ADAM PATKOWSKI, ANDRZEJ DOBEK and DAMIAN LABUDA*  

EFFECT OF SODIUM ION CONCENTRATION ON TRANSFER RNA CONFORMATION IN SOLUTION STUDIED BY RAYLEIGH LIGHT SCATTERING  

Quantum Electronics Laboratory, Institute of Physics, Adam Mickiewicz University,  
Grunwaldzka 6; 60-780 Poznań, Poland, and  
*Department of Biochemistry, Adam Mickiewicz University,  
Fredry 10; 61-701 Poznań, Poland  

The sodium ion concentration dependent conformational changes of transfer RNA (unfractionated tRNA from baker's yeast) have been studied in unbuffered aqueous solutions by Rayleigh light scattering. Changes of the optical parameters of the molecule indicated the following conformational changes of tRNA with increasing NaCl concentration: in salt-free solution tRNA molecules have an irregular hairpin loop-like structure in which the orientation of base rings is not correlated. Upon addition of a small amount of NaCl (0.005 M) an increasing ordering of this structure is observed. In 0.1 M-NaCl the molecule has an extended structure with ordered regions (arms). Further increase of sodium ion concentration up to 2 M results in folding of the extended structure and formation of a compact and rigid conformation in which most of the bases are nearly perpendicular to the symmetry axis of the molecule.  

Measurements of light scattering by solutions of macromolecules, which are small compared to the light wavelength, inform about the values of their effective optical parameters, molecular weight, and their mutual interactions. Measured values of depolarization ratio $D_\omega$, as well as calculated values of the mean effective optical polarizability $\alpha$ and the squared effective optical anisotropy $\delta^2$ provide information concerning the shape and structure of the molecules. So far, Rayleigh light scattering has not been used for characterization of different conformational states of tRNA. The aim of our investigations was to test the scattering in this respect and to find out whether there is any correlation between the observed changes of the measured parameters and different tRNA conformations observed by other techni-
ques (Millar & Steiner, 1966; Zimmerman et al., 1970; Robison & Zimmerman, 1971; Goldstein et al., 1972; Cole et al., 1972; Danchin; 1972, Riesner & Römer, 1973; Coutts et al., 1974; Dobek et al., 1975; Chen et al., 1975; Urbanke et al., 1975; Römer & Hach, 1975; Stein & Crothers, 1976; Augustyniak et al., 1976; Labuda et al., 1977).

Previously, it was found by laser Raman spectroscopy (Dobek et al., 1975) that tRNA in unbuffered aqueous solutions undergoes four conformational transitions caused by changes of base-stacking, base-pairing and configuration of ribosphosphate backbone when NaCl concentration is increased from 0 to 1 M. Therefore, the reported here Rayleigh light scattering measurements were performed for tRNA solutions prepared in the same way.

**THEORY**

For scattering angle 90°, the scattered light intensity can be measured at various configurations. For vertically polarized incident light, \( V_v \) and \( H_v \) stand for partial Rayleigh coefficients corresponding to the polarized and depolarized components of scattered light. \( V_h \) and \( H_h \) denote the same coefficients for horizontally polarized incident light. \( R_u, D_v \) and \( D_u \) stand for total Rayleigh coefficient, and the depolarization ratio for vertically polarized and unpolarized incident light, respectively. For molecules which are small compared to the light wavelength the total Rayleigh coefficient is defined as follows (Cwietkow et al., 1968; Kielich, 1977):

\[
R_u = \frac{1}{2} \left( V_v + H_v + V_h + H_h \right) = \frac{1}{2} \left( V_v + 3H_v \right).
\]  

(1)

