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Arenavirus envelope glycoproteins mimic autoprocessing sites of the cellular proprotein convertase subtilisin kexin isozyme-1/site-1 protease

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ABSTRACT

A crucial step in the arenavirus life cycle is the proteolytic processing of the viral envelope glycoprotein precursor (GPC) by the cellular proprotein convertase (PC) subtilisin kexin isozyme-1 (SKI-1)/site-1 protease (S1P). Here we conducted a systematic and quantitative analysis of SKI-1/S1P processing of peptides derived from the recognition sites of GPCs of different Old World and New World arenaviruses. We found that SKI-1/S1P showed a strong preference for arenaviral sequences resembling its autoprocessing sites, which are recurrent motifs in arenaviral GPCs. The African arenaviruses Lassa, Mobala, and Mopeia resemble the SKI-1/S1P autoprocessing C-site, whereas sequences derived from Clade B New World viruses Junin and Tacaribe have similarities to the autoprocessing B-site. In contrast, analogous peptides derived from cellular SKI-1/S1P substrates were remarkably poor substrates. The data suggest that arenavirus GPCs evolved to mimic SKI-1/S1P autoprocessing sites, likely ensuring efficient cleavage and perhaps avoiding competition with SKI-1/S1P's cellular substrates.

Introduction

The arenaviruses are a large and diverse family of enveloped RNA viruses that includes important emerging human pathogens (Buchmeier et al., 2007). The *Arenaviridae* are currently subdivided into two major subgroups, the Old World arenaviruses and the New World arenaviruses (Clegg, 2002). The Old World lineage contains the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) and the African arenavirus Lassa virus (LASV), which is the causative agent of a severe hemorrhagic fever in humans (McCormick and Fisher-Hoch, 2002). Other African arenaviruses include Mopeia (MOPV), Mobala (MOBV), and Ippy (IPPYV) that, so far, have not been associated with human disease (Emonet et al., 2009). The New World arenaviruses are divided into three Clades, A, B, and C. Clades A and C include non-pathogenic viruses, whereas the Clade B viruses Junin (JUNV), Machupo (MACV), Guanarito (GTOV), and Sabia (SABV) have emerged as causative agents of severe hemorrhagic fevers in South America (Peters, 2002). Human pathogenic arenaviruses emerge on average every 2–3 years, as illustrated by the recent identification of two novel arenavirus species associated with fatal hemorrhagic fevers in humans, Chapare virus in Bolivia and Lujo virus in South Africa. (Briese et al., 2009; Delgado et al., 2008). Each arenavirus species has as a natural host reservoir or a limited number of closely related rodent species, with the possible exception of Tacaribe virus, which has been only isolated from the fruit-eating bat *Artibeus sp* (Emonet et al., 2009). The present phylogenetic diversity of arenaviruses is likely the result of long-term co-evolution between viruses and their corresponding host species reservoir. Human infections occur principally by contact with persistently infected rodents.

Arenaviruses are enveloped negative strand RNA viruses with a non-lytic life cycle. Their genome consists of two single-stranded RNA species, a large segment encoding the virus polymerase (L) and a small zinc finger motif protein (Z), and a small segment encoding the virus nucleoprotein (NP) and glycoprotein precursor (GPC) (Buchmeier et al., 2007; de la Torre, 2009). A crucial step in the arenavirus life cycle is the biosynthesis of the fusion-active envelope GP that is essential for host cell attachment and entry. Maturation of arenavirus GPC requires proteolytic cleavage by the proprotein convertase (PC) subtilisin kexin isozyme-1 (SKI-1)/Site-1 Protease (S1P). Processing of GPC by SKI-1/S1P yields the N-terminal GP1, that is implicated in receptor binding, and the C-terminal transmembrane GP2, which resembles the fusion-active membrane proximal portions of other enveloped viral GPs (Beyer et al., 2003; Kunz et al., 2003; Lenz et al., 2001; Rojek et al., 2008). Processing of arenavirus GPC by SKI-1/S1P is essential for productive infection and viral spread and recent studies identified the SKI-1/S1P-dependent GPC processing as a promising anti-viral strategy to combat arenaviruses (Maisa et al., 2009; Rojek et al., 2010; Urata et al., 2011).

