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Note

Detection of binding between transfer RNA and RNA polymerase by high-performance liquid chromatography

SALLY AMERO*
Division of Plant Science, West Virginia University, Morgantown, WV 26506 (U.S.A.)
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Recent *in vitro* transcription assays indicate that a specific association between *E. coli* transfer RNA and *E. coli* RNA polymerase should be readily demonstrable[1]. Preliminary sucrose density gradient analyses of mixtures of transfer RNA and RNA polymerase suggest that binding between the two molecules does occur. However, examination of this molecular association in the proportions and conditions employed in the original transcription assays requires detection of minute amounts of complex formation in a system that is saturated with transfer RNA, and the density gradient technique affords neither precise measurement of complex formation nor maintenance of isotonic conditions. Therefore, high-performance liquid chromatographic (HPLC) columns were devised which would exclude the bound complex but retain free transfer RNA, so that complex formation could be monitored indirectly as the reduction in the single elution peak from transfer RNA. The simple high performance exclusion technique affords highly reproducible, quantitative results and greater sensitivity and rapidity than is afforded by conventional exclusion chromatography.

EXPERIMENTAL

Sample preparation

E. coli transfer RNA from strain W and *E. coli* RNA polymerase Type 1 (E.C. 2.7.7.6) from strain K12 were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). The polymerase was shipped, at a concentration of 1.69 mg/ml, in a solvent consisting of 60% glycerol and 40% 50 m*M* Tris buffer, pH 8.0, 0.2 m*M* dithioerythritol, 0.1 m*M* EDTA, 10 m*M* $MgCl_2$, and 0.45 *M* $(NH_4)_2SO_4$. This buffer was substituted, with and without glycerol, for polymerase as indicated in the Table. The tRNA was mixed at a concentration of 1 mg/ml in 0.01 *M* Tris–HCl buffer, pH 7.9, with 0.02 *M* $MgCl_2$, 1 m*M* EDTA, and 0.1 *M* KCl. To 100-μl aliquots of tRNA were added 11-μl volumes of (1) Sigma *E. coli* RNA polymerase, (2) Tris buffer without polymerase, (3) Tris buffer with 60% glycerol, (4) maize polymerase of unknown concentration in buffer (prepared as described in ref. 2), and (5) bovine serum albumin (BSA) in Tris buffer.

* Present address: Department of Biology, University of Virginia, Charlottesville, VA 22901, U.S.A.

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HPLC

Quantitation of tRNA was carried out on a Waters Assoc. Model 201 HPLC (Waters Assoc., Milford, MA, U.S.A.) equipped with a Varichrom variable-wavelength detector (Varian Assoc., Palo Alto, CA, U.S.A.) and a Coleman Model 165 strip-chart recorder. A 55 cm × 7 mm I.D. stainless-steel column was dry packed with equal parts of Corning glycophase-G in 100 Å CPG-10 and uncoated 75 Å CPG-10 controlled pore glass (Corning, Medfield, MA, U.S.A.). The mobile phase was 0.01 *M* Tris–HCl buffer formulated as above. All analyses were carried out at a wavelength of 260 nm, a flow-rate of 3 ml/min, and a recorder chart speed of 5 mm/min. Since peak width at half-height was a constant, all data were recorded as peak height in mm.

RESULTS AND DISCUSSION

This study was undertaken to determine whether transfer RNA can bind to, and thereby regulate, *E. coli* RNA polymerase in the context of *in vitro* transcription assays wherein transfer RNA seemingly functions as an effector molecule[1]. It was important that the experimental design accomodate the ionic conditions and the ratio of transfer RNA to polymerase established in the original transcription studies, which contained insufficient polymerase for direct spectrophotometric detection at 280 nm. The single molecular species that could be detected spectrophotometrically was transfer RNA.

As shown in Table I, the peak area of transfer RNA eluted from the HPLC column decreases significantly with the addition of glycerol-stabilized *E. coli* RNA polymerase. Table I further demonstrates that approximately 20% of the initial 27% decrease in peak height may be attributed to the effect of glycerol in the mix. The 20% reduction caused by glycerol, however, is static over a 10× range of glycerol concentrations, and so does not account for the final 7% decrease in the transfer RNA peak. The experiments were repeated with BSA and maize polymerase, with no apparent effect on the transfer RNA peak height.

The glycerol effect has not been characterized for this study. The control experiments with glycerol were performed since viscous solutions such as glycerol may sufficiently reduce operating pressures to affect elution properties[3,4]. The observations that the reductions in the transfer RNA peak are constant over a 10× range of

TABLE I

PEAK HEIGHTS OF VARIOUS TRANSFER RNA MIXTURES

Sample	*Peak height (mm)*	*% of control*
tRNA + water	125.7	120.0
tRNA + buffer	126.0	120.0
tRNA + buffer + glycerol**	104.7	100.0
tRNA + buffer + glycerol + polymerase	96.7	92.3
tRNA + buffer + BSA	128.0	122.0
tRNA + buffer + maize polymerase	128.3	122.0

* Buffer = 50 m*M* Tris, pH 8.0 (see text).

** Control.

glycerol concentrations, and that the retention time for transfer RNA is unchanged, indicate that the glycerol effect does not result primarily from changes in viscosity. The control experiments do indicate that the glycerol effect is observed in the absence of RNA polymerase and must, therefore, involve either transfer RNA or the column matrix. Although it is possible for glycerol to stabilize single-stranded nucleic acids, transfer RNA contains a high degree of secondary structure and its conformation is relatively stable. Glycerol in these concentrations does not affect the absorptivity of transfer RNA in polymerase buffer. However, it is interesting to note the glycophase matrices are coated with glyceropropylsilyl moieties in order to reduce non-specific absorption[4,5], and I speculate that glycerol interacts with the glycophase matrix. On this basis, I would expect other polyalcohols to produce similar effects.

As the association between transfer RNA and RNA polymerase reported here appears quantitatively reproducible within reasonable estimates of error, a molar binding ratio can be calculated. Although the RNA polymerase bound to transfer RNA cannot be directly measured by this monitoring technique, a minimal binding ratio can be estimated by assuming total saturation of polymerase by transfer RNA. Therefore, 7% reduction in the transfer RNA elution peaks represents a molar ratio of 6 transfer RNA molecules per polymerase molecule, assuming molecular weights of 25,000 and 390,000, respectively. On the basis of filter-binding assays, Pongs and Ulbrich[6] report a 1:1 complex between *E. coli* RNA polymerase and *E. coli* fMet-$tRNA_f^{Met}$ and a 5:1 molar ratio of *B. stearothermophilus* rMet-$tRNA_f^{Met}$ and *E. coli* polymerase. As these studies were conducted under much different conditions (3 m*M* $MgCl_2$), and with formylated, aminoacylated tRNA species, a direct analogy to this report is unwarranted. RNA polymerase may be expected to bind to nucleic acids, particularly to template DNA or to RNA products, but it is surprising to encounter such high binding ratios in systems where RNA polymerase is actively transcribing.

Nucleic acids are not generally amenable to HPLC; the macromolecules tend to clog columns and are denatured by sheer forces. However, accommodation of small samples and precise detection of each molecular species involved in a reaction are obvious advantages of HPLC for studies of polynucleotide-binding reactions, compared to conventional methods such as ultracentrifugation, electron microscopy, or filter retention[7,8]. HPLC may therefore be applicable to other analyses of specific polynucleotide interactions, such as template recognition, competitive inhibition, or hybridizations.

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