For the determination of the optical parameters of the molecules from light scattering measurements it is advantageous to know the depolarization ratios, defined by Eqs. (2) and (3)

\[
D_v = \frac{H_v}{V_v} = \frac{3R_{\text{anis}}}{13R_{\text{is}} + 4R_{\text{anis}}} \tag{2}
\]

\[
D_u = \frac{H_u}{V_u} = \frac{2D_v}{1 + D_v} = \frac{6R_{\text{anis}}}{13R_{\text{is}} + 7R_{\text{anis}}} \tag{3}
\]

where \( R_{\text{is}} \) and \( R_{\text{anis}} \) are the isotropic and anisotropic components of the Rayleigh coefficient \( R_u = R_{\text{is}} + R_{\text{anis}} \). Using the measured value of \( R_u \) we are able to calculate \( R_{\text{is}} \) and \( R_{\text{anis}} \) using the results of Cabannes (1929):

\[
R_{\text{is}} = R_u \frac{6 - 7D_u}{6 + 6D_u} \tag{4}
\]

\[
R_{\text{anis}} = R_u \frac{13D_u}{6 + 6D_u} \tag{5}
\]
Let $\alpha_1$, $\alpha_2$, $\alpha_3$ stand for the polarizabilities of the macromolecule in the
directions of its three principal axes. For its mean polarizability we have:

$$\alpha = \frac{\alpha_1 + \alpha_2 + \alpha_3}{3}$$

(6)

Experimentally $\alpha$ value may be obtained in two ways. First, $R_\alpha$ may be
calculated using the measured value of $R_{\text{us}}$ and the following formula:

$$R_{\text{us}} = \frac{1}{4} \pi N \left( \frac{2 \pi}{\lambda} \right)^4 R_\alpha^2,$$

(7)

where $N$ is the number of molecules per unit volume and $\lambda$ is the wavelength
of incident light. Secondly, $\alpha$ value may be obtained from the measured
value of the refractive index increment $\frac{dn}{dc}$ using the formula:

$$n_\alpha = \frac{n_0 M_w}{2 \pi N_A} \frac{dn}{dc},$$

(8)

where $n_0$ is the refractive index of the solvent, $M_w$ is the weight average
molecular weight of the solute, and $N_A$—Avogadro’s number. Likewise, the
squared anisotropy of optical polarizability, defined as:

$$\delta^2 = \frac{(\alpha_1 - \alpha_2)^2 + (\alpha_2 - \alpha_3)^2 + (\alpha_3 - \alpha_1)^2}{2 (\alpha_1 + \alpha_2 + \alpha_3)^2}$$

(9)

can be determined in two ways: by measuring $R_{\text{anis}}$,

$$R_{\text{anis}} = \frac{1}{16} N \left( \frac{2 \pi}{\lambda} \right)^4 R_\alpha^2 \delta^2,$$

(10)

or from the measured value of the depolarization ratio $D_u$,

$$D_\delta = \frac{5D_u}{6 - 7D_u},$$

(11)

Upper case designations $R$ and $D$ to $\delta^2$ denote the way of calculation of
these parameters, i.e. from $R_{\text{anis}}$ and $D_u$, respectively.

MATERIALS AND METHODS

Preparation of the solutions. Measurements of light scattering were carried
out for solutions of unfractionated tRNA from baker’s yeast. The material
was obtained by Holley’s (1963) method and further purified by re-extraction
with phenol, chloroform-isooamyl alcohol mixture and ethyl ether. The pre-
paration was then deaminoacylated (Guderian et al., 1972) and filtered through
Sephadex G-100 (Lindhal & Fresco, 1971). The resulting tRNA solution
(concentration 100 $A_{260}$ units/ml) was extensively dialysed against 0.02
m-EDTA followed by redistilled water, and then lyophilized. As determined by atomic absorption this procedure leaves less than 0.1 mole Mg per one mole of tRNA. After lyophilization tRNA was dissolved in water to a concentration of 100 mg/ml. Dilution series in NaCl or H₂O were obtained from initial 10% solution. Aqueous NaCl-free solutions were prepared by dilution with water. Solutions with determined NaCl concentrations were prepared in two ways:

1) To the 10% aqueous solution of tRNA, an equal volume of NaCl solution was added with a concentration double that required; then, from this 5% solution of tRNA in an NaCl solution of known concentration, a dilution series was obtained using an appropriate solution of NaCl.

2) Starting from 10% aqueous solution, a series of tRNA solutions was prepared at a concentration twice that required, and diluted with equal volumes of appropriate salt solution.

The tRNA concentration of solutions obtained was checked by A₂₆₀ measurements before and after light-scattering measurements. The light-scattering measurements were performed one to ten days after preparation of the solutions.

