# RIBONUCLEIC ACID TOLUIDINE BLUE COMPLEXES STUDIED BY GEL ELECTROPHORESIS AND SPECTROPHOTOMETRY

L.M.POPA* and L.BOSCH**
*Department of Biochemistry, University of Leiden, Leiden, The Netherlands*

Received 10 July 1969

## 1. Introduction

In a recent paper [1] one of us (L.M.P.) showed that TB can form a complex with rRNA (designated complex I) which has the same electrophoretic mobility as the uncomplexed RNA. Complexes of this type, formed from rRNA's of different sizes, can be followed directly during their electrophoretic separation in the gel. At the same time electrophoresis yields interesting information concerning the nature of the bonds that stabilize the dye/RNA complexes. The aim of this paper is to describe further possibilities for the study of dye nucleic acid complexes with the gel electrophoretic procedure and to focus the attention on a few particular aspects of the TB/RNA interaction***.

## 2. Methods

### 2.1. *Gel electrophoresis*

30 ml of gel consisting of 1.5% agarose and 1.25% polyacrylamide was prepared by heating 0.45 g of agarose in 24.3 ml of water at 90° for 20 min. The clear solution was cooled to 48° (solution A).

A solution B was prepared separately by heating 3 ml of 0.2 M tris-citrate buffer, pH 7.9, with 2.5 ml of 15% cyanogum (a mixture of 95 parts of acrylamide and 5 parts of N,N′-methylenebisacrylamide) and 0.015 ml of TEMED (N,N,N′,N′-tetramethylethylenediamine) at 48°. After mixing the solutions A and B, 0.15 ml of a freshly prepared 10% ammonium persulphate solution was added under vigorous stirring and the resulting solution was poured into a glass cell of 17 X 8.5 cm as described by Akroyd [2]. The plasticine and the top glass plate were removed after 1 hr of polymerization, taking care not to disturb gel adhesion to the base plate of the cell. Holes were cut in the gel in order to introduce the nucleic acids. During electrophoresis the gel was in a horizontal position.

### 2.2. *Complex formation of various* RNA *species with* TB *occurring in two ways*

#### 2.2.1. Total saturation of RNA's with TB after electrophoresis

The gel was stained with a 0.01% TB solution [3] for 60 min whereafter the free dye was removed by washing the gel with water overnight.

#### 2.2.2. Partial saturation of RNA's with TB before electrophoresis

RNA and the dye were mixed at the TB/N ratios as indicated in parentheses in figs. 1 and 2 and subsequently submitted to electrophoresis as previously described [1].

* Permanent address Biochemistry Laboratory of the Institute of Inframicrobiology of the SRR Academy, Bucharest, Romania.
** To whom requests for reprints should be addressed.
*** Abbreviations used: TB, toluidine blue, TB/N ratio, TB/nucleotide molar ratio; TYMV-RNA, turnip yellow mosaic virus RNA; rRNA, ribosomal RNA; tRNA, transfer RNA; $MS_2$-RNA, RNA from the phage $MS_2$.

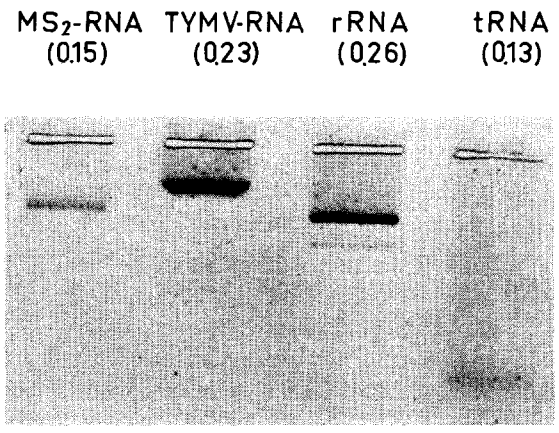


Fig. 1a. Gel electrophoresis of various species of RNA, complexed with TB. RNA was complexed with dye at the TB/N ratios indicated. The concentration of viral RNA and rRNA was 1 mg/ml, that of tRNA 2 mg/ml. Electrophoresis was performed in 0.2 M Tris-citrate buffer (pH 7.9) for 50 min at 150 V and 30 mA at room temperature.

