Recombinant protein purification technology and the usefulness

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Received 26 February 2015; received in received from 27 February 2015; accepted 6 March 2015

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. The acquisition of the protein refinement technology is strongly demanded. However, the protein refinement is very difficult. As for securing quantity of protein which is necessary for analysis, time and cost are required. It is necessary nature and a characteristic of the parkin protein are understood, and to be refined efficiently. An insoluble compartment had the parkin in Escherichia coli expression system. Parkin of 52KDa was able to be refined by solubilizing using a denaturant of guanidine hydrochloride.Furthermore, parkin of 75KDa was able to be purified in the zooblast expression system.

Keywords: Parkin, Klokin 1, mitochondria, biogenesis

Introduction

We conduct a functional analysis of the responsible gene parkin of familial Parkinson's disease. The structure analysis of the protein provides very important information. The acquisition of the protein refinement technology is strongly demanded. However, the protein refinement is very difficult. As for securing quantity of protein which is necessary for analysis, time and cost are required. It is necessary nature and a characteristic of the parkin protein are understood, and to be refined efficiently. Five methods is known to protein expression system primarily [1]. It is important and 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 early paper [2]. The plasmid were expressed in E. Coli BL21. Protein production was induced till OD600, it was added 1mM IPTG. After that it was incubated 20 degrees Celsius over night.

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 cocktail(nacalai tesque, Japan Kyoto) diluted to 1:100.2 And protein was sonicated 7times at 30s, incubated 60min RT in order to solve.

Purification

The sample filutrated filuter 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

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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 SYPRO Ruby protocol (Life Technologies Corp.).

2. Mammarian 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 37?C (5% CO2) 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 was 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.

And protein was sonicated 3times at 5s, incubated 60min RT in order to solve.

Purification

The sample filutrated filuter paper was applied to HaloLinkTM Resin (Promega), incubated RT 2hr. And after washed, eluted a 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 the ECL method3 and stained Anti-PRK8(SIGMA) diluted to 1:50.

