Substrate-Binding Ability of *Escherichia coli* Ribonucleic Acid Polymerase in Relation to its Protein Composition

By AGNETE ROGNES and K. ABRAHAM ABRAHAM
Department of Biochemistry, University of Bergen, 5000 Bergen, Norway

(Received 17 July 1975)

1. Interaction between *Escherichia coli* RNA polymerase and its substrates, the nucleoside triphosphates, was studied by gel-filtration and dialysis-rate-measurement techniques. 2. The holoenzyme bound variable amounts of ATP and GTP. There was no correlation between substrate-binding ability and enzyme activity of different enzyme preparations. 3. The core enzyme bound a maximum of 0.1 mol of ATP/mol of enzyme. The dissociation constant of this interaction was of the order of 1×10^{-5} M. The core enzyme did not bind GTP. 4. A protein of mol.wt. 60000, which was eluted in the first fraction during phosphocellulose column chromatography of the holoenzyme, bound appreciable amounts of ATP. The dissociation constant of this interaction was of the order of 3×10^{-5} – 5×10^{-6} M. 5. Evidence presented shows that this protein, and not the sigma factor, is responsible for the observed variation in the ATP-binding ability of the holoenzyme.

Interaction between *Escherichia coli* RNA polymerase and its substrates, the nucleoside triphosphates, has been studied by several workers. As yet, there is no general agreement with regard to the number of substrate-binding sites, the dissociation constant of the complex, or the specificity of the binding reaction. Ishihama & Hurwitz (1969) by using a gel-filtration technique introduced by Hummel & Dreyer (1962) concluded that there are eight to nine substrate-binding sites of identical binding capacity per mol of enzyme (mol.wt. 340000), and that these sites do not discriminate between the four nucleoside triphosphates. Their analysis of the number of binding sites (carried out by using the dialysis-rate-measurement technique) revealed the presence of three to four nucleoside triphosphate-binding sites per mol of enzyme.

Wu & Goldthwait (1969) found two substrate-binding sites per enzyme molecule, namely a weak site with a dissociation constant of 1.5×10^{-4} M and a strong site with a dissociation constant of 1.5×10^{-5} M. They also found that the enzyme bound the pyrimidine nucleoside triphosphates only weakly.

Abraham (1970) reported the purification of two enzyme preparations from *E. coli*, one able to bind nucleoside triphosphates in the absence of DNA and the other unable to bind substrates in the absence of DNA.

All these early experiments were performed on enzyme preparations of unknown subunit composition. Zillig *et al.* (1970) performed binding studies with core enzyme and sigma factor and concluded that the core enzyme or sigma factor alone had only negligible substrate-binding ability, whereas the

holoenzyme was able to bind three to four purine nucleoside triphosphate molecules per mol of enzyme. Ishihama (1972) isolated a 14S complex containing $\beta'\beta$ and a small amount of α subunit by dissociating the enzyme with *p*-chloromercuribenzoate and urea. This complex had no enzyme activity as one would have expected. The complex, however, was fully active in binding substrates and the binding was of the order of 4–5 nmol of ATP per mg of the complex. This corresponds roughly to 1 mol of ATP/mol of $\beta'\beta$ complex. Even more intriguing was the fact that binding was measured by the gel-filtration technique which revealed eight to nine substrate-binding sites in an earlier investigation (Ishihama & Hurwitz, 1969).

We have investigated the correlation between the substrate-binding ability and the protein composition of different enzyme preparations. Results presented in the present paper offer an explanation for some of these discrepancies.

Experimental

Materials

Chemicals. Unlabelled nucleoside triphosphates were obtained from Schwarz BioResearch (Orangeburg, N.Y., U.S.A.). Streptomycin sulphate and calf thymus DNA were purchased from Sigma Chemical Co. (Kingston-upon-Thames, Surrey KT2 7BH, U.K.). Whatman DEAE-cellulose (DE 23 and DE 52) and phosphocellulose (PC 11) were supplied by Reeve Angel and Co. (London E.C.4, U.K.). [14 C]ATP and [3 H]GTP were obtained from The Radiochemical

Centre (Amersham, Bucks., U.K.) and Schwarz-Mann (Orangeburg, N.Y., U.S.A.) respectively. Standard proteins for molecular weight determination (Combithek) were purchased from Boehringer Mannheim GmbH (Mannheim, West Germany).