The tRNA samples, tested electrophoretically on 5% and 7.5% polyacrylamide gels after Loening (1967), were homogeneous 4S RNA. The electrophoretic pattern and acceptor activity for phenylalanine (Schmidt et al., 1971), measured in preparations before and after light-scattering measurements, were identical. Samples were stored at 0°C. Variations of pH of unbuffered aqueous solutions of tRNA due to dilution and addition of NaCl to initially salt-free tRNA (Patkowski, 1975), were measured separately with the PHM-26 pH-meter (Radiometer, Copenhagen) and are shown in Fig. 1.

In order to eliminate mechanical impurities, the water as well as all solutions were made to pass before use through a Millipore nitrocellulose filter (pore diameter 0.45 µm). The tRNA solutions were filtered again through a Millipore filter into the cuvette immediately before measurements. Light-scattering measurements were also performed for solutions of tRNA from yeast (sodium-potassium salt = Na,K-tRNA), purchased from Boehringer, Mannheim (1971/72 catalogue no. 15363); pH of the solutions ranged from 6.3 to 6.7.

Apparatus and measurements. Measurements of Rayleigh light scattering were carried out with the apparatus schematically represented in Fig. 2. The light source consisted of a He-Ne laser constructed in our Laboratory which emitted a beam of red light, λ = 632.8 nm at a power of about 10 mW. Since the laser power was not stabilized optically, a photodiode connected to a V-527 digital voltmeter (ELPO, Warszawa), was placed behind the mirror of transmittance T = 0%. This enabled us to check the laser power during measurements and to maintain it at the required level within 1%. The laser light was plane-polarized, since the ends of the laser tube were closed off with windows set at Brewster's angle. A Glan-Thompson prism
Fig. 1. Dependence of pH of unbuffered aqueous tRNA solutions on tRNA concentration and NaCl molarity.

Fig. 2. Diagram of apparatus used in Rayleigh light-scattering measurements.

enhanced the degree of polarization of the laser beam and permitted accurate adjustment of the plane electric vector vibrations of the beam. The liquid under study was placed in rectangular, plane-parallel, glass cuvette with 1 x 1 cm square basis. On emerging from the cuvette and traversing the diaphragms (of the diameter of 1.5 and 1.5 mm placed at a distance of 7 mm and 10 cm from the scattering cell, respectively), the scattered light reached the analyser, which permitted the measurement of vertically and horizontally polarized scattered light. The positions of the analyser corresponding to the polarization directions of scattered light were established with an accuracy of 0.01° by the polarizing head of a Hilger polarimeter.
An FEU-17A photomultiplier tube (Moscow, U.S.S.R.) with side window was placed behind the analyser, at a distance of 10 cm from the cuvette. The photomultiplier signal was transmitted to the EZ-10 line Recorder (Laboratorní Pристроje N.P., Praha, Czechoslovakia).

In order to check the apparatus, measurements were made on some, repeatedly distilled, simple organic liquids, namely: benzene, nitrobenzene, cyclohexane, carbon tetrachloride and carbon disulphide. The obtained light scattering parameters, i.e. depolarization ratio and relative Rayleigh coefficients for these liquids were in good agreement with the literature data. As a standard liquid, benzene (B.D.H., England), spectroscopically pure, of refractive index \( n_\mathrm{B} = 1.4941 \), was used, for which abundant, highly accurate literature data are available giving the depolarization ratio, as well as the total Rayleigh coefficient for the wavelength of 632.8 nm (Kaye & Havelik, 1973; Dezelić, 1970; Lalanne & Bothorel, 1970; Cordanone et al., 1972). When calculating the absolute Rayleigh coefficients of the solutions we applied the absolute Rayleigh constant for benzene at 632.8 nm \( R_B = 8.765 \times 10^{-6} \text{ cm}^{-1} \).

All aqueous solutions of tRNA were measured in the same cell at 23°C. The results reported here are averages of at least 10 measurements. The experimental error of the individual measurement was small—never exceeding 2%. Since only relative intensity measurements of light scattered at 90° were performed, the only correction taken into account in addition to the one for the difference in photomultiplier sensitivity for scattered light of various polarization was for the body angle, with regard to the different refractive indices of the standard and studied solutions. Other corrections, related to the geometry of the measuring system, are irrelevant in this relative method.

