Isolation and Characterization of the DNA-dependent RNA Polymerase of *Rhizobium leguminosarum* 300

By W. LOTZ,^{1*} H. FEES,¹ W. WOHLLEBEN² AND H. J. BURKARDT¹

¹ Institut für Mikrobiologie und Biochemie, Lehrstuhl für Mikrobiologie, Universität Erlangen-Nürnberg, D-8520 Erlangen, F.R.G.

² Lehrstuhl für Genetik, Biologie VI, Universität Bielefeld, D-4800 Bielefeld, F.R.G.

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The DNA-dependent RNA polymerase isolated from *Rhizobium leguminosarum* 300 contains the following subunits: β' , β , polypeptide A and α (molecular weights 149 000, 146 000, 93 000 and 42 000, respectively). Polypeptide A is postulated to be the σ factor of the enzyme. The experimental conditions used for the in vitro assay of the enzyme activity, with plasmid pMB9 as template, included: 40 mM-Tris/HCl (pH 7.9), 150 mM-NaCl, 5 mM-MgCl₂, at 34 °C. The isolated enzyme was shown by electron microscopy to bind specifically to the region of early promoters of DNA of the *Escherichia coli* bacteriophage T7. Therefore, *Rhizobium*-specific promoters may have base sequences in common with those of *E. coli*.

INTRODUCTION

The transcription of DNA by the DNA-dependent RNA polymerase from *Escherichia coli* and from other bacterial species has been extensively studied (see Burgess, 1976; Chamberlin, 1976). Within the family *Rhizobiaceae* only the RNA polymerase of *Agrobacterium tumefaciens* has been described (Knopf, 1974), and little is known about transcription in bacteria of the genus *Rhizobium*.

Rhizobia can nodulate Leguminosae and fix N₂ symbiotically as bacteroids (see Vincent, 1977). In the case of rhizobia nodulating *Lotus* species (Pankhurst, 1977) and of *Rhizobium leguminosarum* (Pain, 1979), a significant fraction of mutants resistant to rifampicin have lost the ability to fix N₂. Rifampicin is known to inhibit bacterial transcription (Benveniste & Davies, 1973). In *E. coli* (Rabussay & Zillig, 1969) and *Bacillus subtilis* (Linn *et al.*, 1975) mutations to rifampicin resistance affect the β subunit of the RNA polymerase. Genetic studies of rifampicin-resistant mutants of *B. subtilis* that are unable to sporulate have indicated that the RNA polymerase may have a regulatory function in spore differentiation (see Losick & Pero, 1976). It is therefore possible that the RNA polymerase of *Rhizobium* may play a significant role in the regulation of differentiation of N₂-fixing bacteroids.

In this paper, we report the isolation and characterization of the RNA polymerase from vegetative cells of the wild-type *R. leguminosarum* strain 300 (Johnston & Beringer, 1975) using a method adapted from that of Burgess & Jendrisak (1975). This procedure includes the precipitation of the RNA polymerase from cell extracts by Polymin P, as described by Zillig *et al.* (1970*b*), a DNA affinity chromatography step, and a sizing step by Bio-Gel A5m chromatography. The subunit pattern of the isolated enzyme is compared with that of the RNA polymerase of *E. coli* and of *A. tumefaciens*. In addition, the promoter-specific DNA-binding of the enzyme, and experimental conditions for in vitro transcription assays, are described.

METHODS

Bacterial strains and growth. The wild-type *Rhizobium leguminosarum* strain 300 (Johnston & Beringer, 1975) was provided by Dr J. E. Beringer (John Innes Institute, Norwich) and *Escherichia coli* K12 strain GM4 by Dr F. C. Cannon (ARC Unit of Nitrogen Fixation, Sussex). The latter strain carries plasmid pSA31 which originated from plasmid pBR322 (F. C. Cannon, personal communication) by the insertion of the *Klebsiella pneumoniae* genes *nifH* and *nifD* between the *EcoRI* and *BamHI* cuts of pBR322. *Escherichia coli* strain HB101(pMB9) was provided by Professor A. Pühler (Institut für Genetik, Universität Bielefeld, F.R.G.).

