Vol. 104, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 29, 1982 Pages 733-739

THE EFFECTS OF MAGNESIUM IONS ON THE HYDRODYNAMIC SHAPE, CONFORMATION, AND STABILITY OF THE RIBOSOMAL 23S RNA FROM E. coli

Qing-Ming Yi* and Kin-Ping Wong

Department of Biochemistry
University of Kansas Medical Center
Kansas City, Kansas 66103
and
*Department of Microbiology
Wuhan University, Wuhan
The People's Republic of China

Received December 7, 1981

The hydrodynamic shape, conformation, and thermal stability of ribosomal 23S RNA have been studied by sedimentation velocity analysis, circular dichroism, ultraviolet absorption, and thermal denaturation. The results show that magnesium ion is critically required for maintaining the structural integrity of ribosomal 23S RNA. Upon removal of magnesium ions, the sedimentation coefficient of 21.1 S for native 23 S RNA is reduced to approximately 8.5 S, indicative of a large unfolding of the RNA. A large increase in absorbance at 260 nm in UV spectra of 23S RNA and a decrease in ellipticity near the 265 nm peak which was followed by parallel changes in the 237 nm trough and the 208 nm trough. Furthermore, the melting temperature (T_m) of the 23S RNA is lowered to 50°C from 65°C with a concomitant decrease in the cooperativity of the melting curve of 23S RNA.

INTRODUCTION

Magnesium ions have been shown to be a critical cation for the functioning of RNA (e.g., Hall & Doty, 1959; Tissiere et al., 1959; Edelman, et al., 1960; Fuwa et al., 1960). Several studies also show that it is extremely important for maintaining the structural integrity and functioning of ribosome and ribosomal RNA (e.g., Watson & Kidson, 1969; Cammack et al., 1970; Weiss & Morris, 1973; Wong and Dunn, 1974; Nisbet & Slayter, 1975; Morris et al., 1975; Cox & Hirst, 1976; Muto & Zimmermann, 1978). However, exactly what structural role magnesium ion plays on the various structural aspects of ribosomal 23S RNA is not known. The need to understand the effects of magnesium ion on the 23S RNA is further necessitated by the fact that it plays a pivotal role in the successful reconstitution of the 50S ribosomal subunit (Nierhaus & Dohme, 1974). The present communication describes three types of experiments to study the role

0006-291X/82/020733-07$01.00/0

of the magnesium ion on 23S RNA. The sedimentation velocity analysis was used to find out the change in hydrodynamic property of the 23S RNA, difference circular dichroism and difference absorption spectra were used to monitor conformational change of the 23S RNA upon addition of magnesium ion, and the thermal denaturation of 23S RNA in the presence and absence of magnesium is followed by changes of circular dichroism. The present work shows drastic changes in hydrodynamic shape, conformation, and thermal stability of the 23S RNA as a result of addition of magnesium.

MATERIAL AND METHODS

Buffers. TM_4: Tris 20 mM; $MgCl_2$, 4 mM; pH 7.6.
T: Tris 20 mM; pH 7.6

E. coli cells. E. coli strain MRE 600 (RNase I$^-$) was purchased from Grain Processing Company, Muscatine, Iowa, U.S.A. The E. coli cells had been harvested at one-quarter log phase and were stored at -70°C until needed.

70S Ribosome. Ribosomes were isolated according to the procedure of Nomura and co-workers (Traub et al., 1971) with slight modifications (Dunn & Wong, 1979).

Ribosomal Subunit. The 50S subunits were isolated by centrifugation in a Beckman Ti-15 zonal rotor using a hyperbolic gradient from 7.4 to 38% sucrose according to the method of Eikenberry et al. (1970) with minor modifications (Allen & Wong, 1979).

