CONTINUOUS MONITORING OF TRANSCRIPTION BY LIGHT SCATTERING

Hermann HEUMANN and Wolfram ZILLIG
Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, FRG

Received 22 May 1978

1. Introduction

In vitro transcription is routinely followed by measuring the incorporation of labelled nucleotides into acid insoluble polynucleotides [1,2]. This method (trichloroacetic acid (TCA)-precipitation assay), though successfully used in assembling a large body of information on the transcription process, has some disadvantages:

(i) The rate of RNA synthesis cannot be measured continuously by such a sampling procedure. For monitoring quick changes of transcription rates a continuous method is required.

(ii) The determination of synthesized RNA by the sampling procedure is a multistep process. Experimental errors can occur in every step.

(iii) The sampling procedure requires relatively large amounts of valuable materials, such as labelled nucleosidetriphosphates, RNA polymerase and DNA, especially for kinetics. The samples are consumed by the sampling. In contrast, a continuous method based on the observation of one and the same sample could save materials.

In this paper a convenient optical method overcoming the above disadvantages is presented. The light scattering of nascent RNA is used to continuously monitor RNA synthesis. It can be measured by any fluorescence spectrophotometer or light scattering instrument with the detector 90° to the incident beam.

2. Materials and methods

2.1. *Preparation of RNA polymerase*

RNA polymerase was prepared as in [3]. Concentration was determined as in [4].

2.2. *Preparation of T7 DNA*

T7 DNA was prepared as in [5]. DNA concentration was determined as in [6].

2.3. *Transcription experiments*

The following conditions were used for all transcription experiments:

1. T7 DNA and RNA polymerase were preincubated for 10 min at 37°C in 0.05 M Tris/HCl buffer, pH 7.5, with 0.05 M KCl and 0.007 M MgCl (low ionic strength) or 0.13 M KCl and 0.03 M MgCl (high ionic strength).
2. The reaction was started by addition of prewarmed nucleosidetriphosphates in the same buffer used for the preincubation.
3. The final concentrations of nucleosidetriphosphates were 10^{-3} M.
4. The final concentration of T7 DNA was 0.2 mg/ml for the light scattering measurements and the incorporation test and 0.8 mg/ml for the RNA sizing test on agarose gel.

2.4. *The light scattering test*

To avoid artifactural scattering by dust and bubbles, the solutions were centrifuged for 5 min at 50 000 × *g* and degassed with a water aspirator. The light scattering measurements were performed at 350 nm wavelength and an angle of 90°. A fluorescence quartz cell

(5 × 5 × 25 mm, min vol. 0.5 ml) placed in a thermostatted cuvette holder of a fluorescence spectrophotometer was used. A simple water-jacketed stopped flow apparatus [7] was used for quick mixing of the preincubation mixture with the nucleosidetriphosphates.

2.5. *Agarose-gel electrophoresis*

A slab-gel electrophoresis apparatus was used according to [8]. The conditions for the preparation of the gel are described in [9]. Samples, 50 μl, were taken from the trancription mixture at time intervals given in fig.3 and fig.4. The transcription process was stopped by rapidly cooling the assay to 0°C. DNA was digested for 60 min with DNase (RNase-free, electrophoretically purified, Worthington). The samples were dialysed against 0.01% SDS and 0.05 M EDTA for 1 h and then electrophoresed for 5 h at 50 V.

3. Results

To establish the measurement of the light scattering intensity of nascent RNA for monitoring transcription rates, this method was compared with conventional transcription tests:

(i) The TCA-precipitation test.

