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Selective structural change of bulged-out region of double-stranded RNA containing bulged nucleotides by spermidine

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ABSTRACT

Polyamines are essential for cell growth due to effects mainly at the level of translation. These effects likely involve a structural change, induced by polyamines, of the bulged-out region of double-stranded RNA that is different from changes induced by Mg^{2+}. Structural changes were studied using U6–34, a model RNA of U6 small nuclear RNA containing bulged nucleotides. Binding of NS1–2 peptide derived from the RNA binding site of NS1 protein, to U6–34 was inhibited by spermidine but not by Mg^{2+}. A selective conformational change of the bases in the bulged-out region of U6–34 induced by spermidine was observed by NMR. The selective effect of spermidine was lost when the bulged-out region of U6–34 was removed in U6–34(Δ5). The binding of NS1–2 peptide to U6–34(Δ5) was inhibited both by spermidine and Mg^{2+}. The selective structural change of U6–34 by spermidine was confirmed by circular dichroism.

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Polyamines (putrescine, spermidine, and spermine) play important roles for normal cell growth [1–3]. It is known that polyamines preferentially bind to double-stranded RNA rather than single-stranded RNA and double-stranded DNA [4]. Indeed, polyamines were found mostly in polyamine–RNA complexes when measured in rat liver, bovine lymphocytes and *Escherichia coli* [5,6]. We reported previously that polyamines have not only a sparing effect on the Mg^{2+} requirement of polyphenylalanine and globin synthesis but also a stimulatory effect that cannot be fulfilled by any amount of Mg^{2+} in the absence of polyamines [7]. We also reported that polyamines enhance the synthesis of several kinds of proteins, at the level of translation, that are important for cell growth in *E. coli* [3,8–10]. We propose that a group of genes whose expression is enhanced by polyamines at the level of translation be referred to as "polyamine modulon" [3]. There appear to be several mechanisms underlying polyamine stimulation of the synthesis of various members of the polyamine modulon. First, polyamine stimulation of protein synthesis can occur when a Shine–Dalgarno (SD) sequence in the mRNA is obscure or is distant from the initiation codon AUG. Polyamines cause structural changes of a region of the SD sequence and the initiation codon AUG of the mRNA, facilitating formation of the initiation complex. This is the case for OppA, FecI, Fis, RpoN, and H–NS. Second, polyamines enhance the inefficient initiation codon UUG-(or GUG-)dependent fMet-tRNA binding to *cya* (or *cra*) mRNA–ribosomes. Third, polyamines stimulate readthrough of the amber codon UAG-dependent Gln-$tRNA^{supE}$ on ribosome-associated *rpoS* mRNA, or stimulate a + 1 frameshift at the 26th UGA codon of *prfB* mRNA encoding RF2. We also reported that rat liver Ile-tRNA formation is enhanced by polyamines [11]. Among these polyamine effects, we extensively studied the mechanism of polyamine stimulation of OppA synthesis [8] and Ile-tRNA formation [11]. These results show that polyamine effects likely involve a structural change of the bulged-out region of double-stranded RNA containing bulged nucleotides, and that this is different from changes induced by Mg^{2+}. However, there is little information with regard to the interactions of polyamines and RNA.

U6 snRNA is an essential component of RNA splicing [12], and the non-structural protein 1 (NS1) of influenza virus type A affects several steps of host translation, including the inhibition of nuclear export of mRNAs that contain 3′ poly(A) ends and the inhibition of pre-mRNA splicing by binding to the U6 snRNA [13,14]. RNA binding

Abbreviations: SPD, spermidine; HSQC, hetero single quantum coherence spectroscopy; HOHAHA, homonuclear Hartman–Hahn spectroscopy; CD, circular dichroism; NS1 protein, non-structural protein 1; U6–34, the NS1 binding region of U6 small nuclear RNA.

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properties of the NS1 protein are retained in an amino-terminal fragment containing the first 73 amino acids of NS1 [NS1A (1–73)] as the full length protein consisting of 202–237 amino acid residues [15].

