NMR Reveals the Interplay among the AMSH SH3 Binding Motif, STAM2, and Lys63-Linked Diubiquitin

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Abstract

AMSH [associated molecule with a Src homology 3 domain of signal transducing adaptor molecule (STAM)] is one of the deubiquitinating enzymes associated in the regulation of endocytic cargo trafficking. It shows an exquisite selectivity for Lys63-linked polyubiquitin chains that are the main chains involved in cargo sorting. The first step requires the ESCRT-0 complex that comprises the STAM and hepatocyte growth factor-regulated substrate (Hrs) proteins. Previous studies have shown that the presence of the STAM protein increases the efficiency of Lys63-linked polyubiquitin chain cleavage by AMSH, one of the deubiquitinating enzyme involved in lysosomal degradation. In the present study, we are seeking to understand if a particular structural organization among these three key players is responsible for the stimulation of the catalytic activity of AMSH. To address this question, we first monitored the interaction between the ubiquitin interacting motif (UIM)-SH3 construct of STAM2 and the Lys63-linked diubiquitin (Lys63-Ub_2) chains by means of NMR. We show that Lys63-Ub_2 is able to bind either the UIM or the SH3 domain without any selectivity. We further demonstrate that the SH3 binding motif (SBM) of AMSH (AMSH-SBM) outcompetes Lys63-Ub_2 for binding SH3. Additionally, we show how different AMSH-SBM variants, modified by their sequence and length, exhibit similar equilibrium dissociation constants when binding SH3 but significantly differ in their dissociation rate constants. Finally, we report the solution NMR structure of the AMSH-SBM/SH3 complex and propose a structural organization where the AMSH-SBM interacts with the STAM2-SH3 domain and contributes to the correct positioning of AMSH prior to polyubiquitin chains' cleavage.

Introduction

In the intricate and wide-ranging landscape of the ubiquitin system, deubiquitinating enzymes (DUBs) are specific enzymes that cleave the isopeptide bond either between two ubiquitins involved in polyubiquitin chains or between a ubiquitin and a substrate [1]. DUBs are classified into five families corresponding to different catalytic domain architecture. Ubiquitin COOH-terminal hydrolyses (UCHs), ubiquitin-specific protease (USPs), ovarian tumor proteases (OTUs), and Machado Joseph Disease (MJDs) families rely on cysteine protease, while the Josephins, and the JAB1/MPN/MOV34 (JAMM) family consists of metalloproteases. In the context of endocytosis, membrane proteins to be degraded are primarily mono- or polyubiquitinated through mainly Lys63-linked polyubiquitin chains [2]. Through a process that involves different multiunit complexes and concerted interactions, the ubiquitinated cargo proteins are directed to the lysosome for degradation [3]. Although deubiquitination is not mandatory for cargo sorting, this process is required to maintain a free ubiquitin pool, requested by cargo trafficking [4]. In yeast, this step is ascribed to Doa4, while in mammals, two DUBs, ubiquitin carboxyl-terminal hydrolase 8 (USP8, also called ubiquitin-specific protease Y or UBPY), and AMSH [associated molecule with a Src homology 3

