Human cyclophilin 33 (hCyP33) in T-cell binds specifically to poly(A)$^{+}$RNA (mRNA)

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Abstract  Human cyclophilin 33 (hCyP33), found in 1996, consists of an RNA-binding domain in N-terminus, a cyclophilin domain in C-terminus and a connected part between the two domains. RNA-binding proteins concern functions, such as splicing, modification and transport, after transcription in eukaryotic cells. Cyclophilins (CyPs) possess enzymatic activity, namely peptidyl-prolyl cis-trans isomerase (PPIase). They are involved in folding, transport and interaction of proteins. Cyclosporin A (CsA), an immunosuppressant used by organ transplantation, binds to CyPs and suppresses their enzymatic activity. However, up to now it is unknown that which cellular and physiological roles hCyP33, which possesses the above-mentioned both functions, plays. In this paper the binding specificity of hCyP33 to different cellular RNA is investigated by means of ion-exchange chromatography and affinity adsorption. The results show that it binds specifically to poly(A) tailed mRNA, namely poly(A)$^{+}$RNA.

Keywords: human cyclophilin 33 (hCyP33), ion-exchange chromatography, RNA-binding specificity.
Accordingly, we infer that hCyP33 binds only specifically to one from three kinds of cell RNA, mRNA, namely poly(A)$^+$RNA, which contains polyA tail. Probably, this binding occurs in the polyA tailing sequence AAUAAA. In this work we investigate the binding specificity of hCyP33 to three kinds of cell RNA by means of ion-exchange chromatography and affinity chromatography for proving the first part of our hypothesis.

1 Materials and methods

1.1 Reagents and instruments

Enzymes and reagents: enzymes and normal biological reagents were obtained from Sino-American Biotechnology Company, United Stars Biotechnology Company and Promega, respectively; mRNA purification kit was obtained from Sigma; SP-Sepharose$^{TM}$ Fast Flow S (45—165 µm, ion-exchange capacity: 0.18—0.25 mmol/mL volume of gel) was obtained from Emsershan Pharmacia Biotech Company.

Chromatography system: an LKB 2152 HPLC controller and two LKB 2150 HPLC pumps were used. The size of the column is 9 mm × 100 mm.

1.2 Cloning, expression and isolation of GST-hCyP33 fusion protein

Cloning hCyP33 in pGEX5X1 was performed as described by Sambrook$^{[14]}$. RT-PCR was performed with primers hCyP33f (5′-ccggccgaattcatgccaccaagcgc-3′) and hCyP33r (5′-cccccttcgtctctcac-3′). The PCR product was purified with gel electrophoresis after digestion with restriction enzyme ecru and was ligated with pGEX5X1 plasmid DNA which was cut with restriction enzymes EcoRI and SmaI (ligated by EcoRI/blunt-ends). The recombinant DNA was transferred into competent E. coli cell line DH5α and separated on agar culture-plates with 50 µg/mL Ampicillin. The recombinant plasmid DNA of positive clones would be prepared and defined by DNA-sequencing. Expression and purification GST-hCyP33 fusion protein was performed as described by Smith et al.$^{[15]}$ and improved. The expressional GST-hCyP33 in bacterial extract was affinity adsorbed by glutathione-Sepharose 4B beads. After wash the bound protein would be eluted several times with 400 µL 5 mmol/L reduced glutathione solution (50 mmol/L Tris·HCl, pH 8.0). The purified fusion protein was defined with SDS-PAGE (polyacrylamid gel electrophoresis) and the amount of protein would be quantitatively measured by comparison with a standard protein amount series of BSA-V. The harvest of fusion protein is about 1 mg/L bacterial culture (fig. 1).

1.3 Isolation and purification of RNA

One-step isolation of total RNA from pig liver was performed as described by Celis$^{[16]}$ and improved. The mRNA purification kit was used for separation and purification of poly(A)$^-$RNA and poly(A)$^+$RNA. From total RNA the mRNA which contain a polyA tail would be bound to polyT on the solid carrier to build A-T base-pair and, therefore, would be separated from poly(A)$^-$RNA. The RNAs were measured quantitatively by 260 nm.