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-\(\alpha\) \([2]\). 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 °C 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 \(s = 2\pi \sin(\theta)/\lambda\), where \(\theta\) is the scattering angle and \(\lambda\) 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 (\(R_g\)) values were determined from Guinier plot (\(sR_g < 1.3\)) 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).](image)

(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).](image)
The $R_g$ value of apo form was larger than that of $\text{Ca}^{2+}$-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 $\text{Ca}^{2+}$-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 $\text{Ca}^{2+}$-bound form did not change (Fig. 1C). These results indicate that $\text{Ca}^{2+}$-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 $\text{Ca}^{2+}$-bound hRCN1 at 0.5 and 1 mg/ml, suggesting that molecular shape of apo hRCN1 is different from that of $\text{Ca}^{2+}$-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 $\text{Ca}^{2+}$-bound hRCN1 but not in apo hRCN1, indicating that hRCN1 is folded when bound to $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$-bound form as judged from the large $D_{\text{max}}$ of apo hRCN1. In addition, it is predicted that the $\text{Ca}^{2+}$-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.

Acknowledgement
We thank Drs. Shimizu and Saijo and the PF staff very much for the great support for SAXS experiment.

References

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