domain of signal transducing adaptor molecule (STAM)] have been identified as key players in deubiquitination. It could be inferred from different studies that USP8 and AMSH may operate at different levels and have different roles. AMSH may regulate the ubiquitination status of the sorting complex endosomal sorting complexes required for transport (ESCRT-0) and therefore influences the fate of endocytosed proteins [5,6], removes ubiquitin from receptor at the early stage of endosomal sorting, or competes with Vps4 for ESCRT-III binding [5,7,8]. Conversely, USP8 seems to act on multiple substrates, protecting EGFR from lysosomal degradation [9–11] or being ubiquitinated itself [10]. While USP8 shows a specific ability to process both Lys63- and Lys48-linked chains, AMSH preferentially cleaves Lys63-linked polyubiquitin chains [7,10,12]. In addition to their catalytic activity, USP8 and AMSH have been shown to interact with several components of ESCRT-III [5,13,14] and ESCRT-0[6,15,16]. ESCRT-0 comprises the STAM/ hepatocyte growth factor-regulated substrate (Hrs) heterocomplex [17]. The ESCRT-0/AMSH interaction is achieved through the binding of the SH3 domain of STAM with the SH3 binding motif (SBM) of AMSH [5,18], while the charged multivesicular body proteins (CHMPs) of ESCRT-III use their MIT-interacting motif (MIM) domain to bind the microtubule interacting and transport (MIT) domain of AMSH [19]. Recent findings have revealed the structural and dynamical details regarding the catalytic process by which AMSH-like protein (AMSH-LP) cleaves Lys63-linked polyubiquitin chains [20,21] or the CHMP-III/AMSH interaction [22]. Interestingly, the binding of STAM with AMSH increases its activity toward Lys63-linked polyubiquitin chains [5,23]. Even though the STAM protein harbors three different ubiquitin binding domains (UBDs) [24], namely the Vps27/Hrs/STAM (VHS), ubiquitin interacting motif (UIM), and SH3 domains, in charge of ubiquitin recognition, the minimum requirement to activate AMSH is the presence of the UIM-SH3 domains [7,25]. One of the hypotheses suggests a possible mechanism where the UIM domain would bind the proximal ubiquitin while AMSH would interact with the distal one [25]. However, the question regarding the interaction between the SBM of AMSH and the SH3 domain of STAM is still pending as no structural data exist yet. Several SH3 domains have been shown to bind PxxP sequences [26,27] or non-proline-rich sequence [28,29]. From its side, the SH3 domain of STAM is likely to recognize a novel consensus sequence Px(V/I)(D/N)RxxKP present in AMSH, where the arginine and lysine residues in the RxxK motif are critical to maintain AMSH binding to SH3. The latter motif is also shared by USP8 and is essential for binding the SH3 domain of the Grb2 and STAM protein family [24,30]. Mutation of either arginine or lysine residues disrupts the interaction of AMSH with the SH3 domain of STAM or Grb2 [6]. As can be seen, the foundation of the AMSH activation by STAM has been explored by means of different biological assays. Nonetheless, structural and dynamical data that describe the interplay among STAM, AMSH, and Lys63-linked polyubiquitin chains are missing. In the current article, we aspire to understand if any particular structural organization forms a basis for the activation of AMSH by STAM and allows a better efficiency of its enzymatic process toward Lys63-linked polyubiquitin chains. To address this question, we compared the interaction of the UIM-SH3 construct of the STAM2 protein with the SBM motif of AMSH (AMSH-SBM) or Lys63-linked diubiquitin (Lys63-Ub$_2$). Here, we show that Lys63-Ub$_2$ can bind either the UIM or the SH3 domain without any selectivity and that the AMSH-SBM can outcompete Lys63-Ub$_2$ for binding with the SH3 domain. Our results indicate that Lys63-Ub$_2$ can still bind the UIM domain when AMSH-SBM is bound to the SH3 domain of STAM2. Additionally, we have solved the structure of the UIM-SH3 construct in complex with AMSH-SBM. We show that the latter one is unstructured in the free state and in complex with SH3. We propose a possible organization where the plasticity of STAM2, combined with the flexibility of AMSH, allows its possible interaction with Lys63-linked polyubiquitin chains and induces further chain cleavage.

Results

The SH3 domain of STAM2 binds to Lys63-Ub$_2$

While either deletion or mutation of the STAM-UIM or -SH3 domain prevents the activation of AMSH by STAM (see Fig. 1a for structural details) [25], we were seeking to understand if and how Lys63-Ub$_2$ could interact with the UIM-SH3 domains. To reveal any interaction of the UIM-SH3 construct with Lys63-Ub$_2$, we monitored chemical shift perturbations (CSPs) in ^{1}H,^{15}N-heteronuclear single quantum coherence (HSQC) of either ^{15}N-labeled UIM-SH3 or Lys63-Ub$_2$ upon the addition of the corresponding unlabeled partner. Upon the addition of unlabeled Lys63-Ub$_2$, several residues located on both the UIM and the SH3 domain experience significant CSPs or strong signal broadening, indicative of intermediate-to-slow exchange (Fig. 1b and c). Since the UIM and SH3 domains do not interact with each other in the free state [24], any perturbations seen on these respective domains would result from a specific direct interaction between UIM-SH3 and Lys63-Ub$_2$. Overall, the CSP pattern for the UIM-SH3/Lys63-Ub$_2$ complex is similar to the one seen for the UIM-SH3/Ub binding [24] and does not present additional perturbed residues. On UIM, these perturbations and signal attenuations stretch along one face of the α-helix and mainly