Rhizobium leguminosarum 300 was routinely grown at 29 °C in liquid TY medium (Beringer, 1974) or B + Y medium (Van Brussel *et al.*, 1977). The organism was stored at -20 °C after mixing 1 ml of overnight culture (in TY medium) with 1 ml 90% (v/v) glycerol, or on TY agar plates which were transferred weekly. Nodulation tests with garden peas (Vincent, 1970) and acetylene reduction assays (using a Hewlett-Packard gas chromatograph) were done about every 2 months.

For the isolation of the RNA polymerase, bacteria were grown under aeration in a 70 litre fermenter (Chemap, Zürich, Switzerland) in B + Y medium. The cells were harvested in the late-exponential phase of growth with a Padberg flow-through centrifuge, then frozen in liquid nitrogen and stored at -25 °C. The purity of all fermenter cultures was routinely checked on nutrient agar plates [containing 0.8% (w/v) Difco nutrient broth and 1.5% (w/v) Difco agar] and by sensitivity to *R. leguminosarum* bacteriophage RL-4 (obtained from Dr J. E. Beringer).

Buffers. TGED buffer, grinding buffer and storage buffer were described by Burgess & Jendrisak (1975). TGED/PMSF buffer contained phenylmethanesulphonyl fluoride (PMSF; from Serva) at 23 µg ml⁻¹. To enhance lysis of *R. leguminosarum* 300 the modified grinding buffer contained: 0.05 M-Tris/HCl (pH 7.9); 0.05 M-EDTA (pH 7.9); 0.1 mM-dithiothreitol; 1.0 mM-mercaptoethanol; 0.233 M-NaCl; 5% (v/v) glycerol; 46 µg PMSF ml⁻¹; 1 mg lysozyme ml⁻¹. The incubation buffer for DNA/RNA polymerase binding assays was that of Portmann *et al.* (1974).

Plasmid and phage DNA. Plasmids pMB9 (Rodriguez *et al.*, 1976) and pSA31 were isolated from *E. coli* HB101(pMB9) and *E. coli* GM4(pSA31), respectively, by the method of Guerry *et al.* (1973). At least 80% of the plasmids in these preparations were covalently closed circles as shown on 1% (w/v) agarose gels. The DNA of *E. coli* bacteriophage T7 was a gift from Dr R. Lurz (Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, F.R.G.).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This was carried out as described by Laemmli (1970) on a BioRad model 221 apparatus for plate gels. Staining and destaining of the gels was as described by Burgess & Jendrisak (1975). The molecular weights of the RNA polymerase subunits of *R. leguminosarum* 300 were determined (Dunker & Rueckert, 1969; Weber & Osborn, 1969) by comparison with the SDS-PAGE molecular weight standards from BioRad (range 14 300 to 200 000). In addition, purified RNA polymerase from *E. coli* was run in the same gel; the molecular weights of its subunits were taken as $\beta' = 150\,000$, $\beta = 145\,000$ (Berg *et al.*, 1971), $\sigma = 85\,000$ and $\alpha = 40\,000$ (Zillig *et al.*, 1970 *a*).

DNA-Sepharose 2B column. Sepharose 2B-300 (Sigma) was activated and coupled to DNA as described by Arndt-Jovin *et al.* (1975). A column of 90 ml bed volume, containing about 90 mg coupled calf thymus DNA (Serva), was used.

Isolation of RNA polymerase from *R. leguminosarum* 300. The isolation procedure was adapted from that of Burgess & Jendrisak (1975). All six steps of the following procedure were carried out in the cold room (4 to 6 °C) unless otherwise stated.