Methods

Purification of RNA polymerase. RNA polymerase was isolated from *E. coli* MRE 600 by the method of Chamberlin & Berg (1962) with minor modifications (Abraham, 1968; Abraham *et al.*, 1972). Enzyme fractions eluted from the DEAE-cellulose column with buffer containing 0.15M-KCl (fraction DE-B) and 0.22M-KCl (fraction DE-C) were collected and used in the binding experiments either directly or after dissociation by phosphocellulose chromatography. Some DE-B enzyme was passed through an agarose column as described by Burgess (1969a) and the agarose purified enzyme was collected.

Dissociation of holoenzyme (fraction DE-B). Holoenzyme (fraction DE-B) was dissociated on a phosphocellulose column by the method of Burgess (1969a). Three protein fractions were eluted from the phosphocellulose column with buffer containing 0.10M-KCl (fraction PC 0.10), 0.25M-KCl (fraction PC 0.25), and 0.35M-KCl (fraction PC 0.35) and used in the binding experiments.

DEAE-cellulose chromatography of PC 0.10 fraction. A DEAE-cellulose column (0.9cm × 10cm) was first equilibrated with buffer D [20mM-Tris/H₂SO₄ (pH 7.3)/10mM-MgSO₄/0.2mM-EDTA/5mM-2-mercaptoethanol]. The PC 0.10 protein (25mg), precipitated with (NH₄)₂SO₄, was dissolved in 6ml of the above buffer and dialysed twice against 300ml of the same buffer for a period of 1 h each time. The dialysed protein solution was applied to the column, and washed successively with buffer D, buffer D containing 0.15M-KCl (fraction D 0.15) and 0.25M-KCl (fraction D 0.25).

RNA polymerase assay. RNA polymerase assays were performed with either T₄ DNA or calf thymus DNA as described by Abraham *et al.* (1972).

Measurement of nucleoside triphosphate binding by enzyme fractions. Unless otherwise stated, all binding experiments were performed in a buffer containing 20mM-Tris/HCl, pH 7.9, 250mM-KCl, 10mM-MgCl₂, 0.2mM-EDTA and 2.5mM-2-mercaptoethanol. Substrate binding by the enzyme was measured by the gel-filtration technique introduced by Hummel & Dreyer (1962) and by the dialysis-rate-measurement method of Colowick & Womack (1969). Gel-filtration experiments were performed on a Sephadex G-50 column (0.9cm × 30cm) with the use of substrate concentrations of 10–300 μM. (NH₄)₂SO₄-precipitated protein (10mg in 250 μl of the binding buffer) was first dialysed against the same buffer to remove

all (NH₄)₂SO₄. The dialysed protein solution, mixed with an equal volume of the same buffer containing twice the desired concentration of the nucleoside triphosphate, was applied to a column equilibrated with the binding buffer containing the desired nucleoside triphosphate concentration. Fractions (25, each of vol. 1ml) were collected. The void volume of the column was 8ml and the salt volume 19ml. The flow rate was approx. 15ml/h. A volume of 100–200 μl was used for radioactivity measurements and 10–300 μl for protein determination by the method of Lowry *et al.* (1951). Radioactivity in each fraction was determined in a Nuclear-Chicago liquid-scintillation counter, by using 7ml of Biosolve scintillation liquid [5g of PPO (2,5-diphenyloxazole), 100g of naphthalene, 167ml of Biosolve BBS 3 from Beckman Instruments (Fullerton, Calif., U.S.A.) and 833ml of toluene].

