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SODIUM CHLORIDE-INDUCED CONFORMATIONAL CHANGE IN tRNA AS MEASURED BY CIRCULAR DICHROISM

SZYMON SARBAK, MELANIA KUJAWA, HANNA JURGA-NOWAK, ANDRZEJ DOBEK

Department of Physics, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań,

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The effect of 0.01-1 M sodium ions on the conformation of the folded brewer's yeast $tRNA^{Phe}$ was examined by circular dichroism method in the region 200-350 nm. The minimum peak at about 210 nm for tRNA solution with 50 mM sodium chloride showed a decrease in magnitude by 26-30% in comparison to that recorded for the solution of higher NaCl content. The depths of the peaks at 225 nm and 233 nm for two solutions with the lowest sodium chloride concentrations (c_{NaCl} = 10mM, c_{NaCl} = 50mM) were changed by 3-10% relative to the those in the spectra of other samples, for the 260 nm maximum peak a decrease in height was 21-25%. In the region 300-350 nm no significant difference was observed. The results point to a strong relationship between concentration of sodium ions and stabilization process of secondary and tertiary tRNA structure, which indicates the influence of sodium ions on stacking and base-pairing interactions.

INTRODUCTION

Biological functions of the macromolecules occurring in the living cells are mostly conditioned by their structure and interactions. Present-day research in molecular biology, biochemistry or biophysics is mostly focused on the structure-activity relationship (SAR). Transfer ribonucleic acids (tRNAs) play a vital role in the living organism. They are adaptor molecules with the main function of delivering amino acids to the ribosomes at where the peptide bonds are constructed (in the order specified by mRNA) (Ojala, Merkel, Gelfand & Attardi, 1980; Westhof, Dumas & Moras, 1985; Yokoyama, Watanabe, Murao, Ishikura, Yarnaizumi, Nishimura & Miyazawa, 1985; Kawai G., Ue H., Yasuda M., Sakamato K., Hashizume T. McCloskey JA., Miyazawa T. & Yokoyama S,. 1991). This kind of function is possible due to their highly specific molecular conformation. From all kinds of ribonucleic acids in the cell, 15% is made by transfer RNAs. For each amino acid in the living organism, there is at least one kind of tRNA (in total there are 31 to 40 types of tRNAs – depending on species) (Alberts, Bray, Hopkin, Johnson, Lewis, Raff, Roberts & Walter 2009; Turner, McLennan, Bates & White, 1998). In order to fully understand the biochemical processes taking place on the molecular level in tRNA molecules, it is necessary to examine their physicochemical structure in the crystal state as well as the dependence of this structure on external conditions (e.g. ionic strength, temperature, pH, etc.). Exposure of the tRNA molecule to salt ions in different concentrations has been proved to cause structural changes (Dobek, Patkowski, Labuda, & Augustyniak, 1975; Dobek, Patkowski & Labuda, 1977; Labuda, Ahertle & Augustyniak, 1977; Adams, Lindahl & Fresco, 1967; Dudock, DiPeri & Michael, 1970; Kay & Willick, 1971; Ishida & Sueoka, 1968). The characteristic and complicated structure of tRNA, both secondary (resembling clover leaf) and tertiary (resembling letter L) is a sensitive indicator of even slightest changes in ionic environment (Kay *et al.*, 1971; Ishida *et al.*, 1968; Cantor, Jaskunas & Tinoco, 1966; Doctor, Fuller & Webb, 1969). Literature provides reports that reveal different types of interaction of monovalent and bivalent ions with transfer ribonucleic acids. It has been documented that different character of Mg^{2+} binding to tRNA depends upon the initial conformation of the molecule (Labuda *et al.* 1977). In NaCl-free solution, there are two types of tRNA interaction with Mg^{2+}, a strong one characterized by a binding constant $K_s=1.5x10^5$ $[M^{-1}]$, n=2.4 Mg^{2+}/molecule and a weak independent binding, $K_w=9*10^3[M^{-1}]$. In 0.1 M NaCl solution the two independent types of interaction are weak, since Na^+ is known to be a competitive inhibitor of Mg^{2+} binding. When NaCl concentration increases, then $2Na^+$ /Mg^{2+} binding site is found to be formed and it has been observed that Na^+ destabilizes the tRNA conformation induced by low concentration of Mg^{2+} ions. The results of EPR and NMR (Cohn, Danchin & Grunberg-Manago, 1969; Danchin, 1972) measure-

ments have also exposed two types of binding sites for Mg^{2+} ions in the tRNA molecule: one is a strong cooperative site characterized by the association constant of 5 x 10^5 mol^{-1}, and the other one is weak, characterized by the association constant of 2 x 10^3 mol^{-1}. Analogical results have been obtained calorimetrically (Rialdi, Levy & Biltonen, 1972). In the presence of only Na^+ ions, all binding sites are weak, characterized by the association constant of around 60 mol^{-1} (Danchin, 1972). Structural changes in tRNA can be well observed by means of the chiral optic methods. Circular Dichroism Spectroscopy (CD) permits detection of the presence of different secondary and tertiary structures in biopolymers (Fresco *et al*., 1966; Adams *et. al*, 1967; Ishida *et al.*, 1968; Cantor *et. al*., 1966). The CD spectrum of tRNA in native conditions is characterized by four peaks, corresponding to the following elements of secondary and tertiary structure: 1) double-stranded helix - λ = 210 nm and 224 nm, 2) base-stacking - λ = 233 nm and 264 nm (Basu, Jaisankar & Kumar, 2013). The spectra acquired from the circular dichroism studies are the source of much important information concerning interactions occurring in the transfer ribonucleic acids solutions (Kelly & Pric, 2000; Riazance-Lawrence & Johnson, 1992).

