LASER RAMAN STUDIES OF RNA BACKBONE ORDERING IN *ESCHERICHIA COLI* RIBOSOMES

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Although the primary structures of ribosomal RNA and r-proteins are rapidly being elucidated (1) and the secondary structure of the ribosome is under intense investigation by numerous techniques (2–4), the tertiary structure of the ribosome is largely unknown. Here we present evidence that Laser Raman (LR) spectroscopy may allow the semiquantitative assessment of features of tertiary structure in a variety of environmental conditions.

Ribosomal subunits and detergent-extracted rRNA were purified by conventional means and dialyzed exhaustively against various buffers (Figs. 1 and 2). Samples were excited with the 488-nm line of an argon-ion laser and spectra recorded from a Spex 1400 double-grating spectrometer with Ortec photon counting equipment.

Fig. 1 shows some data from LR heating curve studies, with curves drawn by eye to fit the experimental points. The parameter represented is the ratio of height of the 813-cm^{-1} to the 785-cm^{-1} peak. (The 785-cm^{-1} peak height did not change with temperature and could therefore be used as a normalization standard.) The 813-cm^{-1} peak arises from nucleotides which are in the 3′-endo, "ordered" configuration, so that a higher value of the 813/785 ratio, γ, corresponds to a more ordered configuration of the RNA backbone (5–6).

Ribosomes and rRNA in the presence of EDTA differed very little if at all, but as the Mg^{2+} level was increased, the ribosomes became distinctly more stable than the rRNA to changes induced by increased temperature. Ribosomes also showed progressively sharper, more cooperative changes in LR spectra at higher Mg^{2+} concentrations, i.e., melting occurred

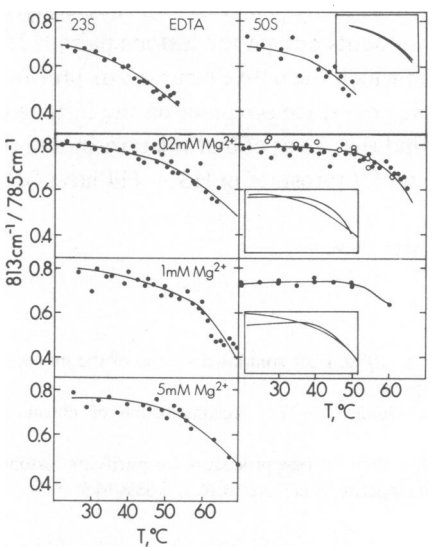

Figure 1 Effect of temperature on the 813 cm^{-1}:785 cm^{-1} Raman signal (γ). Spectra were recorded in 20 mM Tris-CH1, pH 7.5, containing 10 mM EDTA or the indicated Mg^{2+} levels. Results with two preparations are shown with (o) and (•). Insets show superimposed curves for each buffer.

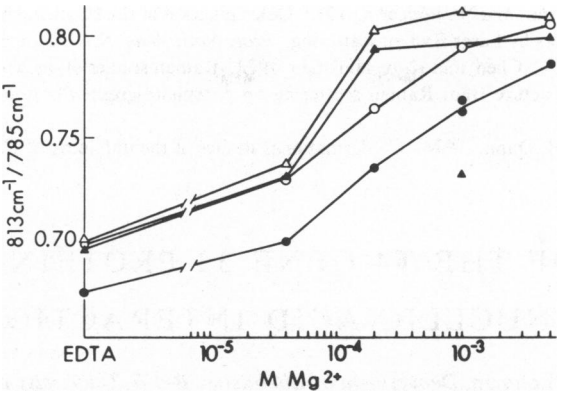

Figure 2 Effect of Mg^{2+} concentration on the 813 cm^{-1}:785 cm^{-1} ratio (γ). 16S rRNA (o); 30S ribosome (•); 23S rRNA (Δ); 50S ribosome (▲).

over a narrower temperature range. Stabilization need not involve more cooperative melting, for the rRNA heating curves showed an increased stabilization over a broad range of temperature without an increase in cooperativity, even in the presence of 0.2 to 1 mM Mg^{2+}, where the stabilization compared to that in EDTA solution was marked (Fig. 1). However, at 5 mM Mg^{2+}, the highest concentration tested, even the melting of the rRNA showed increased cooperativity.

There are several differences between findings from LR and from comparable studies of UV hypochromicity, which measures the extent of base-pairing (1, 4, and our further studies). Changes in the LR parameter γ are seen at lower temperatures than initial changes in hypochromicity. This is consistent with the notion that γ measures at least in part some feature of structure more thermolabile than base-pairing. Also, the LR difference between RNA and ribosome melting are qualitative, except in EDTA (Fig. 1), whereas reported differences in UV hypochromicity are much smaller. Furthermore, the differences seen by LR are maximal at physiological Mg^{2+} levels and are minimal in EDTA, whereas differences in UV hypochromicity are maximal in EDTA. Thus, LR may be detecting a contribution to backbone ordering that is stabilized both by Mg^{2+} ions and by r-proteins.

Fig. 2 shows that γ varies as a function of Mg^{2+} level, again unlike UV hypochromicity. The greatest change in γ occurs over the range of Mg^{2+} concentration in which "ribosome unfolding" occurs (4, 7). Thus, γ may be an index of rRNA backbone ordering which is correlated with tertiary as well as secondary structure.

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KINETICS OF THE T4 GENE 32 PROTEIN-SINGLE-STRANDED NUCLEIC ACID INTERACTION

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The kinetics of the interaction of the T4 bacteriophage coded gene 32 protein (GP32) with single-stranded nucleic acids have been investigated. The GP32 is a helix destabilizing protein necessary for DNA replication, recombination, and repair. GP32 functions through its interaction with single-stranded DNA and RNA to which it binds cooperatively (1). Its main function during replication is thought to be the stabilization of transient single-stranded regions in the replication fork. Kinetic studies are necessary to understand how GP32 interacts with a moving replication fork.

Fluorescence and stopped-flow methods have been used to investigate the association and dissociation kinetics of GP32 with a series of single-stranded homopolynucleotides and intact single-stranded DNA from bacteriophage M13. The time-course was monitored by observing the quenching of the intrinsic tryptophan fluorescence of the GP32 upon its binding to the single-stranded nucleic acids.

ASSOCIATION KINETICS

Polynucleotides and GP32 were mixed (0.1 M NaCl, 10 mM Tris, pH 8.3, 25°C) in a Gibson-Durrum stopped-flow spectrophotometer and the resulting fluorescence decrease was monitored. In all association experiments, the nucleic acid (N.A.) was in great excess over the [GP32] so that isolated binding of the GP32 was the major mode of binding. For poly (rA) and poly(dA) two relaxations are observed (τ_f and τ_s).

The slow relaxation (τ_s) is essentially independent of [N.A.] [for poly(rA) τ_s = 3s], whereas τ_f is a function of [N.A.]. The [N.A.] dependence of τ_f is consistent with the following mechanism for GP32 ($\equiv P$) binding in the isolated mode to single-stranded N.A. ($\equiv D$)

$$P + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PD^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PD. \qquad (1)$$

The observed fluorescence change occurs in the second step presumably involving a conformational change in the GP32 N.A. complex. Evidence of a conformational change has also been obtained from equilibrium studies.[1] The best fit parameters for the scheme in Eq. 1 are

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[1]Kowalczykowski, S., J. Newport, N. Lonberg, and P. von Hippel. Submitted for publication; see also these discussions.