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SKI-1/S1P is an endoplasmic reticulum (ER)/*cis-medial* Golgi membrane-anchored serine protease (Sakai et al., 1998; Seidah et al., 1999). Initially synthesized as an inactive precursor of 1052 amino acids, the primary structure of SKI-1/S1P consists of a signal peptide, an N-terminal prodomain, followed by the catalytic domain, and a P-domain containing a cytokine receptor-like motif (CRM). The transmembrane domain of SKI-1/S1P is followed by a basic cytosolic tail (amino acids 1023–1052). Upon translocation into the ER, SKI-1/S1P undergoes autocatalytic cleavage at three processing sites (B, B′, and C) to generate the active form (Toure et al., 2000; Elagoz et al., 2002). A significant proportion of membrane-bound SKI-1/S1P undergoes autocatalytic cleavage of its ectodomain, resulting in shedding and release of the protease as a soluble protein (Elagoz et al., 2002). In contrast to other basic mammalian PCs, that cleave at the motif (K/R)-(X)n-(K/R) [n = 0,2,4,6; X = any amino acid except Cys], SKI-1/S1P recognizes hydrophobic *consensus* sequences R-X-(hydrophobic)-X↓ (Pasquato et al., 2006). Despite its broad *consensus* sequence, SKI-1/S1P exhibits exquisite substrate specificity and is involved in proteolytic processing of a defined set of cellular proteins. SKI-1/S1P was shown to cleave brain derived neurotrophic factor (BDNF) precursor protein (Seidah et al., 1999) and plays a key role in regulation of lipid metabolism and cholesterol synthesis through the processing of the membrane-associated transcription factors sterol regulatory element-binding proteins SREBP-1 and SREBP-2 (Brown and Goldstein, 1997; Sakai et al., 1998). Another cellular substrate of SKI-1/S1P is the activating transcription factor 6 (ATF6), which is a key player in the cellular response to ER stress (Lenz et al., 2000). Cellular and viral substrates generally share an R-X-(hydrophobic)-X↓ *consensus* sequence (Table 1). However, the presumed SKI-1/S1P processing sites found in GPCs of arenaviruses of different Clades show remarkable diversity, likely as a result of divergent evolution. Considering the importance of GPC processing by SKI-1/S1P for arenavirus infection and its promise as target for future anti-viral therapeutics, the molecular basis of the recognition of viral substrates by SKI-1/S1P is of great importance. In the present study we systematically investigated the ability of human SKI-1/S1P to cleave peptides derived from the GPC processing sites of different arenaviruses and compared them to the cellular substrates ATF6 and SREBP2. Our results reveal remarkable differences between the SKI-1/S1P processing of arenaviral and cellular substrates on the one hand and GPC-derived peptides derived from arenaviruses of different Clades on the other hand.

Table 1
SKI-1/S1P recognition sites found in cellular and viral substrates. Sequences of cleavage sites of viral and cellular precursor proteins cleaved by SKI-1/S1P are given (amino acid, one letter code). Viral substrates derived from GPCs of Old World arenaviruses and the three clades of New World arenaviruses (Clades A, B, and C) are shown. Also included are cellular substrates. Arrow indicates where processing takes place.

	Classification		Precursor protein	Cleavage site sequence							
				P5	P3′			P1P1′		P3	
Viral substrates	Old World		LASV	S	**R**	R	**L**	L↓G		T	F
			LCMV	T	**R**	R	**L**	A↓G		T	F
			LCMV	T	**R**	R	**L**	S↓G		T	F
			MOPV	S	**R**	R	**L**	L↓G		T	F
			MOPV	S	**R**	R	**L**	L↓G		L	F
			IPPYV	S	**R**	R	**L**	M↓S		T	F
			MOBV	S	**R**	R	**L**	M↓G		T	F
	New World	Clade A	PICV	S	**R**	K	**L**	L↓G		F	F
			WWAV	S	**R**	T	**L**	K↓S		F	F
		Clade B	JUNV	R	**R**	S	**L**	K↓A		F	F
			MACV	E	**R**	S	**L**	K↓A		F	F
			TACV	G	**R**	T	**L**	K↓A		F	F
			AMPV	K	**R**	R	**P**	L↓A		F	F
			GTOV	G	**R**	K	**P**	L↓A		F	F
			North American arenavirus	P	**R**	S	**L**	K↓S		F	F
			North American arenavirus	A	**R**	S	**L**	K↓A		F	F
			CHAV	L	**R**	R	**L**	Q↓G		V	F
			SABV	S	**R**	R	**P**	L↓G		I	F
		Clade C	LATV	T	**R**	R	**L**	Q↓A		F	L
			OLVV	T	**R**	R	**L**	Q↓A		F	L
Cellular substrates			hS1P — Site B	F	**R**	S	**L**	K↓Y		A	E
			hS1P — Site C	S	**R**	R	**L**	L↓R		A	I
			hS1P — Site B′	Q	**R**	K	**V**	F↓R		S	L
			hpro-BDNF	S	**R**	G	**L**	T↓S		L	A
			hSREBP2	G	**R**	S	**V**	L↓S		F	E
			hSREBP1a	G	**R**	N	**V**	L↓G		T	E
			hATF6	R	**R**	H	**L**	L↓G		F	S
			hCREBH	S	**R**	T	**L**	H↓N		D	A
			hCREB4	S	**R**	N	**I**	L↓T		H	K
			hOASIS	S	**R**	S	**L**	L↓F		Y	D
			hLuman	S	**R**	Q	**L**	R↓A		L	P
			hBBF2H7	S	**R**	N	**L**	L↓I		Y	E

