Biological Science

NW12A/2006G381

Structural study of K48-linked tetraubiquitin

Tadashi SATOH[1, 2, 3], Eri SAKATA[1], Shunsuke YAMAMOTO[1], Yoshiki YAMAGUCHI[1, 2], Akira SUMIYOSHI[1], Soichi WAKATSUKI[3], Koichi KATO[1, 4*]
[1]Nagoya City University, Nagoya, Aichi 467-8603, Japan,
[2]RIKEN Advanced Science Institute, Wako, Saitama 351-0198, Japan,
[3]Photon Factory, KEK, Tsukuba, Ibaraki 305-0801, Japan
[4]National Institute of Natural Sciences, Okazaki, Aichi, 444-8787, Japan

Introduction

K48-linked polyubiquitin chains serve as a signal for protein degradation by 26S proteasome through interactions using its I44 hydrophobic patches. The individual ubiquitin units of each chain are conjugated through an isopeptide bond between K48 and the C-terminal G76 of the preceding units. K48-linked polyubiquitin has been shown to undergo dynamic conformational changes depending on the association with target proteins. A “closed form” of K48-linked di-ubiquitin units, in which the I44 hydrophobic patches face each other, is transformed into an “open form” to recognize target proteins through the two proximate hydrophobic patches [1].

Here we performed crystallographic study of the tetra-ubiquitin (Ub_4), and provided structural insight into its dynamic nature [2].

Experimental Procedures

To prepare a K48-linked Ub_4, ubiquitin was incubated with E1 and E2-25K. It has been reported that E2-25K catalyzes the formation of cyclic polyubiquitin as well as the non-cyclic variety *in vitro*. Interestingly, cyclic and non-cyclic species were obtained as major and minor products, respectively. Hence, we could solve the crystal structure of cyclic Ub_4 exclusively with native K48-linkages. Crystals of the Ub_4 were obtained in $C222_1$ form by vapor diffusion method. Diffraction dataset was collected at PF-AR NW-12A. The crystal structure was solved by molecular replacement. The refined model of the Ub_4 has an R-factor of 19.4% and R_{free} is 23.9% for data between 50.0 and 1.85 Å resolution (Fig. 1A).

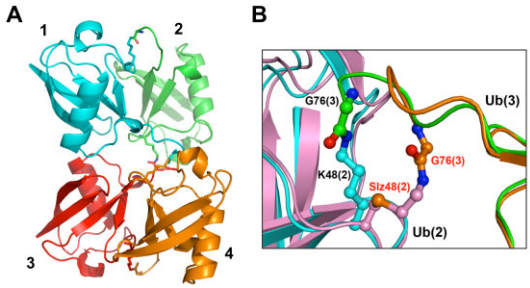

Fig. 1 Structure of K48-linked tetra-ubiquitin.

Results and Discussion

The crystallographic data clearly showed that the synthesized Ub_4 is a cyclic form in which all K48 residues are conjugated to the C-terminal G76 through isopeptide bonds (Fig. 1A). The overall structure is quite similar to the previously reported crystal structure of the closed form of non-cyclic Ub_4. In contrast, the structure of the second and the third ubiquitin units connected through a native isopeptide bond is significantly different from the conformations of the corresponding linkage of the engineered non-cyclic Ub_4 (Fig. 1B). From these observations, we suggest that the flexible nature of the isopeptide linkage thus observed contributes to the structural arrangements of ubiquitin chains exemplified by the closed-to-open conformational transition of K48-linked di-ubiquitin, which is involved in binding to ubiquitin-associated (UBA) domain [1].

References

[1] S. Raasi et al., Nat Struct Mol Biol **12**, 708 (2005).
[2] T. Satoh et al., *Biochem Biophys Res Commun* **400**, 329 (2010).
*kkatonmr@ims.ac.jp