Recombinant protein purification technology and its usefulness

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Abstract

We conduct a functional analysis of the responsible gene parkin of familial Parkinson's disease. The structure analysis of the protein provides very important knowledge. Acquisition of the protein refinement technology is strongly demanded. However, the protein refinement is very difficult. As for securing the quantity of protein which is necessary for analysis, time and cost are required. It is necessary that the nature and characteristics of the parkin protein are understood, and that it is refined efficiently. An insoluble compartment had the parkin in Escherichia coli expression system. Parkin of 52KDa could be refined by solubilizing using a denaturant of guanidine hydrochloride.Furthermore, parkin of 75KDa could be purified in the zooblast expression system.

Keywords: Parkin, Klokin 1, mitochondria, biogenesis,

Introduction

We conducted a functional analysis of the responsible gene parkin of familial Parkinson's disease. The structure analysis of the protein provided very important information. Acquisition of the protein refinement technology is strongly demanded. However, this protein refinement is very difficult. As for securing the quantity of protein which is necessary for analysis, time and cost are required. It is necessary that the nature and characteristics of the parkin protein are understood, and that it is refined efficiently. Five main methods are known to protein expression system [1]. It is essential to choose the methods which was like eyes. We report purified methods of Escherichia coli expression system and the zooblast expression system of the parkin protein.

Materials and Methods

1. Escherichia coli expression system

Materials

Full length His Parkin constructed in pET15b was reported our earlier paper [2]. The plasmid was expressed in E. Coli BL21. Protein production was induced until OD600, and 1mM IPTG was added. After that it was incubated at 20 degrees Celsius overnight.

Solubilization

An insoluble compartment had the parkin protein. Protein was lysed with a lysis buffer containing 20mM Tris-HCl(pH 7.4), 0.3M NaCl, 1% Triton-X100, 5M guanidine hydrochloride and protenainase inhibitor

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cocktail (nacalai tesque, Japan Kyoto) diluted to 1:100 [2]. The protein was sonicated seven times at 30s, and incubated for 60min at RT in order to solve.

Purification

The sample filtrated filter paper was applied to TALON. Metal Affinity Resin (Takara Japan Siga), washed 20mM imidazole and eluted with 150mM imidazole. The eluent was concentrated. The sample was separated by 4-20% SDS-PAGE, and processed for immunoblotting followed by visualization by the ECL method3 and stained Anti-His(GE) diluted to 1:300.

Protein Concentration Assay

Protein concentration was determined according to the SYPRO Ruby protocol (Life Technologies Corp.).

2. Mammalian expression system

Materials

Halo-tagged parkin wild-type was supplied from Kazusa DNA Research Institute (Chiba, Japan). Cells were transfected COS-1 cells using LipofectamineTM LTX Reagent (Life Technologies Corp.)

Cell culture

COS-1 (a cell line derived from kidney cells) was cultured at 37C (5% CO^2) in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-incubated fetal bovine serum (FBS) [3].

Solubilization

Halo-tagged parkin wild-type transfected COS-1 cells were lysed with a lysis buffer containing 100mM Tris-HCl(pH 7.6), 150mM NaCl, 0.05% Triton-X100 and protenainase inhibitor cocktail diluted to 1:100.Protein was sonicated three times at 5s, then incubated for 60min at RT in order to solve.

Purification

The sample filtrated filter paper was applied to HaloLink™ Resin (Promega), and incubated at RT for 2hr. After washed, eluted an elution buffer containing 100mM Tris-HCl (pH 7.6), 150mM NaCl. The eluent was concentrated. The sample was separated by 4-20% SDS-PAGE, and processed for immunoblotting followed by visualization by ECL method 3 and stained with Anti-PRK8 (SIGMA) diluted to 1:50.

Protein Concentration Assay

Protein concentration was determined according to the SYPRO Ruby protocol (Life Technologies Corp.