MATERIALS & METHODS

The measurements were performed on buffered solutions of mixed tRNA (mainly $tRNA^{Phe}$ – a phenylalanine specific tRNA) extracted from brewer's yeast, Boehringer Mannheim GmbH. The samples were additionally purified using size-exclusion chromatography on Sephadex G-100® (Sigma Aldrich®) in order to precisely separate the tRNA molecules from the dye used in the cell isolation procedure, along with the other contaminations. All the buffer salts were of analytical grade or higher. Solutions were freshly prepared using the buffer and kept protected in the dark cold store room. All experiments were conducted using 10 mM Tris buffer of pH = 6.5 containing 100mM NaCl and 1mM EDTA prepared in deionized water. The pH value was adjusted with hydrochloric acid.

SEC Chromatography

Purification was performed using a chromatographic setup consisting of one-meter-long glass column, packed with Sephadex G-100® (Sigma Aldrich®) gel, RediFrac fraction collector and UV-monitor (both from Pharmacia LKB®). Absorbances of collected fractions were measured by means of a Hitachi U2800A spectrophotometer.

Dialysis

After purification, the samples were dialyzed in appropriate buffers, containing Tris(hydroxymethyl) aminomethane, sodium chloride and magnesium chloride in concentrations of: c_{Tris} = 10 mM, c_{NaCl} = 100 mM and c_{MgCl2} = 5 mM, respectively, at pH=6.5. This buffer composition was used in order to obtain stable and the closest to native tRNA molecule structure. Two changes in the buffer with total volume of 5000 cm^3 were performed.

CD Studies

The CD spectra of tRNA solutions with different contents of salt ions were recorded using a CD JASCO J-815 spectrometer provided with Spectra Manager II software. The 0.5 mm optical path length cuvette was used. A scan rate of 50 nm/min, a bandwidth of 1.0 nm and the wavelength range of 200 – 350 nm was set for the experiments. All measurements were performed in room temperature (25˚C). Obtained raw data were normalized by dividing through differing in samples tRNA concentrations.

RESULTS & DISCUSSION

Six samples with tRNA concentrations in the range of $0.48 \leq c_{tRNA} \leq 0.53$ [mg/cm^3] were prepared by adding 10 µl tRNA solution after dialysis to 140 µl of different concentrated NaCl solution. The samples were subjected to CD measurements immediately after preparation. Table 1 presents the Absorbance (A_{260}), concentrations of tRNA (c_{tRNA}) and molar concentration of sodium chloride in buffer (c_{NaCl}) for each solution. The CD spectra of the samples studied are presented in Fig. 1.

Table 1. Absorbance, tRNA concentration and molar concentration of sodium ions in the samples.

Sample number	A_{260}	c_{tRNA} [mg/cm^3]	c_{NaCl} [mM]
1	0.499	0.476	10
2	0.545	0.519	50
3	0.556	0.530	100
4	0.547	0.521	200
5	0.558	0.531	500
6	0.545	0.519	1000

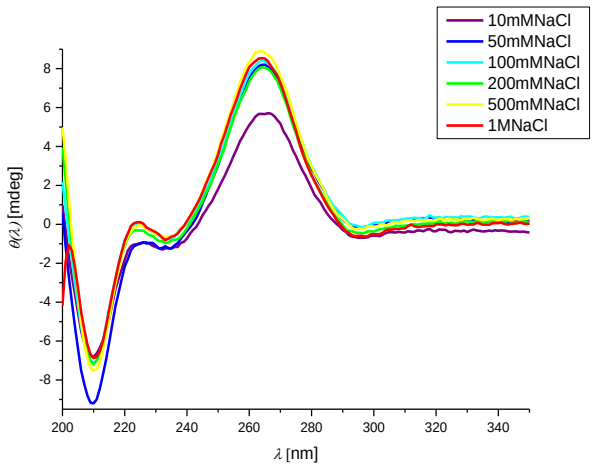

Fig. 1. CD spectra of tRNA samples with different contents of sodium ions (Na^+).

Different solvent polarity caused by the presence of salt ions led to changes in the spectral band position of molecular absorption of the sample. The values of hypso- and bathochromic shifts occurring in the spectra of each sample are shown in Table 2.

Table 2. Position of characteristic peaks of CD spectra of different tRNA samples.