Ribosomal 23S RNA. Ribosomal 23S RNA was extracted by phenol-sodium dodecyl sulfate (SDS) procedure (Nierhaus & Dohme, 1979) with slight modifications. To 50S subunits at a concentration of less than 400 A_{260} unit/ml, 1/10 volume of 10% SDS, 1/20 volume of 2% betonite, and 1.2 volume of 70% (v/v) phenol were added. The mixture is shaken vigorously on a vortex mixer for 8 min and centrifuged for 10 min at 10,000 x g. The aqueous phase is mixed with 1.2 volume of 70% phenol, shaken for 5 min, and centrifuged. The extraction is repeated a third time. The resulting aqueous phase was dialyzed against TM_4 buffer at 4°C until no phenol was detected. The final aqueous solution was stored at -70°C.

Sedimentation Velocity. Sedimentation coefficients were determined in a Beckman Model E analytical ultracentrifuge equipped with Schlieren optics. An aluminum single-sector centerpiece was used with an An-D rotor. All measurements were obtained at 25°C. Photographic plates were read in a Nikon Model 6C microcomparator. The observed sedimentation coefficient, S_{obs}, was obtained from the slope of the logarithm of the radial distance, x, from the center of the rotor to the center of the moving Schlieren peak versus time, t, as given by the equation (Tanford, 1961): $S = 2.303\ d\log x/\omega^2 dt$ where ω is the angular velocity of the rotor in radians/sec. The frictional coefficient ratio, f/f_{min}, and the effective hydrodynamic radius, R_{sed}, were calculated as described previously (Allen & Wong, 1978).

Ultraviolet Absorption Spectrophotometry. A Cary 118 Cx double-beam recording spectrophotometer was used for obtaining ultraviolet absorption spectra and for concentration determination. A Lauda K2-R circulating water bath was used to thermostat the compartments. The results were expressed in terms of molar absorbance, ε, in units of $M^{-1}cm^{-1}$, using the equation: $\varepsilon = A/l \cdot M$ where A is the measured absorbance, l is the pathlength in cm, and M is the molar concentration.

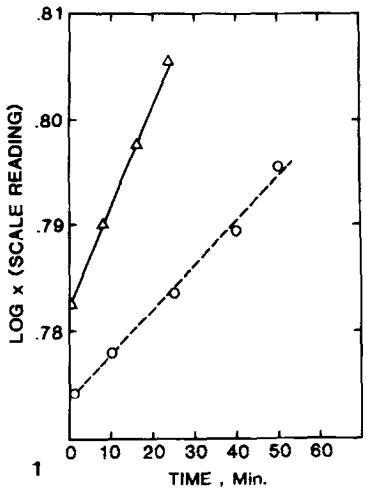
Circular Dichroism. Circular dichroism (CD) was used as a probe to monitor the conformation and stability of 23S RNA in the difference spectroscopy and thermal stability studies. Detailed aspects of CD instrumentation and measurement have been published (Wong, 1974; 1975a,b). CD studies were carried out using a JASCO J-500A CD spectropolarimeter. The temperature of the sample cell was controlled by a water-jacketed aluminum cell holder connected to a Lauda K2-R constant temperature circulating water bath. Absorbances of solutions were typically less than 1.5 at 260 nm. The results were usually expressed in terms of molar ellipticity, $[\theta]$, in units of deg·cm^2·decimole^{-1}, using the equation: $[\theta] = \theta \cdot M/l \cdot c$, where θ is observed ellipticity in degrees, M is the molecular weight, and l and c are the optical pathlength in decimeters and the concentration in gm/ml, respectively. The instrument was routinely calibrated with d-10-camphorsulfonic acid according to Cassim and Yang (1969). The spectropolarimeter was purged continuously with pure nitrogen gas during all measurements.

Thermal Denaturation Studies. Thermal stability studies were carried out with 23S RNA using circular dichroism as probes. CD studies were performed with a JASCO J-20 spectropolarimeter with a jacketed cell holder made from an aluminum block with constant temperature water circulating from a K2-R water bath. Calibration curves were established for the instrument which corrected for the temperature difference between the solution in the spectrophotometric cuvette and the circulating water bath. Silicon oil was layered on top of the samples to prevent evaporation during heating. All samples had an initial absorbance at 260 nm between 0.9 and 1.0 at room temperature. Melting was induced by raising the temperature in 5° to 6° increments. After reaching the desired temperature, each sample was allowed to equilibrate for 20 min before ellipticity was recorded. The melting profiles were represented in terms of hypoellipticity at 265 nm according to the equation (Dunn and Wong, 1979): % hypoellipticity = $[(\theta t°C - \theta 25°C)/\theta 25°C] \times 100$ where $\theta t°C$ is the observed ellipticity at the temperature in question and $\theta 25°C$ is the observed ellipticity at 25°C.