Measurement of substrate binding by the dialysis-rate-measurement technique was carried out in a dialysis cell similar to the one described by Colowick & Womack (1969). Vacuum-stretched dialysis tubing was used as the membrane between the two chambers of the dialysis cell. Unstretched tubing gave a dialysis rate that was too low. The flow rate of the buffer through the lower chamber was 6.25ml/min and fractions of volume 2.5ml were collected every 24s in an LKB Ultrarac fraction collector. A volume of 500 or 1000 μl of each fraction was used for radioactivity measurement as described above. The experiments were started by the addition of 5 μCi of [¹⁴C]ATP (specific radioactivity 490 μCi/μmol) to the upper chamber containing about 20mg of protein in 1.5ml of the binding buffer. After every 12th fraction, 10 μl of non-radioactive ATP of increasing concentration was added to the upper chamber to give the concentrations indicated in the Figures.

Gel electrophoresis. Polyacrylamide-gel electrophoresis was carried out with 5% gels containing 0.1% sodium dodecyl sulphate (Shapiro *et al.*, 1967). Electrophoresis was performed at 8mA per gel (0.6cm × 9.5cm) for about 6h. The gels were stained with Coomassie Brilliant Blue, destained thoroughly with 7.5% (v/v) acetic acid and scanned in an MPS-50 L Shimadzu spectrophotometer at 550nm. Molecular weights of subunits were estimated by using alcohol dehydrogenase, hen's egg albumin, bovine serum albumin and aldolase as markers.

Results

Substrate binding in relation to enzyme activity

Most of the procedures for the isolation of RNA polymerase yield preparations satisfactory for enzymic studies. However, these preparations require further purification to achieve protein purity. Unidentified minor components in the purified enzyme

preparations vary, depending on the isolation procedure. A comparison of the specific activity with the substrate-binding ability of enzyme fractions eluted from the DEAE-cellulose column (the final purification step) and the fractions obtained on dissociating the purified enzyme by phosphocellulose chromatography, is given in Table 1. No correlation could be observed between the amount of substrate bound and the specific enzyme activity. In fact, the amount of ATP bound per mg of protein was highest in the first fraction (PC 0.10) eluted from the phosphocellulose column. The core enzyme (fraction PC 0.35), which showed high enzyme activity in the calf thymus DNA assay, did not bind GTP. This enzyme showed a weak affinity for ATP. Substrate binding determined by dialysis-rate measurement and gel filtration gave similar results.

Specificity of the substrate-binding sites

To study the specificity of the enzyme-substrate interaction, competition experiments were performed with ATP and GTP. As shown in Table 2, CTP was unable to compete for the purine nucleoside triphosphate-binding site. At all substrate concentrations, the enzyme fractions bound ATP more strongly than GTP. The amount of ATP bound by the enzyme was not decreased even in the presence of an excess of GTP. In fact, a slight stimulation of ATP binding was observed in the presence of GTP. The GTP binding, on the other hand, was neither stimulated nor decreased by a ninefold excess of ATP. These results suggest the presence of separate sites for the binding of ATP and GTP.

Substrate binding in relation to protein composition

Coomassie Brilliant Blue is a very sensitive dye for staining proteins, and the amount of dye bound to a

protein, within certain limits, is proportional to the protein concentration (Fazekas de St. Groth *et al.*, 1963). By staining the proteins separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, it is possible to detect as little as 0.1 μ g of protein in a sharp band (Burgess, 1969b). Fig. 1 shows the scanning patterns of the three protein fractions eluted from the phosphocellulose column. Molecular weights of the protein bands estimated by the method of Shapiro *et al.* (1967) are indicated above each peak. Polypeptides of mol.wt. less than 40000 gave diffused weak bands in the gel. These bands, including the omega subunit, were omitted when calculating the amounts of subunits per mg of protein. The relative size of the different peaks in the first fraction (PC 0.10) eluted from the phosphocellulose column showed great variations from batch to batch. Enzyme fractions of low 280/260nm absorbance ratio, obtained from the DEAE-cellulose column at a higher ionic strength (fraction DE-C), did not dissociate on phosphocellulose chromatography. This enzyme fraction, like the core enzyme, showed low affinity for ATP (Table 1) even though it contained the sigma subunit.

Table 2. *Specificity of substrate-binding sites*

Binding of ATP and GTP by different enzyme fractions was measured either alone or in the presence of a large excess of another non-radioactive nucleoside triphosphate. The concentration of the radioactive nucleoside triphosphate was always 30 μ M and that of the unlabelled triphosphate 270 μ M. These experiments were performed by the gel-filtration technique described in the Experimental section.

