Physiological Levels and Properties of Polynucleotide Phosphorylase of *Rhizobium meliloti*

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Polynucleotide phosphorylase activity was demonstrated in free-living and the symbiotic bacteroid forms of *Rhizobium meliloti* and in free-living *Rhizobium japonicum*. Crude extracts of *R. meliloti* F-28 catalysed the polymerization of $3.0 \mu\text{mol ADP (mg protein)}^{-1} \text{ h}^{-1}$. The polynucleotide phosphorylase activity of symbiotic *R. meliloti* was about 50 % of that of free-living *R. meliloti* and remained almost constant throughout the development of the nodules. Partially-purified fractions of free-living *R. meliloti* F-28 catalysed the polymerization of ribonucleoside diphosphates, the phosphorolysis of synthetic homopolymers and natural heteropolymers, and the β -phosphate exchange reaction. The enzyme had a pH optimum of 8.0 and had an obligatory requirement for Mg^{2+} for maximum activity. The phosphorylase was not highly primer-dependent, had low specificity for guanosine-containing substrates, and exhibited cooperative-type kinetics.

INTRODUCTION

Polynucleotide phosphorylase (polyribonucleotide:orthophosphate nucleotidyltransferase, EC 2.7.7.8) catalyses the reversible incorporation of ribonucleoside diphosphates into ribonucleotide polymers. The reverse reaction, the phosphorolysis of polyribonucleotides, is believed to be the principal reaction *in vivo* resulting in the degradation of RNA (Kinscherf & Apirion, 1975; Kaplan & Apirion, 1975). The enzyme has been reported in several bacteria (Godefroy-Colburn & Grunberg-Manago, 1972) as well as in mammalian and plant tissues (See & Fitt, 1972; Brishammar & Juntti, 1974).

Our interest in polynucleotide phosphorylase of rhizobia originated from studies of Dilworth & Williams (1967) who demonstrated that the RNA content in the bacteroid form of *Rhizobium lupini* in lupine nodules decreased significantly during early nodule development. The RNA content decreased from 150 fg per bacteroid in 1-week-old nodules to 20 fg per bacteroid in 6-week-old nodules. A similar change has been observed in developing alfalfa nodules infected with *Rhizobium meliloti* F-28 (A. Paau & J. R. Cowles, unpublished results). The effect of significant RNA degradation by polynucleotide phosphorylase would be that less energy (ATP equivalents) would be required to resynthesize nucleoside triphosphates than if RNA was hydrolysed by RNAase. In addition, the nucleoside diphosphates which are formed can be reduced directly to deoxyribonucleotides and used in DNA synthesis. The enzyme responsible for this reduction, ribonucleotide reductase, has previously been described in *R. meliloti* F-28 (Cowles & Evans, 1968; Cowles, Evans & Russell, 1969).

This paper reports the presence of polynucleotide phosphorylase activity in free-living and symbiotic *R. meliloti* (and in free-living *R. japonicum*), and describes some properties of the enzyme. It is part of an overall study of the biochemical changes involved in the establishment of symbiosis in the rhizobia-legume system.

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METHODS

Materials. Nucleotides, polyribonucleotides and DNA (salmon sperm) were purchased from Sigma. DNA (10 mg) was further purified by treatment with 5 mg RNAase (Sigma) for 1 h and subsequently spooled in propan-2-ol. [$U\text{-}^{14}\text{C}$]Nucleotides and [^{32}P]P_i were purchased from Amersham/Searle (Arlington Heights, Illinois, U.S.A.) or Schwarz/Mann (Orangeburg, New Jersey, U.S.A.), except for [$U\text{-}^{14}\text{C}$]AMP which was prepared by enzymic dephosphorylation of [$U\text{-}^{14}\text{C}$]ATP using potato apyrase (Sigma). Soluble RNA (type III) was purchased from Sigma. Mouse sarcoma mRNA and *R. meliloti* tRNA were gifts from Dr William Jeffery and Mr Lyndon Johnson, respectively, of University of Houston, Texas. Ribosomal RNA from *R. meliloti* was prepared according to Kirby (1965). [$U\text{-}^{14}\text{C}$]Polyadenylic acid (2500 c.p.m. μmol^{-1}) was prepared and isolated by the method of Grunberg-Manago, Ortiz & Ochoa (1956). Sheared poly(A) was prepared by repeated passage of a 0.036 % (w/v) solution through a 25 gauge needle.