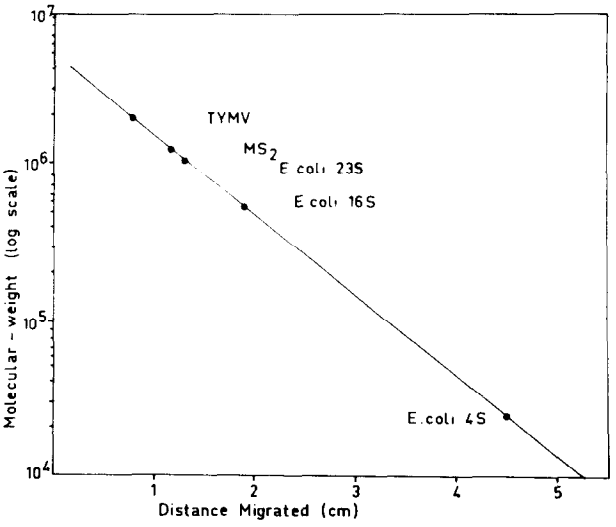


Fig. 1b. Relationship between the molecular weights and the electrophoretic mobilities of various RNA/TB complexes shown in fig. 1a.

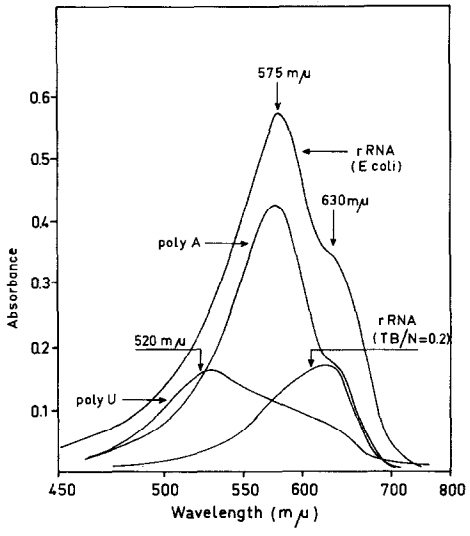


Fig. 2. Absorption spectra of partially or totally complexed RNA. All curves, except the one indicated: rRNA (TB/N= 0.2), were obtained by total saturation of the polynucleotides with dye after electrophoresis according to 2.2.1. The absorption spectra of the complexes in the dried gel were recorded according to 2.3.

### 2.3. *Recording of the absorption spectra of the complexes in the dried gel*

The gel was dried with cooled air, taking care that the formation of air bubbles in the gel was prevented.

The glass plate bearing the dried film was put in a DK2 Beckman spectrophotometer for direct and automatic recordance of the spectra. Similar films without RNA/dye complexes were used as blanks.

## 3. Results and discussion

The complexes formed between TB and various species of natural RNA could be followed during their electrophoretic migration by direct observation (fig. 1a; TB/N ratios $\leqslant 0.26$).

By contrast the synthetic polynucleotides poly U and poly A at comparable TB/N ratios formed complexes which appeared to be unstable under the conditions of the electrophoresis. As a result the free polynucleotides moved away from the origin but the

dye did not migrate. (Migration of the free polynucleotides was demonstrated by staining the latter with TB after electrophoresis according to 2.2.1.).

Complexes between the natural RNA's and the dye formed at the TB/N ratios mentioned above migrate during electrophoresis at the same rate as uncomplexed RNA's. An inverse linear relationship between the logarithms of the assumed molecular weights of various (uncomplexed) RNA's and their electrophoretic mobilities has been reported [4,5]. Such a relationship also exists in the case of the complexed RNA's (fig. 1b). Consequently RNA sizes can now be estimated even more rapidly than before (within 1 hr) as migration can be followed by direct observation.