Enzyme fraction	Radioactive nucleoside triphosphate	Non-radioactive nucleoside triphosphate	Radioactive nucleoside triphosphate bound/mg of protein (nmol)
DE-B	ATP		2.7
	GTP		1.5
	GTP	ATP	1.4
Agarose purified DE-B	ATP		2.0
	ATP	GTP	1.5
	GTP		0.2
	GTP	ATP	0.2
PC 0.10	ATP		3.9
	ATP	CTP	4.1
	ATP	GTP	5.8
	GTP		1.0
	GTP	ATP	0.4
PC 0.25	ATP		1.1
	ATP	GTP	2.5
PC 0.35	ATP		0.4
	GTP		—
D 0.25	ATP		2.4
	ATP	GTP	2.7
	GTP		—

Table 1. *Comparison of ATP-binding ability with RNA polymerase activity of different enzyme fractions*

ATP binding was measured by the gel-filtration technique described in the Experimental section. The concentration of ATP in the binding experiments was 30 μ M. RNA polymerase assays were performed as described previously (Abraham *et al.*, 1972), with calf thymus DNA as template.

Protein fraction	ATP bound/mg of protein (nmol)	AMP incorporated into RNA (nmol/min per mg of protein)
DE-B	2.7	128
DE-C	1.1	87
PC 0.10	2.9	4
PC 0.25	1.0	102
PC 0.35	0.3	107
D 0.25	2.5	—

All enzyme preparations except the core enzyme contained at least two polypeptides of mol.wt. 120000 ± 5000 and 60000 ± 5000 in addition to β' , β , α and σ subunits. Some batches of core enzyme contained small amounts of the 120000 dalton protein. In Table 3, the binding data obtained with different enzyme preparations and their content of 60000 dalton protein (called 'S protein' below), σ and α subunits and $\beta'\beta$ complex are given. The amount of ATP bound by different enzyme preparations showed a positive correlation with the amount of 'S protein', but not with the amount of $\beta'\beta$ complex.

To perform binding experiments with pure σ subunit, we attempted a further purification of the first fraction eluted from the phosphocellulose column. Chromatography of this fraction on a DEAE-cellulose column yielded a fraction containing most of the stimulating activity. This fraction was eluted from the DEAE-cellulose column with

0.25M-KCl (fraction D 0.25) and contained at least four different polypeptides in addition to the σ protein, of which the 'S protein' was the major component (Table 3). A very significant binding of ATP but no binding of GTP was observed with this fraction (Table 2).

Dissociation constants of enzyme-ATP complex

Since protein fraction D 0.25 alone showed significant ability to bind ATP, experiments were performed at different substrate concentrations to determine the binding constants of this interaction. A Scatchard plot of the ATP-binding results obtained by the dialysis rate measurement with D 0.25 protein fraction gave a non-linear biphasic curve with K_s values in the range 3×10^{-5} – 5×10^{-6} M (Figs. 2 and 3). The limiting amount of ATP bound/mg of protein was 3.5nmol. The sample used in this particular experiment contained 5nmol of 'S protein' and 2nmol of σ protein per mg of protein. K_s values of this order were also obtained for the PC 0.10 enzyme fraction. Binding data obtained with a core enzyme preparation (Fig. 4), when plotted as a Scatchard plot, gave a K_s value of the order of 1×10^{-5} M. However, a maximum of only 0.1 mol of ATP was bound per mol of core enzyme.

Discussion

The present experiments were carried out in the presence of 0.25M-KCl and 10mM-MgCl₂ to exclude the non-specific binding of nucleoside triphosphates taking place at low salt concentration (Abraham, 1970).

If the substrate-binding sites of the enzyme molecule are restricted to one or two of the catalytic subunits alone, as shown by Ishihama (1972), the lack of correlation between substrate-binding ability and enzyme activity (Table 1) may be due to a non-stoichiometric existence of the catalytic subunits in our preparations. σ and ω subunits have been shown to exist in a non-stoichiometric ratio in most enzyme preparations (Burgess, 1971; Abraham *et al.*, 1972). β' , β and α subunits are also found non-stoichiometrically in some protein fractions obtained on dissociating the enzyme on a phosphocellulose column. This explanation, however, cannot account for the great variations in substrate-binding abilities of enzyme preparations containing core enzyme subunits in nearly stoichiometric ratio (Table 3).