Miscellaneous. The concentration of the 70S ribosome and its 50S subunit were determined spectrophotometrically using $A_{260}^{0.1\%}$ = 14.5 and 14.5, respectively (Hill et al., 1969). The concentration of RNA was determined by using $A_{260}^{0.1\%}$ = 22.3 (Stanley & Bock, 1965). Molecular weights for the 70S and 50S particles and the 23S RNA were 2.65 x 10^6, 1.55 x 10^6, and 1.18 x 10^6, respectively (Hill et al., 1969; 1970; Tam, et al., 1981). The pH measurements were obtained at room temperature with a Model PHM64 research pH meter equipped with a combined electrode and standardized with pH 7.00 and pH 4.01 standard buffers. Weight measurements were carried out using a Mettler H20T microbalance. All water used was double-distilled and deionized. Dialysis tubing was treated by boiling in 1% Na_2CO_3, 1 mM EDTA for 1 h, followed by thorough washing with distilled water. The prepared tubing retained molecules with molecular weights larger than 3.5 x 10^3. Precautions were taken to avoid ribonuclease contamination. All glassware was acid washed with a 3:1 mixture of nitric acid and sulfuric acid and rinsed thoroughly with distilled and double-distilled, deionized water.

RESULTS

Sedimentation velocity analysis. Fig. 1 shows the sedimentation velocity analysis plotted as log x vs. time for the 23S RNA in the absence (T buffer) and presence (TM_4 buffer) of magnesium ions and then extrapolating to zero concentration. The slope was used to obtain the sedimentation coefficients. The 23S RNA in T buffer yields a sedimentation coefficient of ~8.5 S at a concentration of 4.9 mg/ml. The calculated frictional coefficient ratio, f/f_{min},



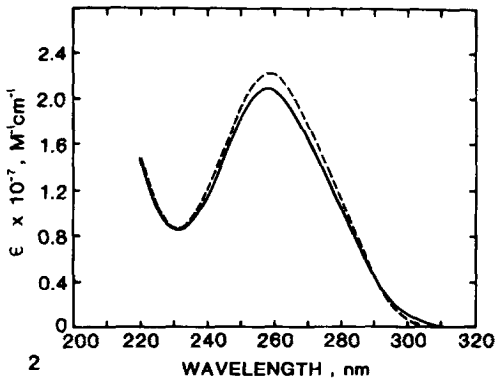


Figure 1. Time-dependent sedimentation velocity plot of 23S RNA in the presence (—△—) and absence (-○-) of magnesium. Rotor, An-D; speed, 40,000 rpm; temperature, 25°C.

Figure 2. Ultraviolet absorption spectra of 23S RNA in the presence (——) and absence (– – – –) of magnesium. Temperature, 25 ± 1°C.

is 7.75. The sedimentation velocity analysis for 23S RNA in TM_4 buffer yields a sedimentation coefficient of ∿21.1 S at the same concentration. The calculated frictional coefficient ratio, f/f_{min}, is equal to ∿3.07. And assuming a spherical hydrodynamic domain generated by the macromolecule, the effective hydrodynamic radius, R_{sed}, was calculated to be ∿452 Å and ∿200 Å for 23S RNA in T buffer and in TM_4 buffer, respectively.

Ultraviolet absorption spectra. Figure 2 shows the ultraviolet absorption spectra for 23S RNA in the absence and presence of magnesium ions. A large decrease at 260 nm is shown upon addition of 4 mM of magnesium. A simultaneous increase in absorbance near 300 nm was also observed.

