CONFORMATIONAL STUDIES OF THE UNFOLDING OF *E. COLI* RIBOSOME

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Received 3 May 1974

1. Introduction

As an initial attempt at deciphering the detailed molecular mechanism of the self-assembly of ribosome we have begun to elucidate the conformational changes of ribosome upon dissociation and unfolding by removal of Mg(II). The hydrodynamic behavior of ribosome upon removal of Mg(II) has been previously studied [1–5]. Below a Mg(II) concentration of 10^{-4} M or in the absence of Mg(II) in the buffer, native ribosome dissociates into its subunits. On further removal of Mg(II) by EDTA, the dissociated subunits unfold into presumably extended and loosened forms. Recently, a thermodynamic study [6] revealed that this unfolding is accompanied by an instantaneous reaction which involves a large endothermic enthalpy change at 25°C and 37°C. Moreover, at 37°C an additional slow reaction which involves an even larger endothermic heat change follows the first instantaneous reaction. No such additional slow reaction was observed at 25°C within the experimental time. The large estimated heat capacity change of the first instantaneous reaction may indicate the exposure of hydrophobic region in the ribosome upon unfolding.

In this communication we report the conformational study on the unfolding of ribosome by circular dichroism (CD) and by ultraviolet absorption spectroscopy. Dissociation of ribosome by exhaustive dialysis against 1 mM Tris appears to result mainly in conformational changes of the ribosomal proteins and not in the RNA's. The major conformational change of ribosome unfolding upon the further removal of Mg(II) by EDTA resides on the RNA's and there are further structural changes observed at 37°C than at 25°C.

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2. Materials and methods

2.1. *Purification of ribosome*

E. Coli MRE 600 was used. The procedure of Nomura and co-workers [7] was employed with minor modifications as described in an earlier paper [6].

2.2. *Preparation of native, dissociated, and unfolded ribosome*

Ribosome sample solutions are obtained by dilution of a stock solution with a concentration of approximately 3 mg/ml in TMA I buffer (Tris, 10^{-2} M; NH_4Cl, 3×10^{-2} M; $MgCl_2$, 10^{-2} M; pH 7.2) to approximately 0.005 mg/ml with the same buffer. This solution is then exhaustively dialyzed against a 10 000-fold volume of TMA I buffer in the cold ($\sim$ 4°C) and used as the 'native' ribosome solution. The 'dissociated' ribosome solution is prepared by exhaustive dialysis of a ribosome sample solution against a 10 000-fold volume of a 1 mM Tris buffer at pH 7.2 in the cold ($\sim$ 4°C). Preparation of the 'unfolded' ribosome solution is done by mixing an appropriate amount of a dissociated ribosome solution with a EDTA solution in 1 mM Tris, pH 7.2, so that the molar ratio of EDTA to ribosome is 3.25×10^3 [5].

2.3. *Ultraviolet absorption spectrophotometry*

A Cary 14 double-beam spectrophotometer equip-

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ped with water-jacketed cell holder was employed. Temperature control was provided by a Haake thermostatted bath.

2.4. *Circular dichroism*

CD spectra were obtained with a JASCO J-20 spectropolarimeter equipped with a jacketed cell holder made from an aluminium block with constant temperature water circulating from a Lauda K2/R water bath. Absorbance of all solutions were maintained below 1.0. The results were expressed in terms of molar ellipticity, $[\theta]$, in units of deg · cm^2 per decimole using the equation: $[\theta] = \frac{M}{dC}\theta$, where θ is the observed ellipticity in degrees, M and C are the molecular weight in g and concentration in g/cc, respectively; and d is the optical path in decimeters. The instrument was calibrated according to Cassim and Yang [8].

2.5. *Miscellaneous*

Ribosome concentration was measured with a Cary 14 spectrophotometer using $A^{1\ mg/ml}_{260\ nm} = 15.5$. A molecular weight of 2.65×10^6 g/mole was employed for ribosome. All pH measurements were performed with a Radiometer Model 26 pH-meter with combined glass electrode. The water used in all experiments was double-distilled and deionized. All measurements at 25°C and 37°C were conducted after the sample solutions have been equilibrated at the specified temperature for at least 4 hr.

3. Results

The native, dissociated, and unfolded state of ribosome as defined respectively by the ribosome in TMA I buffer, 1 mM Tris, and Tris–EDTA was studied at 25°C and at 37°C.