Organisms. *Rhizobium meliloti* strains F-28 and 3D0A1 and *R. japonicum* strain 705 were a gift from Harold J. Evans of Oregon State University. The bacteria were grown on a mannitol medium (Cowles *et al.*, 1969) and harvested by centrifuging when the cultures reached an E_{660} of 0.6 to 0.8, unless otherwise noted. The alfalfa plants were grown, nodules were harvested and bacteroids were isolated as previously described (Pau & Cowles, 1975).

Enzyme preparation. Approximately 20 g rhizobia were washed twice in 40 ml 50 mM-Tris/HCl buffer, pH 8.0, containing 10 mM-2-mercaptoethanol and 0.1 mM-EDTA (buffer G) and then disrupted in a cold French pressure cell. These and subsequent preparation and purification procedures were conducted at approximately 4 °C. The homogenate was centrifuged at 25000 *g* for 20 min and the supernatant was used as a crude extract. For further purification, the crude extracts were diluted to 20 mg protein ml⁻¹ with buffer G and heated at 50 °C for 1 h in a water bath. The coagulated denatured protein was pelleted by centrifuging at 12000 *g* for 10 min. The supernatant was brought to 45 % saturation with ice-cold saturated (NH₄)₂SO₄, pH 8.0. The mixture was equilibrated for another 10 min and then centrifuged at 12000 *g* for 10 min; the pellet was discarded. The (NH₄)₂SO₄ concentration in the supernatant was increased to 65 % saturation and the above procedure was repeated. The resulting pellet was resuspended in 5 mM-Tris/HCl, pH 8.0, containing 10 mM-2-mercaptoethanol and 0.1 mM-EDTA (buffer C).

The fraction precipitated between 45 and 65 % saturation with (NH₄)₂SO₄ (4 ml) was chromatographed on a Sephadex G-200 column (2.5 × 45 cm) previously equilibrated with buffer C. The eluant was collected in 5 ml fractions at a flow rate of 10 ml h⁻¹. Fractions containing at least 60 % of the peak activity were pooled and adsorbed on a 2.5 × 12 cm DEAE-cellulose (Whatman DE-32) column previously equilibrated with buffer C. The column was first eluted with 50 ml buffer C containing 0.2 M-KCl and then with 120 ml of a linear salt gradient (0.2 to 0.5 M-KCl in buffer C). The eluant was collected in 5 ml fractions at a flow rate of 0.75 ml min⁻¹. Fractions containing at least 60 % of the peak activity were pooled, desalted on a column of Sephadex G-25, and stored at 4 °C.

Enzyme assays. A typical polymerization reaction mixture (0.25 ml) contained: 80 μmol Tris/HCl buffer, pH 8.0; 0.4 μmol ADP; 0.1 μmol MgCl₂; 0.025 μCi [$U\text{-}^{14}\text{C}$]ADP (160 μCi μmol^{-1}); 100 μg bovine serum albumin; and enzyme. The reaction was started by the addition of enzyme, incubated for 10 min at 37 °C, and terminated by the addition of 2 ml 5 % (w/v) trichloroacetic acid. After 5 min in an ice bath the acid-insoluble material was collected by filtering through a 2.1 cm diam. Whatman GF/A filter. The filter was washed with 30 to 40 ml 2 % trichloroacetic acid, dried and the radioactivity was determined in a liquid scintillation spectrometer. The vial contained 8 ml scintillation fluid [containing 5 g 2,5-diphenyloxazole and 0.3 g 1,4-di-2-(5-phenyloxazoly)benzene in 1 l toluene]. One unit of activity was defined as the amount of enzyme necessary to incorporate 1.0 μmol nucleoside diphosphate into a polymer in 1 h.