NaCl concentration [mM]	Position of peak 210 nm [nm]	Position of peak 225 nm [nm]	Position of peak 233 nm [nm]	Position of peak 264 nm [nm]
10	210	227	232	266
50	210	226	234	265
100	210	225	235	264
200	210	225	233	264
500	210	225	233	264
1000	210	223	233	264

The CD signal for the solution with the lowest salt ions content (c_{NaCl} = 10 mM) is significantly lower than those for the other samples (the exact values of CD signal are shown in Table 3) which may indicate not completely formed tertiary tRNA structure.

Table 3. Maximal values of ellipticity for characteristic peaks

NaCl concentration [mM]	Wavelength of characteristic peaks 210 nm [mdeg]	225 nm [mdeg]	233 nm [mdeg]	264 nm [mdeg]
10	-6.800	-0.933	-1.296	5.675
50	-9.198	-0.920	-1.237	8.205
100	-7.117	-0.035	-0.717	8.426
200	-7.188	-0.286	-0.965	8.074
500	-7.527	-0.004	-0.699	8.915
1000	-6.730	-0.020	-0.910	8.634

The peaks at 225 nm corresponding to double-helix structure (Wananu & Tor, 2012) are deeper for the solutions with two lowest sodium ions concentrations (c_{NaCl} = 10 mM and c_{NaCl} = 50 mM). It means that this structure is unstable. For the solution with c_{NaCl} = 50 mM the depth of the peak at 210 nm is greater, but considering the decrease in the height of the group of peaks at 225 nm – 236 nm, it cannot be treated as the most stable environment. The spectra of the solutions of other salt concentrations are almost overlapping each other. The differences in CD signal values are between (0.6 - 0.9) mdeg (Table 3.). Fig 2. shows the normalized spectra ($\theta(\lambda)/c_{tRNA}=f(\lambda)$) in order to check the influence of tRNA concentration on a CD signal.

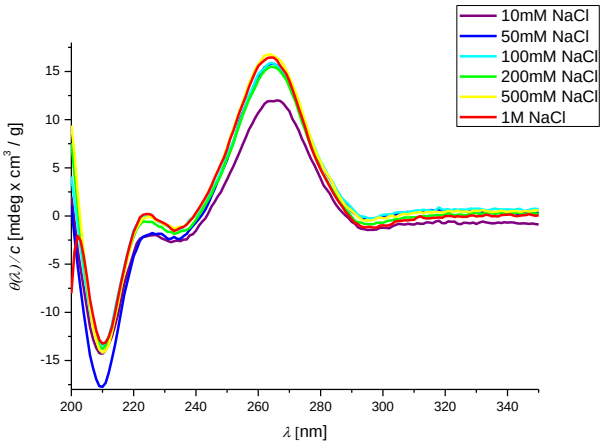

Fig. 2. Normalized CD spectra of tRNA solutions with different NaCl concentration.

In tRNA sample studied here the concentration of Mg ions from dialiysis was amounted to 0.33 mM. According to Labuda *et.al.* 1977, there are three types of Mg^{2+} binding sites (1, 2 and 3) with affinities toward magnesium ions described by constants K_1, K_2, K_3, as well as two forms of tRNA molecule in respect to Mg^{2+} affinities forms X_1 and $X_{1',2,3}$ describing the dependence of Mg^{2+} binding to tRNA upon NaCl concentration in the sample. The authors present that K_1 Mg^{2+} binding may be defined as that which makes possible structural arrangement necessary for the appearance of K_2 Mg^{2+} binding sites. Binding to these sites stabilizes the pre-formed tRNA. Further increase of Mg^{2+} concentration leads to K_3 Mg^{2+} weak binding sites and has no major conformational effect, but it increases the rigidity of the molecule. The effect of K_1 Mg^{2+} binding may be replaced by binding of Na^+ in solution containing 1M NaCl. Increase of NaCl concentration in solution containing Mg^{2+} exerts a destabilizing effect on the tRNA molecule lowering its rigidity, this may promote its biological activity.

CONCLUSION

Analysis of circular dichroism spectra of the tRNA solutions with different sodium ions concentrations revealed a strong positive dependence between the concentration of salt ions and the stabilization process of secondary and tertiary tRNA structures. The character of the CD spectra indicates that the stacking and hydrogen bond interaction between the bases are stronger with increasing Na^+ contents in tRNA solutions. This effect can be explained by the attachment of sodium ions to phosphate groups resulting in the neutralization of the charge of these groups. In the buffer used in this study tRNA occurs in the native folded form, with two classes of independent Mg2+ binding sites. After dissolving folded tRNA in 0.01- 0.05 M NaCl solution, the structure resembles that of unfolded tRNA molecule with one class of Mg^{2+} binding sites. Further results obtained in the presence of 0.1-1 M NaCl reflect the folded but rigid structure. The CD spectrum of the solution with the lowest concentration of NaCl confirms the weakening of stacking and hydrogen bond interactions relative to those occurring in the solutions of higher NaCl concentrations. These results are in agreement with those of the previous investigation by laser Raman spectroscopy (Dobek *et al.* 1975) and light scattering measurements (Dobek *et al.* 1977). After normalization of the CD spectra in relation to tRNA concentration in the solution, no relevant changes between normalized and non-normalized spectra were observed. There is no significant impact of tRNA molecular concentration in solution on circular dichroism signal in the range of tRNA concentrations studied.

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