3.1. *Near ultraviolet CD spectra*

Fig. 1 shows the near ultraviolet CD spectra of ribosome at different conformational states at 25°C, pH 7.2. Native ribosome (70 S) has a CD spectrum characterized by a small but distinct trough at 297 nm with a molar ellipticity of −5, a large positive peak with a maximum molar ellipticity of 106 at 265 nm, and crossovers at 292 nm and 247 nm. The CD curve of dissociated ribosome, other than a negligible small increase around the 265 nm peak and a slight decrease between 290–295 nm region, is virtually identical to that of the native ribosome. However, removal of Mg (II) by EDTA induces some significant changes of the CD spectrum. The 265 nm ellipticity band undergoes a red shift of about 2.5 nm without any change in molar ellipticity. This is accompanied by the complete disappearance of the trough at 297 nm. The near

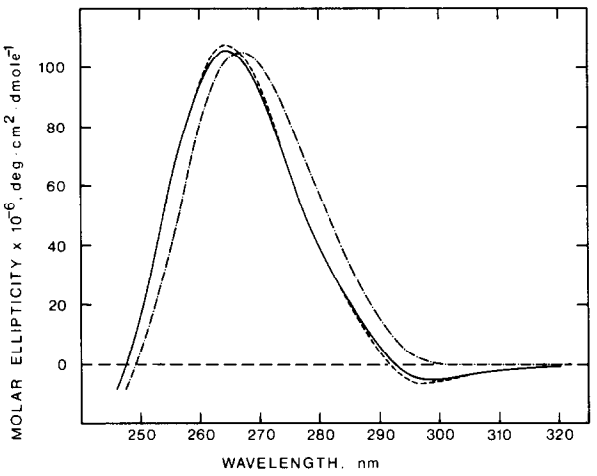

Fig. 1. Near ultraviolet CD spectra of ribosome at 25°C. Solid curve, native ribosome; dotted curve, dissociated ribosome; broken curve, unfolded ribosome. Ribosome concentration, 0.042–0.046 mg/ml; pH 7.2.

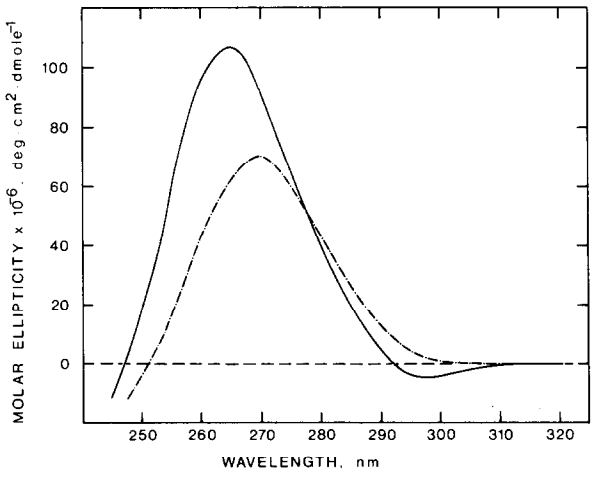

Fig. 2. Near ultraviolet CD spectra of ribosome at 37°C. Solid curve, native and dissociated ribosome; broken curve, unfolded ribosome. Ribosome concentration, 0.042–0.046 mg/ml; pH 7.2.

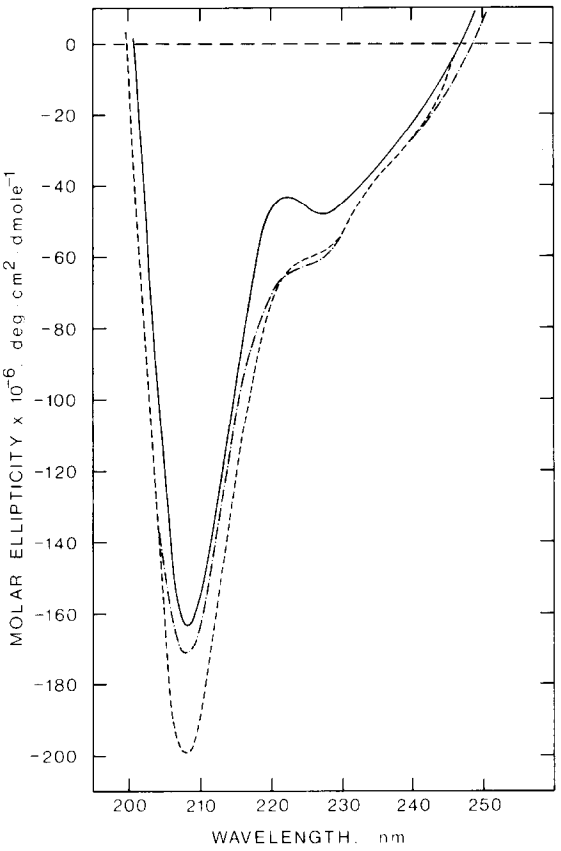

Fig. 3. Far ultraviolet CD spectra for ribosome at 25°C. Solid curve, native ribosome; dotted curve, dissociated ribosome; broken curve, unfolded ribosome. Ribosome concentration, 0.064–0.067 mg/ml; pH 7.2.