Step 1: Cell disruption and enzyme extraction. Cells grown in B + Y medium were used for the isolation of RNA polymerase, since these cells were more sensitive to lysozyme than those grown in TY medium. Frozen cells (100 g) were partially thawed, then placed in a 1 litre Waring blender with 300 ml ice-cold grinding buffer and blended for about 5 min at low speed; the resulting suspension was left at 2 to 5 °C for 2 h. Then 7 ml 4% (w/v) sodium deoxycholate (Merck) was added, the mixture was blended at low speed and left for 40 min at room temperature with occasional stirring. During this period the temperature rose to about 7 °C and, due to cell lysis, the viscosity of the mixture increased. The lysate was placed on ice for 10 min, and then blended at high speed for 30 s. After addition of 400 ml TGED/PMSF buffer containing 0.2 M-NaCl the lysate was blended at high speed for 30 s and then centrifuged (6200 g, 45 min) in 250 ml polycarbonate bottles in a Sorvall RC5-B centrifuge. The resulting supernatant (735 ml) was kept on ice; the pellet (about 46 g) was discarded.

Step 2: Polymyxin P precipitation. Polymyxin P precipitation was performed essentially as described by Burgess & Jendrisak (1975) for the isolation of *E. coli* RNA polymerase. Polymyxin P was a gift of BASF, Ludwigshafen, F.R.G. A 26.7 ml volume of 10% (v/v) ice-cold Polymyxin P (pH 7.9) was added slowly with stirring to the 735 ml supernatant from step 1. Slow stirring on ice was continued for 5 min. The precipitate was then sedimented by centrifugation (3500 g, 15 min). Analysis by SDS-PAGE (not shown) indicated that there was a preferential enrichment of RNA polymerase protein in the precipitate, as judged from the appearance of bands in the chromatogram which probably corresponded to the $\beta'\beta$ subunits of the RNA polymerase (see below).

Step 3: 0.5 M-NaCl wash and 1.0 M-NaCl elution of the Polymyxin P precipitate. The pellet from step 2 was blended for 10 min in 400 ml TGED/PMSF buffer containing 0.5 M-NaCl and the centrifuged (15 min, 3500 g). The resulting pellet was blended at low speed in 400 ml TGED buffer containing 1.0 M-NaCl. This procedure led to the extraction of most of the RNA polymerase. The eluate was freed from the precipitate by centrifugation (3500 g, 30 min).

Step 4: Ammonium sulphate precipitation. $(\text{NH}_4)_2\text{SO}_4$ (133 g) was added in small portions to the 380 ml eluate from step 3 to give 50% saturation, 1 M-NaOH ($5 \mu\text{l g}^{-1}$) being added to maintain constant pH (Burgess, 1969a). The solution was left standing on ice for at least 2 h. The resulting precipitate was sedimented by centrifugation (8800 g, 45 min) and dissolved in 50 ml TGED/PMSF buffer. Then 50 ml TGED/PMSF buffer containing 0.15 M-NaCl was added and the solution was dialysed (2×30 min) against 1 litre TGED/PMSF buffer containing 0.15 M-NaCl. The conductivity of the solution then corresponded to that of the buffer.

Step 5: DNA-Sepharose affinity chromatography. The dialysed solution from step 4 was loaded on to a DNA-Sepharose 2B column, freshly equilibrated with TGED buffer containing 0.15 M-NaCl, and washed with 500 ml of the same buffer. When the A_{280} of collected fractions had reached a value below 0.01, a linear salt gradient from 0.15 to 1.0 M-NaCl (total volume 300 ml) was applied. During loading, washing and elution of the column, 3 ml fractions were collected at a flow rate of 0.4 ml min^{-1} . Both the A_{280} and the RNA polymerase activity of the fractions were measured. Fractions with maximum activity were pooled and concentrated by ultrafiltration (Amicon concentrator, Diaflo UM10 filter) to a total volume of 1 ml.