Recording of the absorption spectra of partially or totally complexed RNA's in the dried gel (compare 2.3) yielded the results illustrated in fig. 2. The absorption spectrum of rRNA totally saturated with dye showed a maximum at 575 mμ with a shoulder at 630 mμ. If rRNA was only partially complexed with TB (TB/N = 0.2) absorption reached maximum values at 630 mμ and a shoulder was apparent at 575 mμ. Striking differences were found between the two synthetic homopolynucleotides when totally saturated with TB. Poly A displayed a maximum at 575 mμ and a shoulder at 630 mμ, whereas poly U had a maximum at 520 mμ which was absent in the case of poly A.

In an attempt to interpret the various spectral characteristics observed with the TB/polynucleotide complexes when recorded directly in the dried gels, we have titrated a number of RNA's (TYMV-RNA, rRNA, tRNA, poly A + poly U, poly A and poly U) with the dye in solution [6].

A representative experiment with TYMV-RNA is illustrated in fig. 3, in which the TB concentration was kept constant and that of the viral RNA varied.

The unbound dye, present as a dimer [1], had an absorption maximum at 600 mμ. At low TB/N ratios (0.01–0.04) this maximum was shifted to 630 mμ (red shift). The corresponding curves all passed through a common isosbestic point. At TB/N ratios of about 0.64 and 1.28 the main maximum was at 575 mμ (blue shift) while shoulders were apparent at 630 and 520 mμ. A second isosbestic point could be detected at about 560 mμ. These data lead us to the following interpretations.

Since the maximum at 630 mμ was also observed

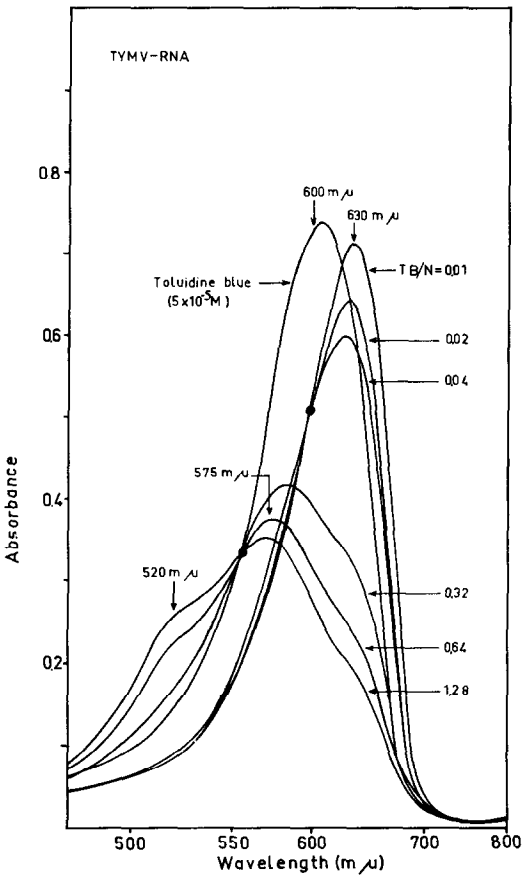


Fig. 3. Spectra of TB in the presence and absence of TYMV-RNA. TB was dissolved in 0.005 M Tris-HCl buffer pH 7, at a concentration of $5 \times 10^{-5}$ M. The formation of different RNA-TB complexes appeared to be better defined under these conditions than in the more diluted dye solution containing NaCl ions [1].

upon interaction of TB with double stranded polynucleotides (like DNA and a mixture of poly U and poly A) but not with a single stranded polynucleotide of low stacking ability (poly U), it was assumed that intercalation of the dye between Watson and Crick type base pairs was responsible for the absorption at 630 mμ. The fact that fully saturated poly A (fig. 2) displayed a shoulder at this wavelength suggested that intercalation may also occur to some extent between stacked bases of a single stranded polynucleotide. The absence of the shoulder at 630 mμ in the case of poly U is in accordance with the lower stack-

ing ability of this polynucleotide as compared with poly A [7,8]. According to common practice the RNA/dye complex resulting from dye intercalation was designated complex I [1,9]. It was generally formed at low TB/N ratios ($\leqslant 0.2$).