Light refractive indices of the tRNA solutions were measured at 23°C for the light wavelength of 632.8 nm by means of an IRF-23 (Moscow, U.S.S.R.) refractometer. Measurements of the refractive index increment were carried out with an ITR-1 (Moscow, U.S.S.R.) difference refractometer at the light wavelength of 550 nm. The measured values were converted to the increments at 632.8 nm using the following formula (Peralmann & Longsworth, 1948):

\[
\left( \frac{dn}{dc} \right)_k = \left( \frac{dn}{dc} \right)_{578} \times \left( 0.940 + \frac{2 \times 10^6}{\lambda^2} \right).
\] (12)

RESULTS AND DISCUSSION

Light refractive indices of the studied tRNA solutions, for the light wavelength of 632.8 nm, are shown graphically in Fig. 3. From the graphs, the refractive index increments \( \frac{dn}{dc} = 0.170 \text{ g}^{-1} \text{ cm}^3 \) and \( \frac{dn}{dc} = 0.166 \text{ g}^{-1} \text{ cm}^3 \) were calculated (\( c \) is the concentration of tRNAs in \( \text{g cm}^{-3} \), and \( c_s \) that of NaCl in \( \text{g cm}^{-3} \)). The value of \( \frac{dn}{dc} = 0.168 \text{ g}^{-1} \text{ cm}^3 \) calculated according
to Eq. (12) from difference measurements of the refractive index increment at
550 nm, is in good agreement with that obtained from absolute refractive
index measurements for red light.

![Graph showing refractive index vs. tRNA concentration and NaCl concentration]

Fig. 3. Refractive index $n$ vs. the tRNA concentration for solutions of tRNA in H$_2$O and in
0.005, 0.1, 1 and 2 M aqueous solutions of NaCl. The broken line denotes the solution
of NaCl in H$_2$O.

Total $R_u$ and partial $H_v$, $V$, Rayleigh coefficients were measured for series
of tRNA solutions of various ionic strength. The intensities of light scattered
by the tRNA molecules in solution were calculated as a difference in the
light intensities scattered by the solution and respective solvent. Table 1 lists,
extrapolated to zero tRNA concentration, values of: mean optical polariz-
abilities $^a\alpha$ (Eq. 8) and $^R\alpha$ (Eq. 7); squared optical anisotropies $^D\delta^2$ (Eq. 11)
and $^R\delta^2$ (Eq. 10); polarizability components $\alpha_1$ and $\alpha_3$ (calculated using
values of $^a\alpha$, and $^D\delta^2$) and their ratio, for tRNA solutions of different NaCl
concentrations. The polarizability components were calculated for assumed
symmetry of an ellipsoid of revolution: $\alpha_1 = \alpha_2 = \alpha_3$. We can compare the
parameters given in Table 1, because the differences in pH between tRNA
solutions of different NaCl molarity may be neglected, due to extrapolation
to zero tRNA concentration.

From light scattering measurements in a DC magnetic field (Dobek
et al., 1977) the optical anisotropy of tRNA molecules is found to be
negative, $\alpha_3 < \alpha_1$, i.e. the polarizability ellipsoid of tRNA molecules is oblate.
This result was obtained on the assumption that the molecules are prolate
ellipsoid of revolution and $\alpha_3$ is parallel to the symmetry axis of the ellipsoid.

Optical polarizability of a molecule is a measure of capability of electric
charge displacement in the electric field of light waves. Therefore $\alpha_1$ and
$\alpha_3$ are the measures of charge displacements in directions 1 and 3 of the
Table 1

Optical parameters of tRNA solutions

Extrapolated to zero tRNA concentration values of: mean optical polarizability \( \alpha \) obtained from refractive index increment measurements — Eq. (8) and \( \alpha \) calculated from the isotropic component of Rayleigh coefficient — Eq. (7), squared optical anisotropy \( \delta^2 \) obtained from the depolarization ratio \( D \) — Eq. (11) and \( \delta^2 \) calculated from the anisotropic component of Rayleigh coefficient — Eq. (10), polarizability components \( \alpha_1 \) and \( \alpha_3 \) calculated using values of \( \alpha \) and \( \delta^2 \) and their ratio, for tRNA solutions of different NaCl concentration.