To study the structural change of the bulged-out region of double-stranded RNA containing bulged nucleotides by spermidine, the NS1 binding site of U6 snRNA, i.e., U6–34, was used as a model system, because its structure has been recently resolved by NMR and the structural change by NS1–2 peptide containing the helix-2 of NS1A was also analyzed by NMR [16,17]. We found that spermidine induces a selective structural change of the bulged-out region of U6–34.

Materials and methods

Preparation of U6–34 and U6–34(Δ5) RNAs. To prepare double-helical oligomers as template DNA for synthesis of U6–34 RNA and U6–34(Δ5) RNA, which lacks A7, A11, A25, A26, and G27 of U6–34, complementary deoxyoligonucleotides of the sequence, 5′-CCTAATACGACTCACTATAGGAACGATACAGAGTTCGCTCGTGAAGCGTTCCA-3′ (S1) and 5′-TGGAACGCTTCACGAGCGAACTCTGTATCGTTCCTATAGTGAGTCGTATTAGG-3′ (A1) for U6–34, and 5′-CCTAATACGACTCACTATAGGAACGTACGAGTTCGCTCGTGCGTTCCA-3′ (S2) and 5′-TGGAACGCACGAGCGAACTCGTACGTTCCTATAGTGAGTCGTATTAGG-3′ (A2) for U6–34(Δ5), were obtained from GE Healthcare Bio-science. The U6–34 RNA and U6–34(Δ5) RNA were synthesized using the above template (S1 + A1 or S2 + A2) by T7 RNA polymerase employing Ampliscribe T7-Flash transcription kit (Epicentre Technologies). Stable-isotopically labeled RNAs were prepared by using [^{13}C/^{15}N] ATP (Taiyo Nippon Sanso). Purification of RNAs was performed with polyacrylamide gel electrophoresis (PAGE) using 30 × 40-cm glass plates (Nihon Eido Co.) under denaturing conditions. RNA samples in H_2O were annealed by heating at 90 °C for 5 min followed by snap-cooling on ice. The formation of the stem–loop structure was confirmed by native PAGE.

RNA binding assay. A synthetic peptide NS1–2, ADRARRDQKSLRGRGSTA, which corresponds to amino acids 19–38 of NS1 protein (underlined Ala was substituted for Leu for solubilization), was obtained from GE Healthcare Bio-science. For RNA binding assay, a reaction mixture (5 μl) contained 10 mM Tris–HCl, pH 7.5, 50 mM KCl, 50 μM U6–34 or U6–34(Δ5) RNA, 0.2 mM NS1–2 peptide, and magnesium acetate and spermidine at the specified concentrations. After incubation at 20 °C for 30 min, 1 μl of 80% glycerol was added to the reaction mixture. Samples of 5 μl were subjected to gel electrophoresis on 8% non-denaturing polyacrylamide gels (59:1% W/V acrylamide:bisacrylamide) using 45 mM Tris–borate, pH 8.3 as a running buffer. After electrophoresis, bound and unbound RNAs were detected using toluidinblau O (Chroma Gesellschaft Schmidt & Co.).

NMR spectra of U6–34 and U6–34(Δ5) RNA. NMR spectra were recorded at a probe temperature of 293 K (20 °C) using Bruker DRX-500 and DRX-600 spectrometers (Bruker Biospin). NMR samples (0.2 ml) contained 10 mM Tris–HCl, pH 7.5, 1.6 mM magnesium acetate, 50 mM KCl, 0.1 mM U6–34 or U6–34(Δ5) RNA, and 5% D_2O. NMR signals of H5–H6 of pyrimidines, H8 and H2 of adenine of U6–34 or U6–34(Δ5) RNA were assigned according to the method of Someya et al. [17]. After NMR spectra were recorded in the presence of Mg^{2+}, spermidine was added to make a concentration of 1.6 mM. Structural change of U6–34 or U6–34(Δ5) RNA by spermidine was monitored with NMR spectroscopy by following the chemical shift changes of H2–C2 and H8–C8 cross-peak of ^{13}C/^{15}N A residue and H5–H6 cross-peak of C and U residues in ^{1}H–^{13}C HSQC [18] and HOHAHA [19] spectra, respectively. All NMR data were processed and analyzed with the program X-WinNMR (Bruker Biospin). As for ^{1}H–^{13}C HSQC experiments, there were no significant changes in the ^{13}C chemical shifts of the RNA in the presence of spermidine; so we only plotted Δ^{1}H for the RNA [20]. Weighted average chemical shift differences of H5–H6 cross-peak of pyrimidine residues were calculated using the equation, $[(\Delta^{1}H5)^2 + (\Delta^{1}H6)^2]^{1/2}$, where Δ^{1}H5 and Δ^{1}H6 are the chemical shift difference (ppm) by spermidine.

