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Calcium-dependent structural changes in human reticulocalbin-1

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1 Introduction

Human reticulocalbin-1 (hRCN1) has six EF-hand motifs and binds Ca^{2+}. hRCN1 is a member of the CREC (Cab45, RCN1, ERC-55, and calumenin) family localized in the secretory pathway. RCN1 was first cloned in mice, and the murine RCN1 (mRCN1) precursor polypeptide consists of 325 amino acid residues [1]. The 23 amino acid N-terminal region is a putative signal peptide, and accordingly, the mature mRCN1 polypeptide consists of 302 amino acid residues (from 24 to 325) [1]. mRCN1 has the endoplasmic reticulum (ER) retention signal HDEL at its C-terminus and was reported to be localized in the ER [1]. Recent studies demonstrated that hRCN1 is also localized on the cell surface in several endothelial cell lines, including bone marrow endothelial cells as well as several prostate cancer cell lines [2]. Moreover, hRCN1 cell surface expression in the endothelial cells is up-regulated by tumor necrosis factor-α [2]. hRCN1 up-regulation has also been reported in several cancer cells [3], but its cellular functions remain unclear. In this study, we observed that overall structure of hRCN1 became more compact upon Ca^{2+} binding by small angle X-ray scattering (SAXS).

2 Experiment

SAXS measurements were carried out at 20 ºC at Beam Line BL10C in the Photon Factory (Tsukuba, Japan). X-ray wavelength was 1.488 Å. Exposure time for each sample was 10 min. SAXS data were recorded using R-AXIS 7 detector (Rigaku) and camera length was 2 m. The samples were placed in a quartz sample cell with a path-length of 1.0 mm. hRCN1 solution was prepared in 25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM $CaCl_2$ and 5% glycerol. $CaCl_2$ solution was added to each hRCN1 solution to give a final concentration of 12 mM for preparation of Ca^{2+}-bound hRCN1, while EDTA solution was added to each hRCN1 solution to give a final concentration of 5 mM for preparation of apo hRCN1. Protein concentrations were adjusted to 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.6 mg/ml for both Ca^{2+}-bound hRCN1 and apo hRCN1. Both apo and Ca^{2+}-buffers were also measured. Reduction of scattering images to one dimensional plots of intensity versus momentum transfer s ($s=4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ the X-ray wavelength), was performed by FIT2D (http://www.esrf.eu/computing/scientific/FIT2D/, ESRF). Buffer subtraction was performed by Excel (Microsoft office). Buffer subtracted data were then analyzed using the ATSAS software package [4, 5]. Radius of gyration (Rg) values were determined from Guinier plot ($sRg < 1.3$) using PRIMUS [4, 6] and pair distance distribution function, P(r), was computed with the program GNOM [7]. Definition of parameters used in this analysis is according to a literature [8].

3 Results and Discussion

Structural changes of hRCN1 in solution upon Ca^{2+}-binding were examined by SAXS. Figure 1A shows the scattering curves of apo and Ca^{2+} bound hRCN1 in protein concentrations between 0.5 to 3.6 mg/ml. The scattering curves of apo form showed more straight and flat lines than those of Ca^{2+}-bound form and especially the curves of 3.6 mg/ml of apo hRCN1 showed higher intensities in low s values compared to those of Ca^{2+}-bound form (Fig. 1A).

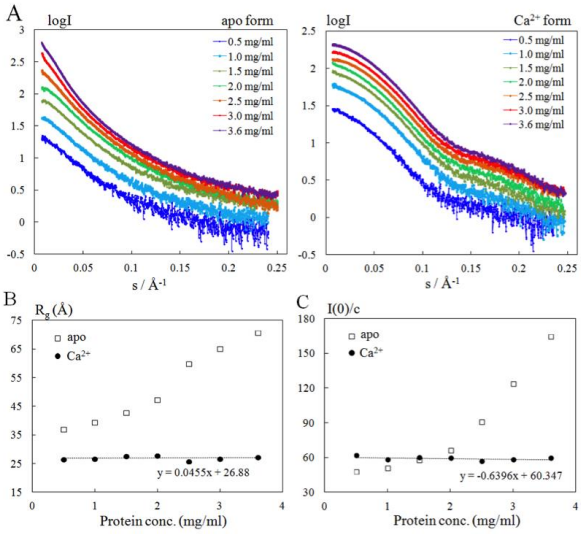

Fig. 1: SAXS data of human reticulocalbin 1 (hRCN1). (A) Scattering patterns of apo and Ca^{2+}-bound hRCN1 from 0.5 mg/ml (bottom line) to 3.6 mg/ml (top line), (B) R_g plots against protein concentration. (C) $I(0)/c$ plots against protein concentration.

R_g and I(0) were obtained by Guinier plot and Gnom analysis and R_g and I(0)/c were plotted against the protein concentration (Fig. 1, B and C, respectively).

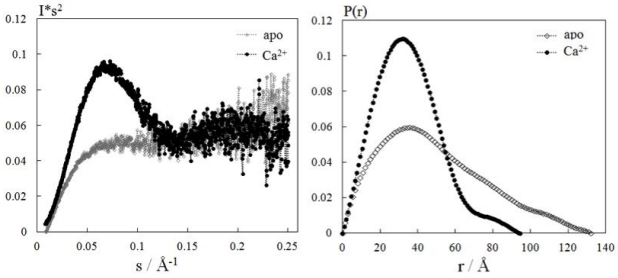

Fig. 2: Kratky plots (left panel) and pair distribution functions (right panel).

The R_g value of apo form was larger than that of Ca^{2+} bound form at any concentration examined and increased in a protein concentration-dependent manner (Fig. 1B). The value of I(0)/c is proportional to molecular weight. The I(0)/c values at 1.5 were similar to each other in both apo and Ca^{2+}-bound forms, but from 2 to 3.6 mg/ml the values of apo form increased in a protein concentration-dependent manner while the values of Ca^{2+}-bound form did not change (Fig. 1C). These results indicate that Ca^{2+}-bound hRCN1 is a monomeric protein and does not form aggregates at high protein concentrations such as more than 3 mg/ml. On the other hand, apo hRCN1 is a monomeric protein with larger R_g value than that of Ca^{2+}-bound hRCN1 at 0.5 and 1 mg/ml, suggesting that molecular shape of apo hRCN1 is different from that of Ca^{2+}-bound hRCN1. At high concentration at more than 2 mg/ml apo hRCN1 is not a monomer and might form aggregates. The Kratky plot in Figure 2 is derived from the data at 1 mg/ml and a prominent peak at low angle is shown in Ca^{2+}-bound hRCN1 but not in apo hRCN1, indicating that hRCN1 is folded when bound to Ca^{2+} but is unfolded or only partially folded in the apo form [9]. Pair distance distribution function (Fig. 2) shows that apo hRCN1 has elongated shape compared to that of the Ca^{2+}-bound form as judged from the large D_{max} of apo hRCN1. In addition, it is predicted that the Ca^{2+}-bound form has mainly globular part but there is a projecting part. While the existence of small amount of the species often causes projection, this was a reproducible result.

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