C4a desArg and destroys all biological activities of C4a. C4a desArg is an unglycosylated polypeptide containing 76 amino acids with a molecular mass of 8,603 daltons.

Physical Characteristics & Structure

Molecular weight: 8,603 calculated molecular mass. Observed mass (MALDI-TOF) is 8,606 $\pm$ 9 mass units. pI = 9.0 to 9.5 (Gorski, J.P. et al. (1981))

Amino acid sequence (76 amino acids): NVNFQKAINE KLGQYASPTA KRCCQDGVTR LPMMRSCEQR AARVQQPDCR EPFLSCCQFA ESLRKKSRDK GQAGLQ

C4a and C4a desArg are thought to be structurally very similar to C3a and C5a to which they are homologous. Thus the 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 desArg is functionally inactive form of C4a. 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 even before inactivation to C4a desArg.

Assays

C4a desArg is the inactivated form of C4a, which as described above, exhibits orders of magnitude less activity than C3a and C5a. Thus, no biological activity is thought to be exhibited by C4a desArg.

ELISA kits for the assay of C4a levels (or more correctly C4a desArg levels) in blood and other fluids are commercially 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 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 desArg (~30 µg/mL). Due to the low biological activity of C4a and C4a desArg 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 and C4a desArg 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 to make C4a desArg. Because C4a desArg does not bind to the C3a/C4a receptor it is probably not captured, internalized and digested like the other anaphylatoxins. Filtration by the kidney is a likely mode of clearance.

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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Hugli, T.E., Gerard, C., Kawahara, M., Scheetz, M.E. 2nd, Barton, R., Briggs, S., Koppel, G., and Russell, S. (1981) Isolation of three separate anaphylatoxins from complement-activated human serum. Mol. Cell. Biochem. 41, 59-66.

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Murray, I., Kohl, J. and Cianflone, K. (1999) Acylation-stimulating protein (ASP): structure-function determinants of cell surface binding and triacylglycerol synthetic activity. Biochem J. 342:41-48.

Nettesheim, D.G., Edalji, R.P., Mollison, K.W., Greer, J. and Zuiderweg, E.R. (1988) Secondary structure of complement component C3a anaphylatoxin in solution as determined by NMR spectroscopy: differences between crystal and solution conformations Proc Natl Acad Sci U.S.A. 85:5036-5040

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