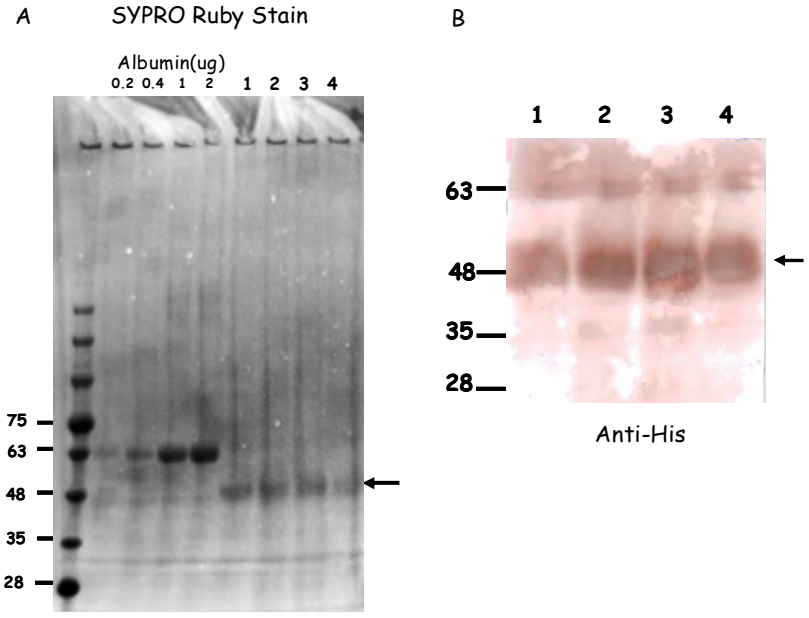

Figure 1. Purification of Parkin protein in Escherichia coli expression system . A. Protein of 100ug could be refined. B. In immunoblot, a band of 52KDa stained in Anti-His was detected. It was confirmed to be His-Parkin.

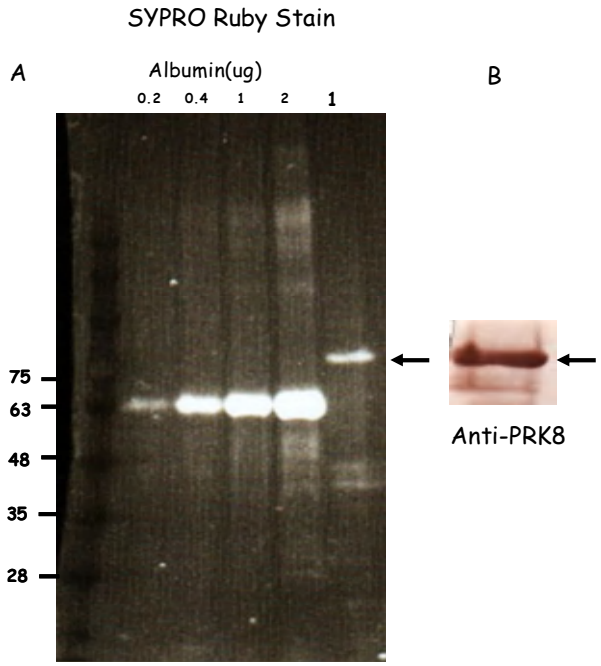

Figure 2. Purification of Parkin protein in mammalian expression system. A. Protein of 50ug could be refined. A band of 75KDa was detected by SYPRO Ruby staining. B. This band was stained with anti-Parkin antibody, PRK8. It was confirmed that this was Halo-Parkin.

Results

In the Escherichia coli expression system refinement, SYPRO Ruby staining was conducted with 1.2.3.4 sample. Protein of 100ug could be refined.
(Figure 1A). In immunoblot, a band of 52KDa stained in Anti-His was detected. It was confirmed to be His-Parkin (Figure 1B). Protein of 50ug could be refined in the zooblast expression system. A band of 75KDa was detected by SYPRO Ruby staining (Figure 2A). This band was stained with anti-Parkin antibody, PRK8. It was confirmed that this was Halo-Parkin (Figure 2B).

Discussion

As Parkin protein refinement methods, Escherichia coli expression system and zooblast expression system were performed. In the Escherichia coli expression system, the target parkin protein of 52KDa could be obtained. Furthermore, the target 75KDa parkin protein was detected in the zooblast expression system. Establishment of the protein manifestation refinement law is a manual skill that is very important to a functional analysis of the protein. Protein refinement will be conducted tenaciously in future. We hope this will lead to the discovery of new knowledge in the near future.

References

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