<table>
<thead>
<tr>
<th>Concentration of NaCl C_1 (M)</th>
<th>Depolarization ratio ( D_\alpha )</th>
<th>Mean optical polarizability ( \alpha \times 10^{21} ) (cm³)</th>
<th>Squared optical anisotropy ( \delta^2 )</th>
<th>Polarizability components ( \alpha_1 \times 10^{21} ) (cm³) ( \alpha_3 \times 10^{21} ) (cm³)</th>
<th>Polarizability components ratio ( \alpha_3/\alpha_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.051</td>
<td>1.58</td>
<td>1.55</td>
<td>0.045</td>
<td>0.045</td>
</tr>
<tr>
<td>0.005</td>
<td>0.088</td>
<td>1.58</td>
<td>1.64</td>
<td>0.082</td>
<td>0.083</td>
</tr>
<tr>
<td>0.1 (Proc. 1)</td>
<td>0.028</td>
<td>1.58</td>
<td>1.77</td>
<td>0.024</td>
<td>0.025</td>
</tr>
<tr>
<td>0.1 (Proc. 2)</td>
<td>0.028</td>
<td>1.58</td>
<td>1.77</td>
<td>0.024</td>
<td>0.022</td>
</tr>
<tr>
<td>1</td>
<td>0.211</td>
<td>1.59</td>
<td>1.83</td>
<td>0.233</td>
<td>0.233</td>
</tr>
<tr>
<td>2</td>
<td>0.198</td>
<td>1.60</td>
<td>1.84</td>
<td>0.215</td>
<td>0.216</td>
</tr>
<tr>
<td>Na,K-tRNA</td>
<td>0.041</td>
<td>1.58</td>
<td>1.67</td>
<td>0.038</td>
<td>0.033</td>
</tr>
</tbody>
</table>

tRNA ellipsoid, respectively. The polarizabilities of the bases and also phosphate groups provide the main contribution to total tRNA polarizability. In aromatic components, as the bases are, the component of polarizability parallel to the ring plane is larger than the perpendicular one. In phosphate groups it should be expected that the polarizability component parallel to the plane of PO_2-double bond would have the highest value. Since the polarizability is an additive quantity, \( \alpha_1 \) is a sum of the polarizability components of the bases and phosphate groups in a direction perpendicular to the symmetry axis, and \( \alpha_3 \) in a direction parallel to symmetry axis. The lowest value of the \( \alpha_3 \) component will be observed when all the bases are parallel to each other and perpendicular to the \( \alpha_3 \) direction. This would be the case of a hairpin loop-like structure with the maximal number of pairing and stacking interactions. In this configuration the value of the squared optical anisotropy will be the highest and the \( \alpha_3/\alpha_1 \) ratio will reach its minimum.

The results reported by Goldstein et al. (1972) led them to suggest three conformational forms of tRNA: a salt-free coil, a "clover leaf" at about 0.1 M-NaCl with all secondary structure elements present, and a highly ordered conformation appearing at 2 M-NaCl. This pattern of tRNA conformational changes as a function of Na⁺ concentration was recently confirmed by laser Raman spectroscopy studies (Dobek et al., 1975) of unbuffered aqueous
solutions of tRNA, together with the postulated (Goldstein et al., 1972) intermediate conformation occurring between salt-free coil and the "clover-leaf" structure. Similarly, UV-studies of Na\(^+\) concentration-dependent tRNA melting profiles enable four thermodynamically distinct conformations to be distinguished in the 0 to 0.17 M-Na\(^+\) and 4° to 88°C interval (Cole et al., 1972).