Step 6: High salt Bio-Gel A5m chromatography. The 1 ml solution from step 5 was loaded on to a Bio-Gel A5m column (2×55 cm; mesh 200 to 400, preswollen, from Bio-Rad). The column was run with TGED buffer containing 0.5 M-NaCl and fractions of 2 ml were collected at a flow rate of 0.14 ml min^{-1} . The A_{280} and the RNA polymerase activity of the fractions were determined. The fractions with maximum RNA polymerase activity were pooled, dialysed against storage buffer and stored at -20°C .

RNA polymerase assay. The general RNA polymerase assay conditions were adapted from those of Burgess (1969a). The standard *in vitro* transcription assay contained (in a total volume of 100 μl): 40 mM-Tris/HCl (pH 7.9); 5 mM-MgCl₂; 0.4 mM-potassium phosphate (pH 7.5); 150 mM-NaCl; 0.1 mM-EDTA; 0.1 mM-dithiothreitol; 0.1 mM each of GTP, CTP, ATP, and UTP; 3 μCi [³H]UTP [45 Ci mmol^{-1} ($1.665 \text{ TBq mmol}^{-1}$); Amersham]; 3 μg pMB9 DNA or 2 μg pSA31 DNA; 10 μg enzyme. The potassium phosphate was routinely added to inhibit any polynucleotide phosphorylase activity possibly present during some steps of enzyme purification (Burgess, 1969a). Glycerol, which is known to stabilize the RNA polymerase (Burgess, 1976); was not added to the assays because the purified enzyme was stored in buffer containing 50% (v/v) glycerol (giving a final glycerol concentration in the assay of 5%). For the determination of the activity of the isolated RNA polymerase the unlabelled UTP was replaced by 0.1 mM-[³H]UTP [1 Ci mmol^{-1} (37 GBq mmol^{-1})]⁻¹. One unit of RNA polymerase activity is defined as the amount of enzyme required to catalyse the incorporation of 1 nmol UMP into trichloroacetic acid-precipitable material in 10 min.

The *in vitro* assays (done in duplicate or triplicate) were routinely incubated at 34°C for at least 20 min, then chilled on ice and precipitated with 2 ml ice-cold 5% (w/v) trichloroacetic acid. After about 10 min the precipitates were collected on nitrocellulose filters (0.45 μm ; Sartorius), and washed five times with about 2.5 ml and then once with about 5 ml ice-cold 5% trichloroacetic acid. The filters were dried under an infrared lamp and counted in a scintillation counter (Beckman LS-230) in 5 ml Scintimix (Koch-Light). In controls either the DNA template or the RNA polymerase was omitted.

RNA polymerase-DNA binding studies. The general methods for the binding of RNA polymerase to DNA were those of Portmann *et al.* (1974) and Koller *et al.* (1978). About 25 ng T7 DNA was mixed with 100 μl incubation buffer, then 1 μl RNA polymerase solution (2.7 mg ml^{-1}) was added and the sample was incubated at 37°C for 10 min. Glutaraldehyde fixation (10 min at 37°C) was done by adding 20 μl 0.5% (v/v) glutaraldehyde freshly prepared in 20-fold diluted incubation buffer. The fixed sample was spotted on to the surface of a clean plastic Petri dish and the droplet was touched with a piece of freshly cleaved mica. The mica was washed twice by touching droplets of double-distilled water, and the sample was stained by floating for 30 s on 1% (w/v) uranyl acetate. After two additional washings, the sample was dehydrated stepwise in 50% and 90% (v/v) ethanol. The air-dried mica was rotary shadowed with platinum/iridium (80:20) at an angle of 5.5° and covered with a carbon layer by carbon casting using carbon rods pretreated with 0.5 M-NaCl. The carbon film was floated on to a water surface and parts were collected with copper grids.