Results

Design of substrate peptides derived from arenavirus GPCs and cellular SKI-1/S1P substrates

For our systematic comparative study of the SKI-1/S1P processing of peptides derived from the cleavage sites of different arenavirus GPCs and cellular substrates, we employed a reliable and quantitative biochemical assay based on fluorogenic peptides. This well-controlled *in vitro* enzymatic activity assay allows quantitative assessment of the preference of a protease towards specific peptide sequences representing processing sites within their target protein. Previous studies analyzed the activity of SKI-1/S1P using intramolecularly quenched fluorigenic (IQF) peptides derived from Lassa virus (LASV) GPC, in particular LASVGP Q-GPC251–263: o-aminobenzoic acid-DIYISRRLL-GTFTW-3-nitrotyrosine-A-amide (Basak et al., 2002). This peptide was shown to be efficiently cleaved by SKI-1/S1P. However, since cleavage at any amide bond may release fluorescence, enzymatic impurities may give rise to false positive signals (Fig. 1A), which was of considerable concern in the context of the present study. To address this issue, we opted for peptidyl methyl coumarides, which are routinely used as fluorogenic substrates to assess the activity of proprotein convertases (PCs). Methyl coumaride (MCA)-peptides have the advantage to release fluorescence only when cleavage takes place at the putative processing site at the sessile bond C-terminal to MCA (Fig. 1B). Upon processing, 7-amino-4-methylcoumarin (AMC) is released. The shift of fluorescence can be easily detected at 460 nm, thus monitoring the kinetics of cleavage. Furthermore, the high variability of amino acid at P′ positions of SKI-1/S1P recognition sites (Table 1), ranging from hydrophobic/aromatic to hydrophilic/basic, suggests that P1′–P3′ may not strictly require specific characteristics. Thus, the MCA aromatic group should not drastically affect substrate–enzyme recognition. Envelope GPCs of arenaviruses derived from different Clades and cellular SKI-1/S1P substrates were taken as template to generate a series of MCA-peptides (Table 2). For our studies we chose peptides derived from the GPC processing sites of the African arenaviruses LASV, MOBV, and MOPV. For the South American arenaviruses, we selected peptides derived from Pichinde virus (PICV) belonging to Clade A, and Oliveros (OLVV) from Clade C. To represent the two major sub-groups of the more complex Clade B, we chose peptides derived from the GPCs of TACV and JUNV on the one hand and peptides derived from the pathogenic GTOV and its non-pathogenic relative Amapari virus (AMPV) on the other hand. The recognition sequence RRLL found in the GPCs of the African arenaviruses LASV and MOPV is identical to the RRLL site found in site C of SKI-1/S1P autoprocessing (Elagoz et al., 2002; Lenz et al., 2001; Seidah et al., 1999), whereas the recognition sites derived from the other selected arenavirus GPCs contain a number of amino acid substitutions generating a remarkable diversity (Table 1). Our study further included MCA-peptides derived from the SKI-1/S1P processing site of human SREBP2 and ATF6 (Table 2). In addition to peptides corresponding to the minimal recognition sequence containing amino acids P1–P4, we also designed longer substrates containing residues upstream of P4 to assess their potential role in SKI-1/S1P recognition.