Kinetics of RNA synthesis from T7 DNA at low and high ionic strengths at different enzyme/DNA molar ratios are shown in fig.1,2. The background scattering of RNA polymerase and DNA was subtracted from the measured light scattering intensities. The

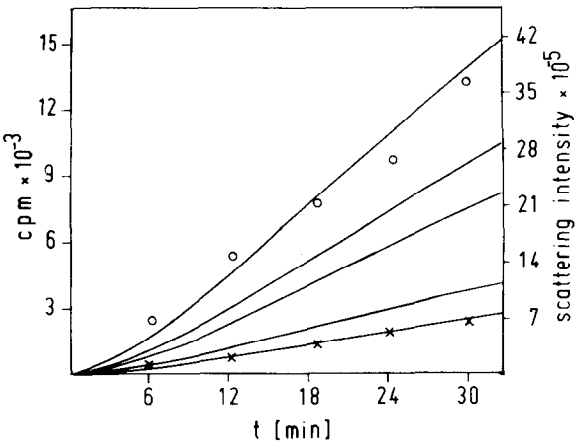

Fig.2. Transcription kinetics at high ionic strength (reinitiation conditions) measured by light scattering (——) at intervals of 3 s at enzyme/DNA molar ratios of 1, 1.8, 2.5, 3.2 and 5 (from bottom to top) and by the TCA-precipitation test using an ATP with ^{14}C spec. act. 0.1 Ci/mol at an enzyme/DNA molar ratio of 1 (×) and 5 (○).

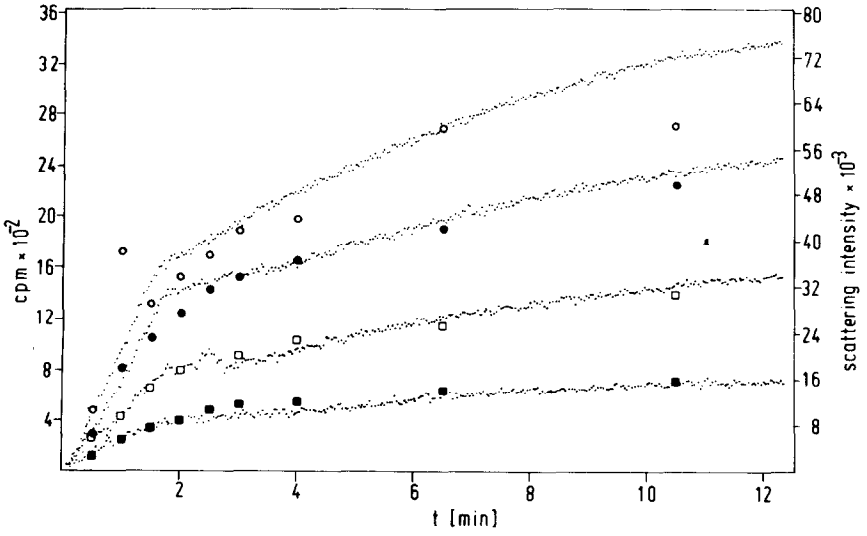

Fig.1. Transcription kinetics at low ionic strength measured by light scattering and by determination of the incorporation of radioactively-labelled ATP with ^{14}C spec. act. 1 Ci/mol. The enzyme/DNA molar ratio was: 1 (■), 2 (□), 3 (●), 4 (○). The corresponding scattering intensities were determined at 3 s intervals at an angle, $\delta = 90°$ and a wavelength, $\lambda = 350$ nm.

scale of the light scattering intensity is normalized so that the kinetics measured by the TCA-precipitation test at low ionic strength at an enzyme/DNA molar ratio of 2 optimally fits the kinetics measured by light scattering under the same conditions.

(ii) The time-dependent analysis of RNA size by agarose-gel electrophoresis.

The size of the early T7 mRNA synthesized at low ionic strength has been analysed for its time dependence on an agarose gel (fig.3). The low salt kinetics measured by light scattering shows a rapid increase followed by a slower second phase (fig.1). The break point between these phases is at 110 s. This value corresponds to the time required for synthesizing the early T7 mRNA with mol. wt 2.2×10^6 [10] (fig.3). Thus the averaged growth rate is 65 nucleotides/s/chain. A part of the RNA products of the slow second phase has a molecular weight larger than 2.2×10^6 and must have been synthesized by RNA polymerase molecules reading through the '20%-terminator' [11]. Another part of the products has mol. wt $< 2.2 \times 10^6$ and must have been synthesized by reinitiating or slowly starting polymerase molecules.