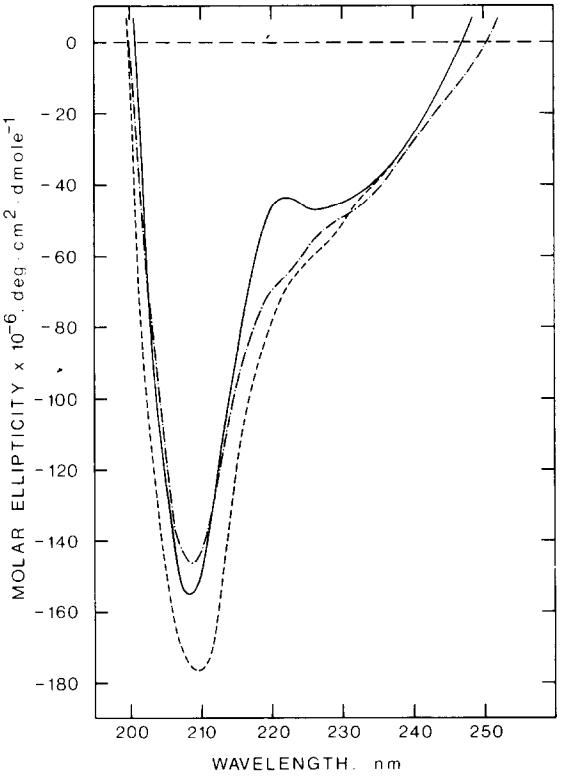

Fig. 4. Far ultraviolet CD spectra of ribosome at 37°C. Solid curve, native ribosome; dotted curve, dissociated ribosome; broken curve, unfolded ribosome. Ribosome concentration, 0.064–0.067 mg/ml; pH 7.2.

ultraviolet CD spectra of ribosome at 37°C are shown in fig. 2. Both native and dissociated ribosome have the same CD curves which are in turn indistinguishable from their corresponding CD curves at 25°C. However, the removal of Mg(II) by EDTA causes a drastic change in the CD spectrum. The 265 nm peak undergoes a much larger red-shift to 270 nm with a concomitant large decrease in molar ellipticity as shown in fig. 2. This change is also accompanied by a complete disappearance of the 297 nm trough.

3.2. *The far ultraviolet CD spectra*

The CD spectra below 250 nm at 25°C are shown in fig. 3. Dissociated ribosome, which has near CD spectrum identical to that of the native ribosome, undergoes a drastic change below 240 nm. The molar ellipticity at 222 nm increases from -43×10^6 for the native ribosome to -66×10^6 for the dissociated ribosome. A similar large increase in molar ellipticity of about -36×10^6 is observed at the 208 nm trough. In comparison to this large change, the change of the spectra upon removal of Mg(II) by EDTA is less dramatic. There is a slight increase of negative ellipticity around 222 nm, and a large decrease in the negative trough at 208 nm. The far ultraviolet CD spectra at 37°C are shown in fig. 4. The pattern of change observed at this temperature is similar to those seen at 25°C. After exhaustive dialysis against 1 mM Tris, a large change of the spectra occurs below 240 nm. Unfolding of the ribosome by addition of EDTA results in negligible change at 222 nm and a larger decrease in the 208 nm trough.

3.3. *Ultraviolet absorption spectra*

At 25°C, both native and dissociated ribosomes give identical spectra. However, the spectrum of unfolded ribosome undergoes a hyperchromic effect with a molar absorbance increase of $3.5 \times 10^6 \cdot M^{-1} \cdot cm^{-1}$ at 257 nm. At 37°C, the spectra of native and dissociated ribosome are the same and are indistinguishable from the corresponding spectra at 25°C. However, unfolding of ribosome at 37°C results in a larger hyperchromic effect at the 257 nm peak. The molar absorbance change is about 2 times of that observed at 25°C.