Electron micrographs were taken with a Philips EM400 electron microscope at a magnification of 3500. Determinations of the sites of RNA polymerase binding and measurements of T7 DNA lengths were made with a Brühl LM1 digitizer at a final magnification of 50800. The computerized evaluation method of Burkhardt *et al.* (1980) was used with the following modifications: the resolution power of the program was 0.5% of the total DNA length, and the RNA polymerase molecules on the DNA were enlarged mathematically to a size of 1% total DNA length. A total of 60 molecules of T7 DNA with bound *R. leguminosarum* 300 RNA polymerase were analysed by this method.

Fig. 1

Fig. 2

Fig. 1. Isolation of RNA polymerase from *R. leguminosarum* 300 by DNA-Sepharese 2B chromatography: ○, absorbance at 280 nm; ●, RNA polymerase activity; —, NaCl concentration.

Fig. 2. Purification of RNA polymerase from *R. leguminosarum* 300 by Bio-Gel A5m chromatography: ○, absorbance at 280 nm; ●, RNA polymerase activity.

RESULTS AND DISCUSSION

Isolation of the RNA polymerase from R. leguminosarum 300

The maximum RNA polymerase activity was found in fractions 30 to 55 of the eluate from the DNA-Sepharese 2B column (Fig. 1). Fractions 35 to 54 were pooled, concentrated to 1 ml by ultrafiltration, and then loaded on to a Bio-Gel A5m column. The A_{280} profile of fractions from this column showed three prominent peaks (Fig. 2). Only fractions 43 to 49, corresponding to the middle peak, showed significant RNA polymerase activity.

The polypeptides present in this peak were analysed by SDS-PAGE on 10% gels (Fig. 3). Fractions 44 to 51 showed bands in the molecular weight range of the β' and β subunits of the RNA polymerase from *E. coli*, which was run in the same gel. Fractions 44 to 55 contained a polypeptide with a molecular weight somewhat higher than that of the α subunit of the *E. coli* RNA polymerase. Two other major bands were visible in the gel – A (present in fractions 44 to 48) and B (in fractions 49 to 55). The molecular weight of the A polypeptide was somewhat higher, and that of the B polypeptide somewhat lower, than the σ factor of the *E. coli* RNA polymerase. The three fractions with maximum RNA polymerase activity (45, 46 and 47) were pooled, dialysed against storage buffer and then stored at -20°C .

From the subunit pattern obtained with SDS-PAGE (Figs 3 and 4) the RNA polymerase was judged to be about 95% pure. The yield of the enzyme was relatively low [1 mg (100 g cells) $^{-1}$; activity 310 units mg $^{-1}$]. Using the same isolation procedure, we obtained an 18-fold higher yield of RNA polymerase from *E. coli* (not shown). *Rhizobium leguminosarum* 300 is much more refractory to lysis than *E. coli*, regardless of the method applied (e.g. cell grinding with aluminium oxide, treatment with ultrasound, or washing with TGED buffer containing 0.2 M-EDTA before the lysozyme/deoxycholate treatment). We conclude that the main reason for the relatively low yield of RNA polymerase from *R. leguminosarum* 300 was due to the difficulty in obtaining optimal cell lysis and enzyme extraction.

Subunits of the RNA polymerase

The isolated RNA polymerase from *R. leguminosarum* 300 was characterized by SDS-PAGE on 10% gels using the RNA polymerase from *E. coli* as a reference (Fig. 4). The polypeptide corresponding to band B in Fig. 3 was removed by the Bio-Gel A5m

Fig. 3. SDS-polyacrylamide gel after electrophoresis of fractions from the Bio-Gel A5m column of Fig. 2. Samples of 10 μ l from fractions 42 to 55, which contained the maximum of RNA polymerase activity, were applied to the gel.

chromatography and was probably a contaminating polypeptide. The molecular weights of the two largest subunits of the RNA polymerase were determined as 149 000 and 146 000. These two polypeptides correspond to the β' and β subunits of the *E. coli* RNA polymerase (molecular weights 150 000 and 145 000, respectively; Berg *et al.*, 1971). Separation of the two large subunits of the rhizobial enzyme could only be demonstrated when relatively low concentrations of RNA polymerase were applied to a gel; at higher enzyme concentrations both subunits tended to run as one band (compare Fig. 3, fractions 44 and 45; and Fig. 4, lanes *b* and *c*), probably because of the relatively small difference in molecular weight.