At high ionic strength under reinitiation conditions the kinetic values measured by both the light scattering and the TCA-precipitation test are the same within experimental error (fig.2). The kinetics for both tests are linear after an initial lag phase. The amount of the 2.2×10^6 dalton RNA pieces, estimated after electrophoretic separation, shows the same time course (fig.4).

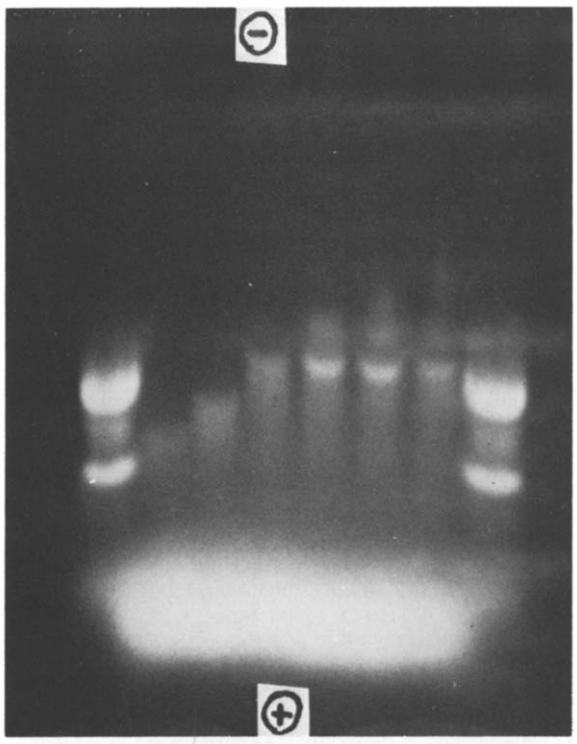

Fig.3. Analysis of the size of T7 mRNA synthesized at low ionic strength at an enzyme/DNA molar ratio of 3 on 1% agarose gel. Tracks 2–7 show samples which were taken after incubation times of: 0.5 min, 1.0 min, 1.5 min, 2.0 min, 4.0 min and 10 min. Tracks 1 and 8 show rRNA from rat liver as a reference. The molecular weights are 1.8×10^6 (28 S), 6.9×10^5 (18 S) and 4×10^4 (5 S) [13]. The RNA was stained by ethidium-bromide. The low molecular weight particles are DNA-pieces which were not completely digested.

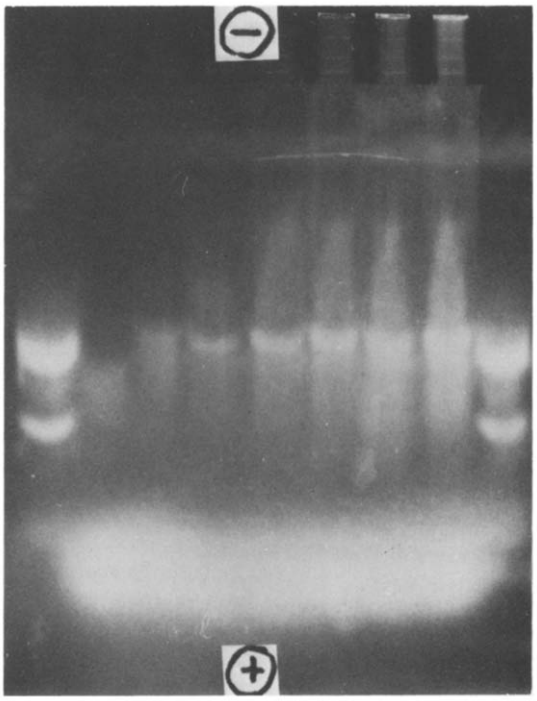

Fig.4. Analysis of the size of T7 mRNA synthesized at high ionic strength under the same conditions used for fig.3. The samples in tracks 2–8 were taken after 1.0 min, 2.0 min, 4.0 min, 8 min, 20 min, 30 min, 40 min incubation period. In tracks 1 and 9 are shown rRNA from rat liver.