Circular dichroism (CD). Figure 3 shows the CD spectra. The addition of magnesium ion to 23S RNA results in an increase of ellipticity near the 265 nm peak. Changes in the 237 nm trough and the 208 nm trough are also observed. In the latter case, the addition of magnesium to 23S RNA actually results in an increase in ellipticity at 208 nm trough, while a less negative trough near 237 nm is seen.

Thermal denaturation studies. The melting profile for 23S RNA in Tris and in the presence of 4 mM magnesium ion is shown in Figure 4. It is

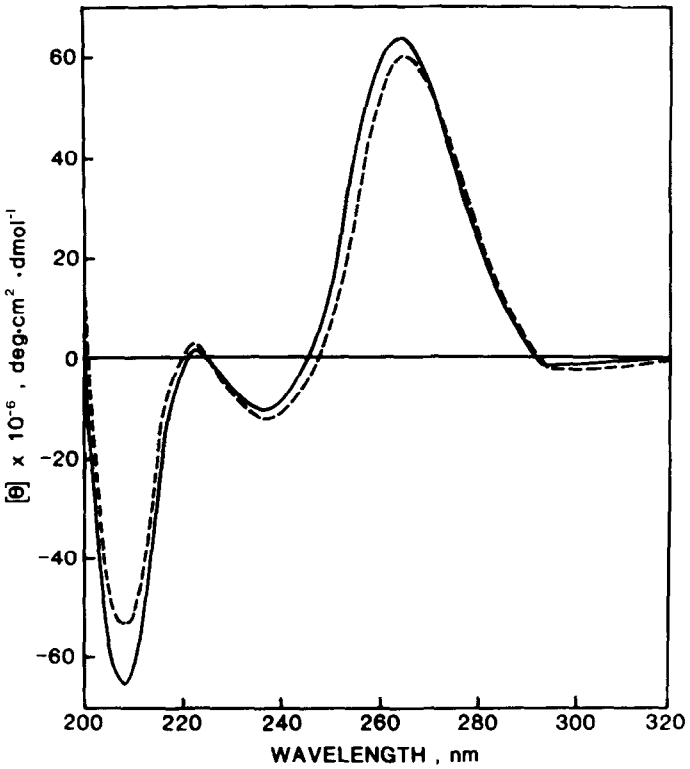


Figure 3. Ultraviolet CD spectra of 23S RNA in the presence (———) and absence (— — — —) of magnesium. Temperature, 25 ± 1°C.

obvious that the presence of magnesium results in a more cooperative melting profile, as detected by changes in ellipticity at 265 nm. On the other hand, the 23S RNA in the absence of magnesium shows a less cooperative curve with an almost linear increase in hypoellipticity from 27°C to 65°C. The melting temperature, T_m, is 50°C and 65°C for 23S RNA in the absence and presence of magnesium, respectively

DISCUSSION

The present study shows that magnesium ion is critically required for the maintenance of the hydrodynamic shape, conformation, and thermal stability of the ribosomal 23S RNA. Upon removal of magnesium ion, the sedimentation coefficient of 23S RNA reduces from 21.1 S to approximately 8.5 S. This corresponds to a drastic unfolding of the RNA molecule from a relatively compact form to an extended one. In addition, the hyperchromicity from UV absorption studies indicate that the removal of magnesium ions results in a large decrease of base pairing and base stacking in the 23S RNA molecule. This is further supported

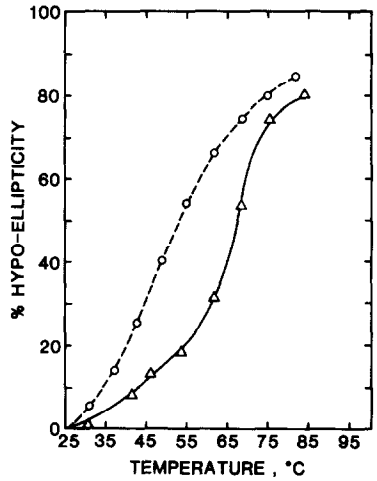


Figure 4. Thermal denaturation profile of 23S RNA in the presence (—Δ—) and absence (–○–) of magnesium.