It remains to be determined which of the two large subunits of the isolated enzyme corresponds to ' β '. By definition, the subunit responsible for the rifampicin sensitivity of the RNA polymerase is named ' β ' (Burgess, 1976). Preliminary *in vitro* experiments have shown that the RNA polymerase from *R. leguminosarum* 300 is rifampicin-sensitive, in agreement with the *in vivo* rifampicin sensitivity of *R. leguminosarum* (Johnston & Beringer, 1975).

The band in Fig. 4 corresponding to the α subunit of the rhizobial RNA polymerase has a higher molecular weight (42 000) than that of the α subunit of the *E. coli* RNA polymerase [determined as 39 000 by Burgess (1969*b*) and 40 000 by Zillig *et al.* (1970*a*)].

The molecular weight of polypeptide A of the *R. leguminosarum* RNA polymerase was 93 000 (based on a molecular weight of 85 000 for the σ factor of the *E. coli* enzyme; Zillig *et al.*, 1970*a*). We postulate that polypeptide A, which was present in the enzymically active fractions (44 to 48) of the Bio-Gel A5m column, is the σ factor of the *R. leguminosarum* 300 RNA polymerase. Its molecular weight is similar to that of the postulated σ factor of the RNA polymerase from *A. tumefaciens* (χ_1 = 98 000; Knopf, 1974).

The ratio of holoenzyme to core enzyme without σ was not determined. In comparison to the bands corresponding to the β' and β subunits of the *R. leguminosarum* RNA polymerase, band A was relatively weak and therefore the enzyme preparation must also contain a

Fig. 4. SDS-polyacrylamide gel after electrophoresis of RNA polymerase from *E. coli* (a, 5 μ g) and from *R. leguminosarum* 300 (b, 1 μ g; c, 2 μ g) after purification by Bio-Gel A5m chromatography.

significant fraction of core enzyme. The σ factor may have been partly lost from the polymerase during the isolation and purification. In addition, it has been shown that only 0.3 to 0.4 mol of σ are present in vivo in *E. coli* per mol of core polymerase (Burgess, 1976). The situation may be similar in the case of *R. leguminosarum* 300.

RNA polymerase preparations from *E. coli* have often been found to contain a small polypeptide, named 'omega', of unknown function (molecular weight 9000 to 12000; Burgess, 1976). Such a polypeptide was not detected in fractions 41 to 55 of the Bio-Gel A5m column by SDS-PAGE on 15% gels, even though the ω polypeptide of the *E. coli* RNA polymerase was demonstrated on the same gel (not shown). An ω polypeptide was also absent from the RNA polymerase of *A. tumefaciens* (Knopf, 1974).

Properties of the purified enzyme

RNA polymerase activity, assayed with pMB9 DNA as template, was dependent on the presence of divalent cations, maximum activity occurring at 2.5 to 5.0 mM MgCl_2 . Therefore, all further in vitro assays with the RNA polymerase from *R. leguminosarum* 300 were performed in the presence of 5 mM- MgCl_2 .

The temperature optimum was between 30 and 35 $^{\circ}\text{C}$. There was a linear increase in activity between 20 and 30 $^{\circ}\text{C}$ by a factor of 2.6. All further in vitro assays with this RNA polymerase were done at 34 $^{\circ}\text{C}$. Rhizobia are soil bacteria and generally grow best between 25 and 30 $^{\circ}\text{C}$ (Vincent, 1970). This is also the temperature range for optimal growth of the related genus of *Agrobacterium* (Beiderbeck, 1977). The relatively high temperature (41 $^{\circ}\text{C}$;

Fig. 5. Electron micrograph of a complex between the RNA polymerase holoenzyme from *R. leguminosarum* 300 and T7 DNA. Three molecules of RNA polymerase (arrowed) are bound close to one end of the DNA filament. The bar marker represents 0.5 μ m.