4. Discussion

The above results show that light scattering can be used for continuously monitoring transcription. The two established transcription tests, the TCA-precipitation test and the gel electrophoretic analysis of the size of the RNA gave the same results within experimental error as the light scattering test. Beyond 30 min (not shown) the high salt kinetics measured by light scattering remain linear and possess higher values than the kinetics measured by the TCA-precipitation test. This is probably due to an increasing amount of RNA molecules with mol. wt $> 2.2 \times 10^6$, synthesized by RNA polymerase molecules reading through the '20%-terminator'.

For a more exact understanding of the scattering data a theoretical scattering function for the RNA synthesis has been developed (in preparation). The complete theoretical evaluation of the scattering data is very complex.

In contrast to the precipitation test, where only the nucleosidetriphosphates incorporated into the RNA contribute to the determined radioactivity, the light scattering intensity consists of the scattering of all components of the incubation mixture, namely the DNA, the RNA polymerase, the RNA 'in statu nascendi' and the RNA after release. The nucleosidetriphosphates do not contribute to the scattering intensity significantly. The main contribution to the time dependent scattering intensity is given by the RNA 'in statu nascendi' and the RNA after release. The parameters which determine the time-dependent scattering intensity are the concentration, the molecular weight and the form factor [12] of the RNA. In addition, there are scattering intensities due to interferences between the nascent RNA and the RNA polymerase, and between nascent RNA and DNA in the elongation complex.

The following problems complicate the evaluation of the scattering function for RNA synthesis:

1. The form of the RNA during elongation is unknown.

2. The two interference terms are difficult to determine.

3. The contribution of the nascent RNA to the scattering intensity is not directly proportional to the molecular weight and the form factor of the RNA. Therefore, the degree of synchrony of transcription must be taken into account for the interpretation of the experimental curves.

In spite of these difficulties, light scattering appears to be an appropriate empirical method for monitoring transcription in vitro continuously and for the determination of growth rates of RNA. Moreover, light scattering is suitable for studying the binding of RNA polymerase to DNA and the ionic strength dependent monomer–dimer equilibrium of holoenzyme [9]. Light scattering measurements might be valuable for studying the termination process, provided the interference of the nascent RNA with both the DNA and the RNA polymerase during elongation can be determined.

References

[1] Weiss, S. B. and Gladstone, L. (1959) J. Am. Chem. Soc. 81, 4118–4126.
[2] Hurwitz, I., Bresler, A. and Kaye, A. (1959) Biochem. Biophys. Res. Commun. 1, 3–12.
[3] Zillig, W., Zechel, K. and Halbwachs, H. J. (1970) Hoppe Seyler's Z. Phys. Chem. 351, 221–227.
[4] Heil, A. and Zillig, W. (1970) FEBS Lett. 11, 165–170.
[5] Fuchs, E., Millette, R. L., Zillig, W. and Walter, G. (1967) Eur. J. Biochem. 3, 83–94.
[6] Dische, Z. (1930) Mikrochemie 8, 4–7.
[7] Quast, U., Engel, J., Heumann, H., Krause, G. and Steffen, E. (1974) Eur. J. Biochem. 13, 2512–2523.
[8] Studier, F. W. (1973) J. Mol. Biol. 79, 237–248.
[9] Heumann, H. (1978) Thesis, University of Munich.
[10] Millette, R. L., Trotter, C. D., Herrlich, P. and Schweiger, M. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 135–142.
[11] Schäfer, R. and Zillig, W. (1973) Eur. J. Biochem. 33, 215–226.
[12] Guinier, A. and Fournet, G. (1955) Small angle scattering of X-rays, John Wiley and Sons, London.
[13] Wellauer, P. and David, J. (1973) Cold Spring Harb. Symp. Quant. Biol. 38, 525–535.