Structure
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The Crystal Structure of PCSK9: A Regulator of Plasma LDL-Cholesterol

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SUMMARY

Proprotein convertase subtilisin kexin type 9 (PCSK9) has been shown to be involved in the regulation of extracellular levels of the low-density lipoprotien receptor (LDLR). Although PCSK9 is a subtilase, it has not been shown to degrade the LDLR, and its LDLR-lowering mechanism remains uncertain. Here we report the crystal structure of human PCSK9 at 2.3 Å resolution. PCSK9 has subtilisin-like pro- and catalytic domains, and the stable interaction between these domains prevents access to PCSK9's catalytic site. The C-terminal domain of PCSK9 has a novel protein fold and may mediate protein-protein interactions. The structure of PCSK9 provides insight into its biochemical characteristics and biological function.

INTRODUCTION

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a serine protease involved in regulating the levels of the low-density lipoprotein receptor (LDLR) protein (Horton et al., 2007; Seidah and Prat, 2007). In vitro experiments have shown that adding PCSK9 to HepG2 cells lowers the levels of cell surface LDLR (Benjannet et al., 2004; Lagace et al., 2006; Maxwell et al., 2005; Park et al., 2004). Experiments with mice have shown that increasing PCSK9 protein levels decreases levels of LDLR protein in the liver (Benjannet et al., 2004; Lagace et al., 2006; Maxwell et al., 2005; Park et al., 2004), while PCSK9 knockout mice have increased levels of LDLR in the liver (Rashid et al., 2005). Additionally, various human PCSK9 mutations that result in either increased or decreased levels of plasma LDL have been identified (Berge et al., 2006; Kotowski et al., 2006; Zhao et al., 2006). PCSK9 has been shown to directly interact with the LDLR protein, to be endocytosed along with the LDLR, and to coimmunofluoresce with the LDLR throughout the endosomal pathway (Lagace et al., 2006). Degradation of the LDLR by PCSK9 has not been observed, and the mechanism through which it lowers extracellular LDLR protein levels is still uncertain.

PCSK9 is a prohormone-proprotein convertase in the subtilisin (S8) family of serine proteases (Seidah et al., 2003). Humans have nine prohormone-proprotein convertases that can be divided between the S8A and S8B subfamilies (Rawlings et al., 2006). Furin, PC1/PC3, PC2, PACE4, PC4, PC5/PC6, and PC7/PC8/LPC/SPC7 are classified in subfamily S8B. Crystal and NMR structures of different domains from mouse furin and PC1 reveal subtilisin-like pro- and catalytic domains, and a P domain directly C-terminal to the catalytic domain (Henrich et al., 2003; Tangrea et al., 2002). Based on the amino acid sequence similarity within this subfamily, all seven members are predicted to have similar structures (Henrich et al., 2005). SKI-1/S1P and PCSK9 are classified in subfamily S8A. Sequence comparisons with these proteins also suggest the presence of subtilisin-like pro- and catalytic domains (Sakai et al., 1998; Seidah et al., 1999, 2003). In these proteins the amino acid sequence C-terminal to the catalytic domain is more variable and does not suggest the presence of a P domain.

Prohormone-proprotein convertases are expressed as zymogens, and they mature through a multistep process. The function of the prodomain in this process is two-fold. The prodomain first acts as a chaperone and is required for proper folding of the catalytic domain (Ikemura et al., 1987). Once the catalytic domain is folded, autocatalysis occurs between the prodomain and catalytic domain. Following this initial cleavage reaction, the prodomain remains bound to the catalytic domain, where it then acts as an inhibitor of catalytic activity (Fu et al., 2000). When conditions are correct, maturation proceeds with a second autocatalytic event at a site within the prodomain (Anderson et al., 1997). After this second cleavage event occurs, the prodomain and catalytic domain dissociate, giving rise to an active protease.

Autocatalysis of the PCSK9 zymogen occurs between Gln152 and Ser153 (VFAQ|SIP) (Naureckiene et al., 2003) and has been shown to be required for its secretion from cells (Seidah et al., 2003). A second autocatalytic event at a site within PCSK9's prodomain has not been observed. Purified PCSK9 is made up of two species that can be separated by nonreducing SDS-PAGE: the prodomain at

Table 1. Data Collection and Refinement Statistics

Crystal Name	Form 1-Native	Form 1-Hg	Form 1-Xe	Form 2-Native
Data Collection				
Wavelength (Å)	1.0717	1.0063	2.0	1.0
Space group	$P2_13$	$P2_13$	$P2_13$	$P2_12_12_1$
Cell dimensions				
a, b, c (Å)	a = b = c = 139.77	a = b = c = 140.33	a = b = c = 140.15	63.11, 70.71, 150.69
α, β, γ (°)	α = β = γ = 90	α = β = γ = 90	α = β = γ = 90	α = β = γ = 90
Resolution (Å)	40–3.1 (3.21–3.1)[a]	40–3.35 (3.47–3.35)	40–3.15 (3.26–3.15)	40–2.3 (2.38–2.3)
Completeness	99.8 (99.9)	99.9 (100)	99.9 (100)	97.3 (81.7)
Redundancy	5.3	12.1	5.8	4.9
R_{sym}	6.2 (50.3)	8.4 (46.7)	7.3 (43.7)	12.4 (37.6)
I/σI	20.1 (2.5)	30.9 (5.1)	19.3 (4.0)	10.8 (2.2)
Refinement				
Resolution (Å)				40–2.3
Molecules/ASU				1
Reflections				
Total				29,916
Working set				28,405
Test set				1511
R_{work}/R_{free}				0.196/0.232
Number of atoms				
Protein				4,359
Ion				10
Water				215
B factors				
Protein				35.83
Ion				95.54
Water				38.06
Rms deviations				
Bond lengths (Å)				0.005
Bond angles (°)				0.846

[a] Numbers in parenthesis are for the highest resolution shell.

17 kDa and the catalytic plus C-terminal domains at 65 kDa. PCSK9 has not been isolated without its inhibitory prodomain, and measurements of PCSK9's catalytic activity have been variable (Naureckiene et al., 2003; Seidah et al., 2003). In order to gain insight into the regulation of PCSK9's catalytic activity and how PCSK9 lowers the level of the LDLR protein, we have determined the crystal structure of full-length wild-type PCSK9.

RESULTS AND DISCUSSION

Overall Structure of PCSK9

We solved the structure of PCSK9 by multiple isomorphous replacement with anomalous scattering and refined it to 2.3 Å resolution (see Experimental Procedures and Table 1). The crystal structure reveals that PCSK9 has subtilisin-like pro- and catalytic domains, and a novel C-terminal domain (termed V domain) (Figure 1). Although full-length PCSK9 (31–692) was used for crystallization, electron density is present only for residues 61–683. The prodomain extends from residues 61 to 152, and autocatalysis has broken the peptide bond between Gln152 and Ser153. The catalytic domain extends from residues 153 to 447 but has two loops lacking electron density. The V domain extends from residues 452 to 683 with two disordered regions. While there is no electron density for the polypeptide chain that connects the catalytic domain to the V domain, the placement of the V domain in the overall