CD (circular dichroism) measurement. CD spectra were recorded over 200–320 nm on a Jasco J-820 spectropolarimeter (Jasco International Co.) using a 0.1 cm path-length cuvette at 25 °C [21]. Scan speed was 100 nm/min, and CD sam-

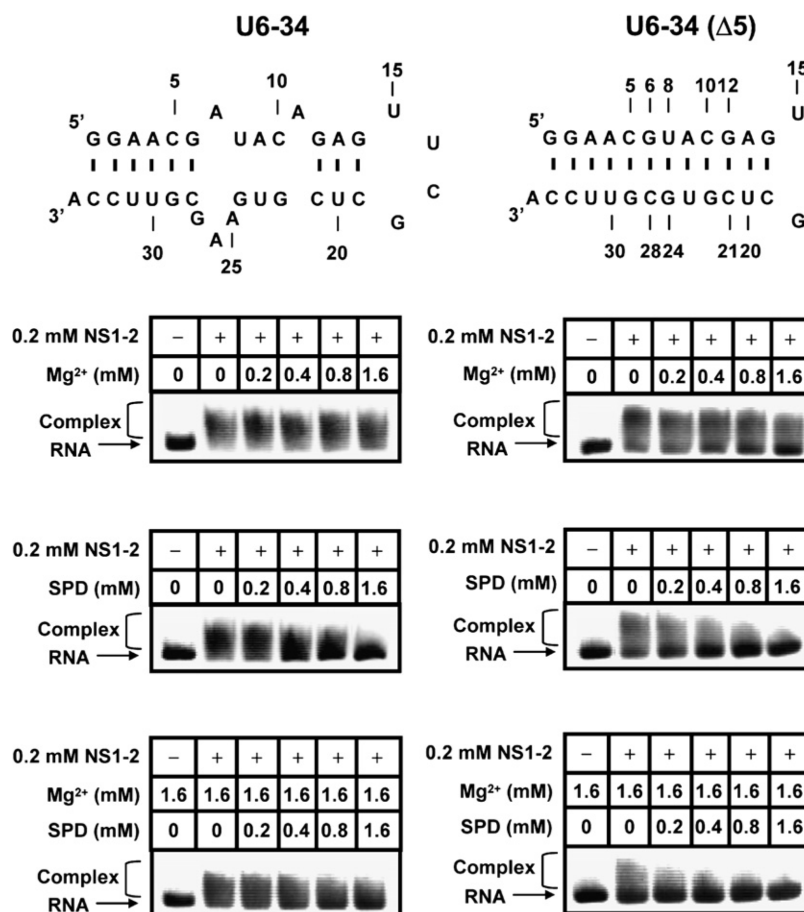

Fig. 1. Effect of spermidine and/or Mg^{2+} on the interaction between U6–34 or U6–34(Δ5) and NS1–2 peptide. RNA binding assay was performed as described in Materials and methods. NS1–2-bound RNA (shown as complex) and unbound RNA were separated by 8% native polyacrylamide gels, and RNAs were stained with toluidinblau O. Secondary structures of U6–34 and U6–34(Δ5) are shown in the upper part of figure. SPD, spermidine.

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