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X-ray Structures of the Microglia/Macrophage-specific Protein Iba1 from Human and Mouse

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Introduction

Iba1 (ionized calcium binding adaptor molecule 1) with 147 amino acid residues has been identified as a novel calcium (Ca^{2+})-binding protein, expressed specifically in microglia/macrophages. Iba1 has two EF-hand motifs, a feature common to a large family of Ca^{2+}-binding proteins, known as the EF-hand proteins. The classical EF-hand proteins, troponin C and calmodulin, show 33 and 32 % sequence identity with Iba1 between the EF-hands, respectively, but outside of the EF-hands, the N-terminus and C-terminus, show no sequence similarity with any known proteins. Iba1 was reported to be involved in the signaling pathways of Ca^{2+} and a Rho family small GTPase, Rac, which is essential for regulating the reorganization of actin cytoskeleton in membrane ruffling. Iba1 enhances membrane ruffling and the activation of Rac through phospholipase C-γ-dependent pathways. Interestingly, a study of Iba1-interacting molecules as possible targets of Iba1 revealed that Iba1 has actin-binding and actin-cross-linking activities, suggesting that it may directly interact with filamentous actin. To obtain new insights how Iba1 interacts with targets at an atomic level, the three-dimensional structure of Iba1 needs to be elucidated. We have reported the crystal structure of human Iba1 (H-Iba1) in Ca^{2+}-free form and of mouse Iba1 (M-Iba1) in Ca^{2+}-bound form.[1,2]

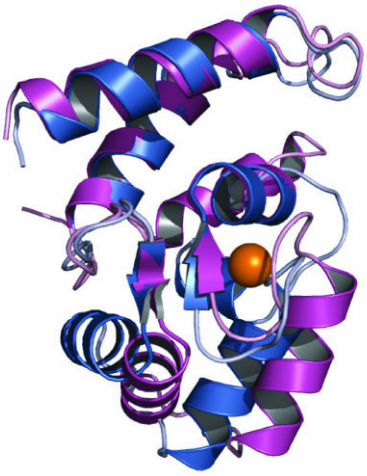
Materials and Methods

Crystals of the Au-derivative of M-Iba1 were obtained by soaking the crystals in a reservoir solution containing 1 mM $KAuCl_2$ for one day. Data for native H-Iba1 and M-Iba1 were collected on a beam line NW-12, and MAD data for the Au-derivative of M-Iba1 were collected on a beam line BL-5A at Photon Factory, using an ADSC/CCD detector system at 100K. The data were processed using the program HKL2000. Phasing calculations, and initial model building were carried out using the programs SOLVE/RESOLVE. Models were corrected on the (2|Fo|-|Fc|) electron density map using the program Xfit. Calculations of structure refinement were carried out using the program CNS. The structure of native M-Iba1 was determined by isomorphous replacement using the structure of the Au-derivative of M-Iba1. An attempt was made to solve the structure of H-Iba1 with a molecular replacement method using the structure of M-Iba1 as a probe model, but the correct solution for the rotation and translation functions could not be obtained. Using the model with a truncated region between the two EF-hands (Asp36 – Glu99), a plausible candidate was obtained with the program MOLREP in CCP4 program suite.

Results and Discussion

X-ray structures of Iba1 revealed a compact, single-domain protein with two EF-hand motifs, showing similarity in overall topology to partial structures of the classical EF-hand proteins troponin C and calmodulin, as shown in Figure 1. In M-Iba1, the second EF-hand contains a bound Ca^{2+}, but the first EF-hand does not, which is often the case in S100 proteins, suggesting that Iba1 has S100 protein-like EF-hands. The molecular conformational change induced by Ca^{2+}-binding of Iba1 is different from that found in the classical EF-hand proteins and/or S100 proteins, which demonstrates that Iba1 has an unique molecular switching mechanism dependent on Ca^{2+}-binding, to interact with target molecules.

Figure 1. Overall structure of H-Iba1 (blue) and M-Iba1 (magenta) illustrated by the program PyMol. The bound Ca^{2+} in M-Iba1 is shown in orange.



References

[1] M. Yamada et al., Acta Cryst. D 60, 569-571. (2004).
[2] M. Yamada et al., J. Mol. Biol. 364, 449-457(2006).
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