by significant decrease of the circular dichroism spectra at 265 nm which indicates a decrease of base stacking and a decrease in the asymmetric structural elements (e.g., helical structures). The change in thermal stability is even more dramatic. The highly cooperative melting profile of the 23S RNA in the presence of magnesium ion becomes less cooperative upon removal of magnesium ions. Drastic changes of the melting temperature from 65°C to approximately 50°C (a decrease of 15°C) upon removal of magnesium indicate a large decrease in thermal stability of the RNA molecule. It is obvious from these studies that magnesium affects all levels of the three-dimensional structure of the 23S RNA molecule.

ACKNOWLEDGEMENTS

This work has been supported by NIH Grant GM 22962. One of us (Q.M.Y) is the recipient of a research fellowship from the Government of The People's Republic of China.

REFERENCES

Allen, S.H. & Wong, K.-P. (1978) J. Biol. Chem. 253, 8759-8766.

Allen, S.H. & Wong, K.-P. (1979) Arch. Biochem. Biophys. 195, 112-120.

Cammack, K.A., Miller, D.S., & Grinstead, K.H. (1970) Biochem. J. 117, 745-755.

Cassim, J.Y., & Yang, J.T. (1969) Biochemistry 3, 1947-1951.

Cox, R.A., & Hirst, W. (1976) Biochem. J. 160, 505-519.

Dunn, J.M. & Wong, K.-P. (1979) Biochemistry 18, 4380-4385.

Edelman, I., Ts'o, P.O.P., & Vinograd, J. (1960) Biochem. Biophys. Acta 43, 393-403.

Eikenberry, E.F., Bickle, T.A., Traut, R.R., & Price, C.A. (1970) Eur. J. Biochem. 12, 113-116.

Fuwa, K., Wacker, W.E.C., Druyan, R., Bartholomay, A.F., & Vallee, B.L. (1960) Biochemistry 46, 1298-1307.

Hall, B.D., & Doty, P. (1959) J. Mol. Biol. 1, 111-126.

Hill, W.E., Anderogg, J.W., & Van Holde, D.E. (1970) J. Mol. Biol. 53, 107-121.

Hill, W.E., Rosetti, G.P., & Van Holde, K.E. (1969) J. Mol. Biol. 44, 263-277.

Morris, D.R., Dahlberg, J.E., & Dahlberg, A.E. (1975) Nucl. Acid Res. 2, 447-459.

Muto, A., & Zimmermann, R.A. (1978) J. Mol. Biol. 121, 1-15.

Nierhaus, K.H., & Dohme, R. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 4713-4717.

Nierhaus, K.H., & Dohme, R. (1979) Methods Enzymol. 59, 445-446.

Nisbet, J.H., & Slayter, H.S. (1975) Biochemistry 14, 4003-4010.

Stanley, W.M., & Bock, R.M. (1965) Biochemistry 4, 1302-1311.

Tam, M.F., Dodd, J.A., & Hills, W.E. (1981) FEBS Lett. 130, 212-220.

Tanford, C. (1961) in Physical Chemistry of Macromolecules, p. 710, John Wiley and Sons, Inc., New York.

Tissiere, A., Watson, J.W., Schlessinger, D., & Hollingworth, R.B. (1959) J. Mol. Biol. 1, 221-233.

Traub, P., Mizushima, S., Lowry, C.V., & Nomura, M. (1971) Methods Enzymol. 20, 391-407.

Watson, J.D., & Kidson, C. (1969) J. Mol. Biol. 43, 331-335.

Weiss, R.I., & Morris, D.R. (1973) Biochemistry 12, 435-441.

Wong, K.-P. (1974) J. Chem. Ed. 52, A573-A575.

Wong, K.-P. (1975a) J. Chem. Ed. 52, A9-A13.

Wong, K.-P. (1975b) J. Chem. Ed. 52, A83-A88.

Wong, K.-P., & Dunn, J.M. (1974) FEBS Lett. 44, 50-54.