Protein Concentration Assay

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

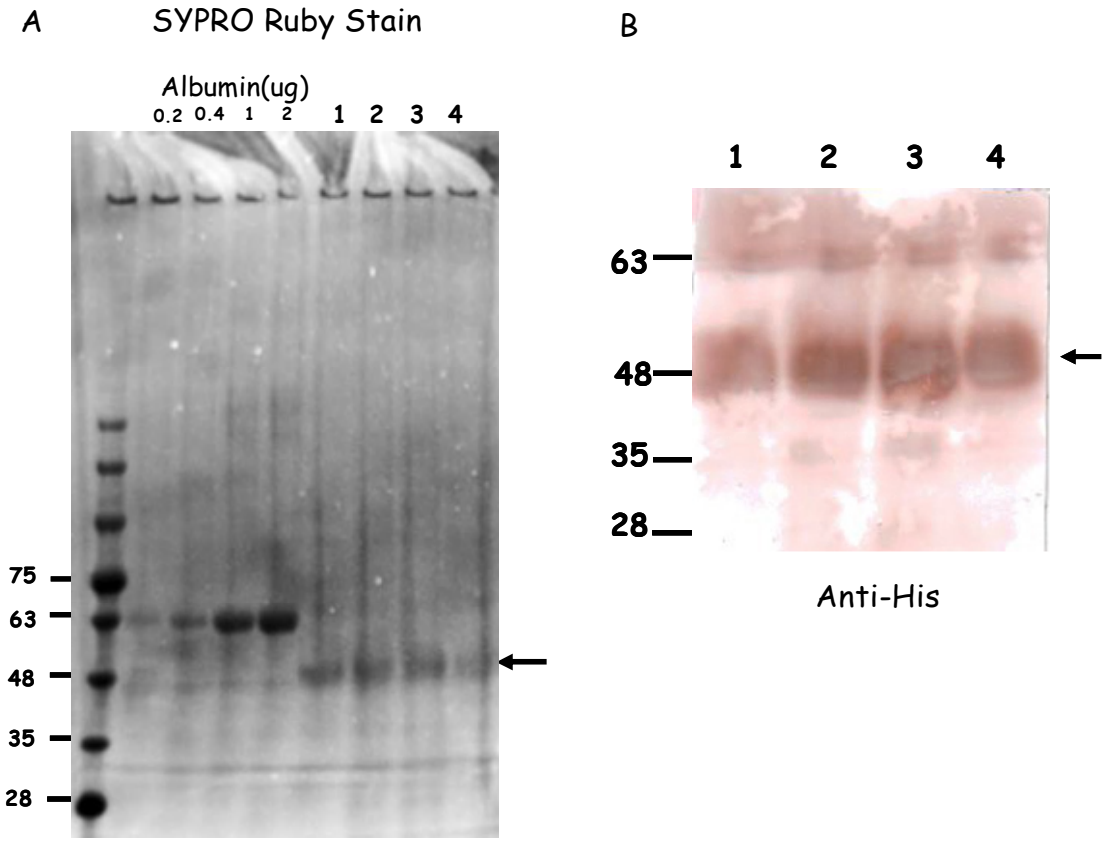

Figure 1. Purification of Parkin protein in Escherichia coli expression system . A. Protein of 100ug was able to be refined. B. In immunoblot, a band of 52KDa stained in Anti-His was detected. It was confirmed to be His-Parkin.

Results

In the Escherichia coli expression system refinement, SYPRO Ruby staining was conducted with 1.2.3.4 sample. Protein of 100ug was able to 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 was able to be refined in 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, parkin protein of 52KDa to be aimed for was able to be obtained. Furthermore, 75KDa parkin protein to be aimed for was detected in the zooblast expression system. Establishment of the protein manifestation refinement law is the manual skill that is very important to a functional analysis of the protein. The protein refinement is going to be conducted tenaciously sequentially in future. That we will lead to discovery of new knowledge in the near future is prayed for.

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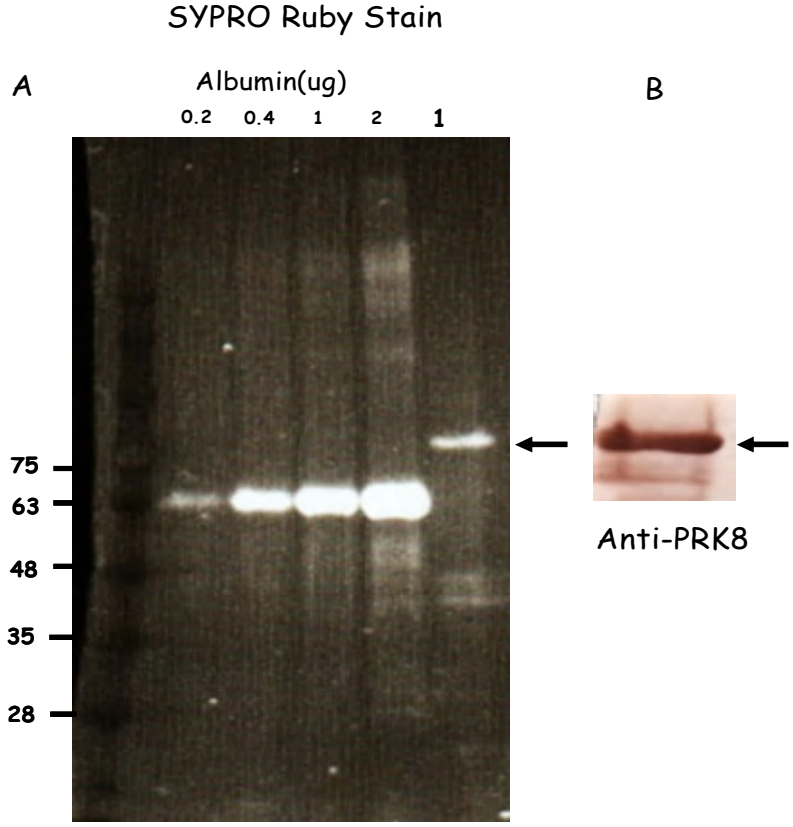

Figure 2. Purification of Parkin protein in mammalian expression system. A. Protein of 50ug was able to be refined. A band of 75KDa was detected by SYPRO Ruby staining. B. This band was stained with anti-Parkin antibody, PRK8. It was confirmed that this was Halo-Parkin