It is also possible that although core enzyme or σ factor alone is unable to bind substrate, the potential binding sites of the core enzyme might become functional in the presence of σ factor. Even so, since most enzyme preparations are not able to synthesize RNA in the absence of DNA, only the sites

Fig. 1. 550nm absorption profiles of stained polyacrylamide gels

Electrophoresis, staining, destaining and scanning of the gels were performed as described in the Experimental section. (a) PC 0.35 protein; (b) PC 0.25 protein; (c) PC 0.10 protein. Marker proteins used for molecular weight estimations are given in the Experimental section. Molecular weights corresponding to migration distance are shown.

Table 3. ATP bound by different enzyme preparations and their content of 'S protein', σ , α and β' β subunits

Substrate binding was measured by the gel-filtration technique, by using 30 μ M-[¹⁴C]ATP as described in the Experimental section. The amount of 'S protein', σ , α and β' β subunits in each enzyme preparation used for the binding experiment was determined by measuring the area under the corresponding peaks from the 550nm absorption profiles of the stained gels as described in the Experimental section. The data shown are the average for three different experiments with each enzyme fraction. Great variations in the amount of ATP bound and the subunit composition were observed for DE-B and PC 0.10 enzyme fractions.

Enzyme fraction	ATP bound (nmol/mg of protein)	Subunit mol.wt.	Subunit composition (amount/mg of protein)							
			... 'S protein' ...		σ		α		$\beta'+\beta$	
			60000		95000		40000		320000	
			nmol	mg	nmol	mg	nmol	mg	nmol	mg
DE-B	2.5		3.4	0.20	0.7	0.07	4.2	0.17	1.1	0.35
PC 0.10	1.9		4.6	0.28	1.2	0.11	2.8	0.11	0.7	0.22
PC 0.25	1.1		2.0	0.12	0.2	0.02	4.0	0.16	2.0	0.64
PC 0.35	0.3		—	—	—	—	4.1	0.16	2.3	0.73
D 0.25	2.5		5.2	0.31	1.2	0.11	3.2	0.13	0.1	0.03

Fig. 2. Measurement of ATP binding at various ATP concentrations by D 0.25 protein fraction

Upper curve, radioactivity in the diffusate without protein; lower curve, radioactivity in the diffusate with 18mg of D 0.25 protein/ml in the upper chamber. Dialysis was started in both experiments by the addition of 5 μ Ci of [¹⁴C]ATP (specific radioactivity 490 μ Ci/ μ mol). The concentration of ATP (μ M) at different intervals during dialysis is indicated at the arrows. Radioactivity was determined in 1 ml samples of each fraction, as described in the Experimental section.

responsible for the binding of the first nucleoside triphosphate, but not the polymerization site, can be functional in the holoenzyme. Therefore 3–4mol of ATP bound/mol of enzyme monomer (Ishihama, 1972) would imply simultaneous initiation of several

RNA chains per enzyme molecule. It is now well established that each enzyme molecule makes only one RNA chain at a time. Even the binding of one substrate molecule per core enzyme raises the question of specificity of the initiation site, because chain

Fig. 3. Plot of ATP-binding data derived from the steady-state values in Fig. 2

From the vertical-axis intercept, the limiting amount of ATP bound per mg of protein is 63/18 nmol or 3.5 nmol. From the slope, the dissociation constant (K_s) was in the range 3×10^{-5} – 5×10^{-6} M.

initiation both *in vitro* and *in vivo* takes place both with ATP and GTP, and the number of chains initiated with either of these nucleotides depends on the type of template. Our results show that polypeptides other than β' , β , α or σ (either alone or together) are responsible to a great extent for binding of ATP and GTP by free enzyme.