The salt-free structure is characterized by the lowest values of ellipticities at the wavelength of the major positive circular dichroism band and for the weak negative band near 295 nm, as well as by the highest hyperchromicity with respect to the ordered states (Goldstein et al., 1972). Raman spectroscopy reveals some pairing and stacking interactions and low content of the A form characterizing conformation of the ribosophosphate backbone, as indicated by the relative intensities at 815 and 615 cm\(^{-1}\). Recently, it has been found by photon correlation spectroscopy studies (Patkowski et al., 1978) that the melted form of tRNA molecule occurring at high temperatures can be approximated by a long prolate ellipsoid of revolution with the axial ratio of 8 and is not a random coil, as it was postulated previously. Since a decrease in cations concentration has a similar effect on tRNA conformation as an increase of solution temperature, we may expect a similar elongated structure for the ion-free solution. However, from our measurements one can see that the squared optical anisotropy (Table 1) for this solution is small, the \(\alpha_3/\alpha_4\) ratio is quite high and this means that the orientation of the planes of base rings is almost random.

As the sodium ion concentration is raised to 0.005 M, the squared optical anisotropy becomes twice as high as before, and the \(\alpha_3/\alpha_4\) ratio is lower. The conclusion is that a small amount of sodium ions present in solution, partially neutralizing the electrostatic repulsion of the phosphate groups, allows for some stacking and pairing interactions, which create some kind of order within the molecule. Since the orientation of the bases is more correlated, the squared optical anisotropy is higher. This is consistent with observations that the tRNA structure appearing at 0.005 M-Na\(^+\) exhibits a significant hypochromic effect at 260 nm with respect to that in salt-free solution (Goldstein et al., 1972) and to that occurring at elevated temperatures (Cole et al., 1972). It is characterized by relatively low \(T_m\), high intrinsic viscosity, as well as low number of exchangeable protons and high initial rate of hydrogen exchange (Goldstein et al., 1972). At 0.1 M-NaCl the value of squared optical anisotropy has its minimum and the \(\alpha_3/\alpha_4\) ratio has its maximum. Theoretically, there are two ways to explain this observation. One is that the orientation of bases is less correlated and more random than in the salt-free solution. However, this possibility is excluded because it is known from other measurements (Goldstein et al., 1972; Cole et al., 1972; Dobek et al., 1975) that at 0.1 M-NaCl there exist almost all pairing and stacking interactions characteristic for the native form of tRNA. Upon addition of NaCl up to 0.1 M, a large decrease in intrinsic viscosity and
a large decrease in initial rate of hydrogen exchange together with an increase in the number of slowly exchangeable hydrogens (from about 40 to 80 in the case of mixed E. coli tRNAs) is observed (Goldstein et al., 1972). Also $T_m$ value and hypochromic effect are increased and circular dichroism spectra as well as UV-melting profiles are altered with respect to low-salt solutions (Goldstein et al., 1972; Cole et al., 1972). In this context, the other explanation is that an extended clover-leaf-like structure is formed and some of its arms are perpendicular to the symmetry axis of the molecule, but the bases are ordered (parallel to each other) within each of the arms.

A further increase of NaCl concentration up to 1 M causes a significant increase of the squared optical anisotropy and a decrease of the $\alpha_3/\alpha_1$ ratio. This can be explained by the creation of a more compact tertiary structure in which the arms of the clover-leaf structure are folded together and the planes of the rings of most of the bases are nearly perpendicular to the symmetry axis of the molecule. The same holds true for 2 m-NaCl solution and the small differences in measured parameters between 1 m and 2 m-NaCl solutions may be due to further ordering of the structure. This is consistent with the previous findings suggesting that Na$^+$ concentration of 2 m is required to obtain the structure equivalent to the tRNA conformation appearing at low concentrations of Mg$^{2+}$ (Goldstein et al., 1972). This conformation, in turn, was found, in the case of phenylalanine tRNA (yeast) to be identical with that predicted for crystalline tRNA by X-ray analysis (Chen et al., 1975). If this is so, the polarizability values obtained for tRNA in 1 m and 2 m solutions characterize the folded, T-shaped (Ladner et al., 1975) or L-shaped (Kim et al., 1974) structure of tRNA observed in crystalline state. The oblate ellipsoid of optical polarizabilities, exhibiting the highest asymmetry in this case, points to highly ordered and rigid structure of tRNA in 1 and 2 m-NaCl solutions. This structure presumably contains long helical fragments arranged mainly parallel with respect to the symmetry axis of the ellipsoid approximating the shape of the molecule.