4. Discussion

While the hydrodynamic studies of the native, dissociated, and unfolded ribosome give information on the overall hydrodynamic shape or gross conformation of the ribosome particle at different states, the spectroscopic studies reported here provide the more subtle conformational information which reflect the changes of the secondary and tertiary structure of the component proteins and RNA's. The CD spectrum of native ribosome possesses at least four parameters which are sensitive to some conformational aspects of the component RNA's and proteins. The near ultraviolet CD curve is presumably due solely to the RNA's since the proteins do not contribute to the CD at this region. There is a small but distinct negative trough around 297 nm and a large positive peak centered around 265 nm. This curve is characteristic of many RNA's [9–12] and is probably a resultant curve of conservative and non-conservative interactions [13]. The presence of the small trough was reported by Yang and co-workers [14], but its structural origin is unknown. They found that the trough disappeared upon melting, and they also suggested that it might arise from the η–π^* transition. The large positive CD peak centered around 265 nm probably originated from the conformational state of the bases of the RNA's [9,15]. In the far ultraviolet region, the CD curve of native ribosome shows a broad negative trough around 220–225 nm and a large trough centered around 208 nm. The broad trough at 220–225 nm probably reflects contribution mainly from the helical structure of the proteins since RNA's normally have zero or negligible ellipticity around 222 nm. The 208 nm trough probably arises from both the RNA's and the proteins. It has been suggested that this trough is sensitive to tilting and to conformational changes of nucleic acids as well as conformational changes of the proteins. The other spectroscopic property we used here is the hypochromicity observed in the ultraviolet absorption spectrum of ribosome which is primarily due to the complementary hydrogen bonding and the stacking of the bases in the double helical structure of the RNA's [16].

Dissociation of native ribosome by exhaustive dialysis against 1 mM Tris at 25°C and at 37°C does not result in conformational changes of the RNA's. This is suggested by the lack of significant CD changes at the 297 nm trough and at the 265 nm peak in the near ultraviolet spectral region. This conclusion is further supported by the absence of any hyperchromic effect at the 257 nm peak of the absorption spectrum. Dissociation of native ribosome does, however, give a drastic change in the far ultraviolet CD spectra. The increase of molar ellipticity around 222 nm suggested a conformational change which may be related to an increase of secondary structure of the proteins. The increase of magnitude of the 208 nm trough is probably a manifestation of the same phenomenon. These changes in the conformation of the proteins at 25°C and 37°C are similar though not completely identical. Early optical rotatory dispersion (ORD) studies suggested that association–dissociation of ribosome does not involve any significant conformational changes of the component proteins and RNA's [17,18]. This is contradicted by recent CD studies [19,20] which indicate a change in magnitude of the 265 nm peak. Our results show that dissociation of ribosome by exhaustive dialysis with 1 mM Tris does not result in conformational change of the RNA. Furthermore, large changes of the CD parameters which are presumably related to the conformation of the proteins have been observed at 25°C and 37°C. This conformational change may be related to the conformation of proteins involving in subunit association via protein–protein and protein–RNA interactions. Some such interactions have recently been identified and inferred [21,22].

Further removal of Mg(II) by addition of EDTA to yield the unfolded ribosome results in significant conformational change of the RNA's. At 25°C, this change is manifested in the near ultraviolet CD as a

red-shift of the 265 nm peak without any change in ellipticity and accompanied by the disappearance of the 297 nm trough in agreement with the results of Adler et al. [23]. Similar ORD change has been suggested to be due to breakage of the complementary base pairs of the double helical conformation with base-stacking remaining undisturbed [24–26]. The hyperchromicity observed in ultraviolet absorption spectrum of unfolded ribosome reinforces this conclusion that the secondary structure of the RNA has undergone some changes. At 37°C further conformational change of the RNA's involving unstacking of the bases in the helical regions occurs as observed in a large hyperchromic effect on the ultraviolet absorption spectrum around 257 nm, and a more drastic change of the near ultraviolet CD spectrum involving a large red-shift of the 265 nm peak and a large decrease in magnitude of this peak. This type of large redshift of the 265 nm peak with a simultaneous large decrease in ellipticity was alleged to originate from a drastic unstacking and destruction of the complementary base pairing of the helical structure of the RNA's [9,15,25]. This further conformational change which occurs only at 37°C may account for the larger enthalpy change at 37°C and/or the additional slow second reaction observed in the microcalorimetric study reported earlier [6]. Kinetic experiments are being conducted to investigate these possibilities.

Although the present work represents only a diagnostic technique for following the conformational changes of the proteins and the RNA's in the ribosome, it does, however, provide some rather interesting results and promise to be a potentially useful technique for other conformational studies of the ribosome. Attemps in deciphering the structural origin of each of the CD parameters and the application to conformational change of the RNA's and proteins upon re-assembly are currently in progress.

Acknowledgements

The financial support of Damon Runyon Fund (DRG 1165) is gratefully acknowledged. The recording CD spectropolarimeter was purchased through a Research Corporation grant (#6014). We thank Miss Susan Allen for helpful discussions.

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