Most enzyme preparations contain variable amounts of other proteins besides the ones mentioned above. These include M factor (Davison *et al.*, 1969), ψ factor (Travers *et al.*, 1970), ρ factor (Roberts, 1969), CAP or cyclic AMP receptor protein (Chambers & Zubay, 1969) and new sigma factors (Fukuda *et al.*, 1974; Wickner & Kornberg, 1974). Significant binding of ATP and GTP by the first fraction eluted from the phosphocellulose column (fraction PC 0.10) also suggests that substrate binding by the free enzyme is not solely due to the formation of primary initiation complex between core enzyme and nucleoside triphosphate. The correlation between enzyme-bound ATP and the amount of 'S protein' in different enzyme samples (Table 3) suggests that a major portion of the substrate binding by these enzyme preparations is most probably due to this protein. These results, however, do not eliminate the possibility that some of the observed substrate binding

Fig. 4. Measurement of ATP binding by core enzyme (PC 0.35) at various ATP concentrations

This experiment was performed with 30 mg of core enzyme/ml and $5 \mu\text{Ci}$ of $[^{14}\text{C}]\text{ATP}$ (specific radioactivity $490 \mu\text{Ci}/\mu\text{mol}$). The concentration of ATP (μM) during dialysis is indicated at the arrows. Details of the experimental set-up are described in the Experimental section.

is caused by other polypeptides. It could also be argued that the substrate binding taking place in the absence of DNA is caused by an unmasking of the binding site(s) by β subunits splitting (King *et al.*, 1974; Lill & Hartmann, 1975). This possibility has not been studied in the present investigation.

A polypeptide of mol.wt. 56000 has been identified in *E. coli* RNA polymerase preparations (Fukuda *et al.*, 1974). This protein, the so-called second σ factor, catalysed the synthesis of unprimed poly(A)·poly(U) by the free enzyme. To explain this unprimed RNA synthesis, Fukuda *et al.* (1974) proposed that this factor is probably highly reactive with substrates in the absence of DNA (Iwakura *et al.*, 1974). It is not clear at present whether the 'S protein' found in our enzyme preparations and the second σ factor reported by Iwakura *et al.* (1974) are identical. Our enzyme preparations containing the 'S protein' synthesized poly(A) and poly(A)·poly(U) (J. Petterson, unpublished work), whereas RNA polymerase II made poly(A)·poly(U) but not poly(A) (Iwakura *et al.*, 1974). It is noteworthy that Ishihama used the same isolation procedure to obtain enzyme for his binding studies (Ishihama, 1972) and to demonstrate the presence of RNA polymerase II in *E. coli* (Fukuda *et al.*, 1974). In a previous paper (Abraham *et al.*, 1972), we have indicated the presence of an unidentified component of similar mobility in sodium dodecyl sulphate/polyacrylamide gels.

The low substrate-binding ability of core enzyme reported above and in Table 2 indicates that the substrate-binding sites of the enzyme are not functional in the absence of DNA. However, a maximum of 0.1 mol of ATP bound/mol of core enzyme is rather intriguing. If it is assumed that core enzyme can exist in two or more different conformations, the observed binding by core enzyme may represent the fraction with a conformation suitable for interaction with substrate. Conformational changes in the enzyme molecule as a result of interaction with DNA, may produce active sites for the binding of substrates. Such template-specified conformational changes will also explain the changing specificity of the initiation and polymerization sites for substrates.

Although disulphide bonds are not involved in the interaction between RNA polymerase subunits, it seems reasonable to assume that thiol groups do play an important role in maintaining the conformation of the enzyme molecule. Therefore conformational changes produced by the blocking of these groups may expose the hidden substrate-binding sites of the enzyme. The $\beta'\beta$ complex used in the binding experiments by Ishihama (1972), was isolated by dissociating the enzyme with *p*-chloromercuribenzoate and urea. Inability of this complex to function in any of the other reactions catalysed by the enzyme again suggests that the results obtained by freezing

a part of the enzyme molecule in a particular configuration have to be considered with great reservation.