Our investigations have been carried out on unfractionated tRNA solutions. The assignment of a given tRNA conformation, as salt-free structure, intermediate structure, “clover-leaf” or folded structure was performed for a population of tRNAs which differ in details of conformational transition induced by Na$^+$. Our conclusions, therefore, are only of a general character, but they are consistent with the overall picture of tRNA conformational changes as a function of Na$^+$ appearing from previous findings (Goldstein et al., 1972; Cole et al., 1972; Dobek et al., 1975; Patkowski & Chu, 1979). Many experiments have shown that although the detailed nature of tRNA structural transitions is strongly sequence-dependent, the general conformational behaviour of all tRNAs is nearly identical, consistent with their common function as adaptor molecules (Millar & Steiner, 1966; Goldstein et al., 1972; Cole et al., 1972; Danchin, 1972; Riesner & Römer, 1973; Dobek
et al., 1975; Römer & Hach, 1975; Urbanke et al., 1975; Stein & Crothers, 1976). The data on salt-free coil are available only from studies of hypochromic effect, circular dichroism, photon correlation spectroscopy, Raman spectra and from deprotonation studies (Dobek et al., 1975; Goldstein et al., 1972; Cole et al., 1972; Augustyniak et al., 1976). To observe formation of ordered structure starting from salt-free conformation, our measurements were intentionally made on unbuffered solutions to avoid the presence of any ions in the solution without NaCl. The data on low-salt solutions are more abundant in the literature. Low-salt aberrant structures (Urbanke et al., 1975) and particularly extended forms of tRNA proposed by Cole et al. (1972) fit very well with the observed flattening of polarizability ellipsoid at 0.005 M-NaCl. Differential melting studies have shown that the structure appearing in 0.1 M-NaCl possesses the majority of features (elements of secondary and tertiary structure and interactions) characteristic for folded conformation (Riesner & Römer, 1973; Urbanke et al., 1975; Römer & Hach, 1975). This is supported by observations on the binding behaviour of monovalent cations to tRNA and the influence of Na⁺ on the Mg²⁺ binding (Augustyniak et al., 1976; Labuda et al., 1977).

Summarizing, we conclude that light-scattering measurements are applicable to structural studies as a sensitive probe of tRNA conformation in solution. The four different tRNA conformations observed in the present work may be compared with those appearing upon sequential melting of tRNA in the absence of Mg²⁺ (Cole et al., 1972; Stein & Crothers, 1976). The method described here permits to monitor changes in the shape of the tRNA molecule and particularly the changes of orientation of the structural elements within the molecule. This may be related to the capability of interaction with other macromolecules. We feel, therefore, that light-scattering measurements may appear important in characterization of tRNA conformations in respect to structural requirements for and during interactions of biological importance.

We are indebted to Professors F. Kaczmarek and J. Augustyniak for the helpful comments and discussions.

RÉFÉRENCES


---

**Wpływ stężenia jonu sodu na konformację tRNA w rozwozie badany przy pomocy rozpraszania światła Rayleigha**

**Streszczenie**

Zmiany konformacyjne transferowych kwasów rybonukleinowych (tRNA), wywołane jonami sodu o różnym stężeniu, badano za pomocą rozpraszania światła Rayleigha. Obserwowano wraz ze wzrostem stężenia NaCl zmiany parametrów optycznych tRNA mogą wskazywać na następujący model przejść konformacyjnych:

- w rozworze bezsolnym tRNA posiadają nieregularną strukturę typu spinki do włosów (hairpin-like structure) o nieskorelowanej względnej orientacji pierścień zasad.
- dodatek małej ilości NaCl (0.005 M) powoduje wzrost uporządkowania tej struktury.
- w rozworze o stężeniu 0.1 M-NaCl następuje dalsze uporządkowanie i zorganizowanie struktury, dotyczące najprawdopodobniej "ramion" cząsteczki tRNA,
- zwiększenie stężenia soli do 2 M prowadzi do upukowania tej struktury, w wyniku czego tworzy się zwarta i ozjutrz konformacja, w której przeważająca liczba zasad zajmuje położenie prawie prostopadle do osi symetrii cząsteczki tRNA.

Received 9 July, 1980; revised 16 May, 1981.