At higher ratios an additional binding process took place in which non-hydrogen bonded nucleotides are probably involved, yielding a so-called complex II with a maximum of absorption at 575 mμ. This type of interaction, presumably between TB and the negatively charged phosphate groups of TYMV-RNA (fig. 3), was also observed with totally charged poly A (fig. 2).

Evidently absorption at 520 mμ, visible as a maximum in the spectrum of poly U (fig. 2) and as a shoulder in that of TYMV-RNA (fig. 3) must be due to a different type of interaction. Possibly this absorption may also be ascribed to a binding of TB to the exterior of the RNA chain. In this case, however, unstacked nucleotides in the more amorphous part of the chain may be involved and interaction between adjacent dye ions may result in aggregation or stacking of TB on the surface of the polyanion. Evidence for TB binding to amorphous regions was derived from the following observations. The shoulder at 520 mμ, which was observed with a number of natural RNA's [6] could only be detected at high TB/N ratios and under particular conditions of ionic strength and dye concentration (compare legend of fig. 3). The conditions in the dried gel for instance (fig. 2) did not permit detection of the shoulder in the case of rRNA.

Titration of poly A under the favourable conditions of fig. 3 did not reveal any absorption at 520 mμ in agreement with the stacking of bases which is characteristic for this polynucleotide.

By contrast absorption at 520 mμ is most clearly demonstrated in the experiment with the polynucleotide of low stacking ability (poly U, fig. 2) in which even maximal absorption is reached at this wavelength. Highly ordered structures like DNA and poly A plus poly U do not show absorption at 520 mμ.

## 4. Conclusions

(1) Natural RNA's like $MS_2$-RNA, TYMV-RNA, tRNA and rRNA form stable complexes with TB. During gel electrophoresis these complexes migrate with the same rate as the uncomplexed RNA's.

(2) The electrophoretic migration rate permits an estimation of RNA sizes, which is more rapid than that of similar previously reported procedures.

(3) Poly U and poly A form complexes with the dye, which are unstable under the conditions of electrophoresis.

(4) At low dye concentrations natural RNA's form a so-called complex I, in which intercalation of dye between Watson and Crick type base pairs is assumed to occur. At higher dye/nucleotide ratios two types of interaction with the phosphate groups may take place; one probably involves mainly non-hydrogen bonded nucleotides in stacked conformations and the second unstacked nucleotides.

## Acknowledgement

One of us (L.M.P.) is indebted to the Netherlands Ministry of Education and Sciences for financial support.

## References

[1] L.M.Popa and R.Repanovici, Biochim. Biophys. Acta 182 (1969) 158.
[2] P.Akroyd, Anal. Biochem. 19 (1967) 399.
[3] W.McIndoe and H.N.Munro, Biochim. Biophys. Acta 134 (1967) 458.
[4] A.C.Peacock and C.W.Dingman, Biochemistry 72 (1968) 668.
[5] U.E.Loening, J. Mol. Biol. 38 (1968) 355.
[6] L.M.Popa and L.Bosch, in preparation (1969).
[7] W.Fuller and A.Hodgson, Nature 215 (1967) 817.
[8] I.Tinoco Jr., R.C.Davis and S.R.Jaskaunas, in: Molecular Associations in Biology, ed. B.Pullman (Academic Press, New York, 1968), p. 77.
[9] M.Semmel and M.Daune, Biochim. Biophys. Acta 145 (1967) 561.

## Note added in the proof

The agarose used was a Behringwerke A.G. product. The composite gel prepared by the procedure described in this paper is very useful for radioautographic determinations.

The toluidine blue was a product from Chroma Gesellschaft, Schmidt und Co. Stuttgart. This dye appears to have a high association ability.