The biphasic nature of the Scatchard plot (Fig. 3) is most probably due to the presence of two or more ATP-binding components in this protein sample. The correlation between ATP-binding ability and the concentration of 'S protein' in different enzyme fractions, however, suggests that this protein is responsible to a great extent for the observed ATP binding. The dissociation constant (3×10^{-5} M) for the weak ATP-binding site in this protein fraction was of the same order as that (1×10^{-5} M) observed with the core enzyme and could be due to the presence of core enzyme subunits. These K_s values are close to each other, and also to the K_s value of 1.5×10^{-5} M found by Wu & Goldthwait (1969) for their enzyme preparation. All these values are from experiments performed in the presence of Mg^{2+} .

The low binding of GTP by partially purified 'S protein', compared with holoenzyme (Table 2), indicates that this protein is not responsible for the binding of GTP. The major GTP-binding component of the holoenzyme samples, also present in the first fraction eluted from the phosphocellulose column (fraction PC 0.10), is removed from the 'S protein'-containing fraction (D 0.25) during DEAE-cellulose chromatography.

From these results it is concluded that the native enzyme, unable to synthesize RNA in the absence of DNA, cannot bind more than a maximum of one purine nucleoside triphosphate per enzyme molecule. This interaction is rather weak even in the presence of Mg^{2+} ions, and is of the order of 1×10^{-5} M, a value very close to that reported by Wu & Goldthwait (1969).

This work was part of a thesis submitted by A. R. to the University of Bergen.

References

- Abraham, K. A. (1968) *Eur. J. Biochem.* **5**, 143–146
- Abraham, K. A. (1970) in *Lepetit Colloquium on RNA Polymerase and Transcription* (Silvestri, L., ed.), pp. 1–9, North-Holland Publishing Co., Amsterdam and London
- Abraham, K. A., Andersen, K. J. & Rognes, A. (1972) *Biochem. J.* **129**, 291–299
- Burgess, R. R. (1969a) *J. Biol. Chem.* **244**, 6160–6167
- Burgess, R. R. (1969b) *J. Biol. Chem.* **244**, 6168–6176
- Burgess, R. R. (1971) *Annu. Rev. Biochem.* **40**, 711–740
- Chamberlin, M. & Berg, P. (1962) *Proc. Natl. Acad. Sci. U.S.A.* **48**, 81–94
- Chambers, D. A. & Zubay, G. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **63**, 118–122
- Colowick, S. P. & Womack, F. (1969) *J. Biol. Chem.* **244**, 774–777

- Davison, J., Pilarski, L. M. & Echols, H. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **63**, 168-174
- Fazekas de St. Groth, S., Webster, R. G. & Datyner, A. (1963) *Biochim. Biophys. Acta* **71**, 377-391
- Fukuda, R., Iwakura, Y. & Ishihama, A. (1974) *J. Mol. Biol.* **83**, 353-367
- Hummel, J. P. & Dreyer, W. J. (1962) *Biochim. Biophys. Acta* **63**, 530-532
- Ishihama, A. (1972) *Biochemistry* **11**, 1250-1258
- Ishihama, A. & Hurwitz, J. (1969) *J. Biol. Chem.* **244**, 6680-6689
- Iwakura, Y., Fukuda, R. & Ishihama, A. (1974) *J. Mol. Biol.* **83**, 369-378
- King, A. M. Q., Lowe, P. A. & Nicholson, B. H. (1974) *Biochem. Biophys. Res. Commun.* **59**, 38-43
- Lill, H. R. & Hartmann, G. R. (1975) *Eur. J. Biochem.* **54**, 45-53
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Roberts, J. W. (1969) *Nature (London)* **224**, 1168-1174
- Shapiro, A. L., Vinuela, E. & Maizel, J. V. (1967) *Biochem. Biophys. Res. Commun.* **28**, 815-820
- Travers, A. A., Kamen, R. & Schleif, R. F. (1970) *Nature (London)* **228**, 748-751
- Wickner, W. & Kornberg, A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4425-4428
- Wu, C. W. & Goldthwait, D. A. (1969) *Biochemistry* **8**, 4458-4464
- Zillig, W., Zechel, K., Rabussay, D., Schachner, M., Sethi, W. S., Palm, P., Heil, A. & Seifert, W. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 47-58