Knopf, 1974) for maximal in vitro activity of the RNA polymerase from *A. tumefaciens* was therefore unexpected. We assume that the difference in apparent temperature optima for the RNA polymerases from *R. leguminosarum* 300 and *A. tumefaciens* are due to differences in the in vitro assay conditions (e.g. salt concentrations and types of templates used).

The time course of in vitro RNA synthesis catalysed by the RNA polymerase from *R. leguminosarum* 300 was studied over a period of 30 min using pSA31 as a DNA template. The incorporation of [3 H]UMP into trichloroacetic acid-precipitable material was linear with time for at least 25 min. A linear increase in radioactivity in trichloroacetic acid-precipitable material was also obtained with increasing RNA polymerase concentration (up to 15 μ g per 100 μ l assay), indicating that the enzyme preparation was free of major inhibitors of RNA synthesis.

Promoter-specific DNA binding of the RNA polymerase

Only the holoenzyme, not the σ -free core enzyme, of the *E. coli* RNA polymerase can bind selectively to promoter sites of a DNA template (for review, see Chamberlin, 1976). If polypeptide A corresponded to the σ factor of the *R. leguminosarum* 300 RNA polymerase, we expected the enzyme to recognize promoter sequences. Since *Rhizobium*-specific promoters have not been described, we used purified DNA of *E. coli* phage T7, which has four RNA polymerase binding sites in the early promoter region at its left-hand end (Portmann *et al.*, 1974; Koller *et al.*, 1978).

The RNA polymerase of *R. leguminosarum* 300 bound to this region of the T7 genome (Fig. 5). The frequency of RNA polymerase molecules attached at given positions along the T7 DNA was determined quantitatively by computer analysis. The profile (Fig. 6) shows that the major peak of the RNA polymerase binding frequency is within the left 2% of the T7 DNA molecule. A similar distribution was obtained with *E. coli* RNA polymerase (not shown). The peak of RNA polymerase binding (Fig. 6) was not resolved further; in the case of the *E. coli* enzyme, four separate sub-peaks can be demonstrated if special experimental conditions are chosen (Koller *et al.*, 1978).

The small peak of RNA polymerase binding at the right-hand side of the T7 genome (Fig. 6) is located at about 92% of the total molecule length. It may correspond to the peak VII of binding sites for *E. coli* RNA polymerase (Koller *et al.*, 1978), determined at 92.5% (Delius *et al.*, 1973). We conclude that the RNA polymerase from *R. leguminosarum* 300 can bind selectively to *E. coli*-specific promoter sites.

Fig. 6. Histogram of *R. leguminosarum* 300 RNA polymerase molecules bound to T7 DNA. The left-hand side of the histogram corresponds to the left side of the genetic map of bacteriophage T7. A total of 60 T7 molecules with bound RNA polymerase were analysed.

Wiggs *et al.* (1979) have shown that the early promoters of bacteriophage T7 can be utilized for the initiation of transcription by RNA polymerases of a great variety of bacterial orders and suggested that 'some common element in promoter sites remains conserved in all bacteria'. Although promoter sequences from the genus *Rhizobium* have not been described, the binding of *R. leguminosarum* RNA polymerase to the T7 promoters supports this hypothesis and is consistent with the expression of the *trp* genes of *E. coli* (localized on the broad host range plasmid RP4) in *R. leguminosarum* 300 (Nagahari *et al.*, 1979). In addition, a cell-free 'coupled' transcription/translation system from *R. leguminosarum* 300 synthesized colicin E1 when the *E. coli*-specific ColE1 plasmid was added as a template (Laue-Schuler & Lotz, unpublished results).

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