A typical phosphorolysis reaction mixture (0.1 ml) contained: 40 μmol Tris/HCl buffer, pH 8.0; 0.1 μmol poly(A) (calculated as AMP); 1.0 μmol MgCl₂; 1.0 μmol K₂HPO₄; [^{32}P]P_i (250000 to 500000 c.p.m.); and enzyme. The reaction was started by the addition of enzyme and incubated for 15 min at 37 °C. The reaction was terminated and the orthophosphate was precipitated by the method of Sugino & Miyoshi (1967). A 0.1 ml sample of the supernatant was added to a scintillation vial containing 6 ml scintillation fluid (as above) and 4 ml 95 % (v/v) ethanol, and the radioactivity was determined in a liquid scintillation spectrometer. One unit of activity was defined as the amount of enzyme necessary to incorporate 1.0 μmol phosphate into nucleoside diphosphates in 1 h.

The β -phosphate exchange reaction was measured using the phosphorolysis assay procedure except 0.4 μmol ADP and 0.1 μmol MgCl₂ were substituted for poly(A) and 1.0 μmol MgCl₂ in the reaction mixtures. One unit of activity was defined as the amount of enzyme necessary to exchange 1.0 μmol phosphate with terminal phosphate in 1 h.

Other procedures and determinations. Adenosine nucleotides were separated by ascending paper chromatography on Whatman 3MM paper, using 95 % ethanol/1.0 M-ammonium acetate pH 5.0 (7:3, v/v) as solvent.

This method was used to analyse the products of the phosphorolysis reaction and in the preparation of [U-¹⁴C]AMP; in both these procedures the reaction was stopped by adding an equal volume of ice-cold ethanol.

Gel electrophoresis was carried out as described by Davis (1964) and Ornstein (1964), using 7 % (w/v) polyacrylamide gels. Electrophoresis in sodium dodecylsulphate gels was according to Weber & Osborn (1969). The gels were stained for protein using Coomassie blue or for polynucleotide phosphorylase activity by the method of Thang, Thang & Leautey (1967).

DNA and RNA concentrations were determined with diphenylamine and orcinol, respectively (Ashwell, 1957). Protein concentration was estimated by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

RESULTS

Polynucleotide phosphorylase activity

The highest level of polynucleotide phosphorylase activity (ADP polymerization) was found in young free-living cultures of *R. meliloti* F-28 (Table 1). Activity decreased to 50 % in older cultures. Chromatography of the products of the reaction with [¹⁴C]poly(A) as substrate showed the principal product (70 %) to be ADP. Polynucleotide phosphorylase activity in bacteroids of alfalfa nodules was about 50 % of that of a free-living culture of *R. meliloti* F-28 with an E_{860} of 0.7. The level of phosphorylase activity did not change appreciably in symbiotic rhizobia isolated from nodules of alfalfa plants grown to different ages. Polynucleotide phosphorylase activity of *R. japonicum* was approximately 40 % of that of *R. meliloti* F-28. Very little polynucleotide phosphorylase activity was detected in crude extracts of *R. meliloti* 3DOa1, a strain ineffective in establishing symbiosis. However, in this system the primary reaction product was adenosine and not ADP; purification of the extracts with ammonium sulphate removed competing factors, and increased the specific activity of the phosphorylase to 3.4 units (mg protein)⁻¹.

Purification of polynucleotide phosphorylase

Polynucleotide phosphorylase in crude extracts of free-living *R. meliloti* F-28 was partially-purified (see Table 2) and used to examine properties of the enzyme. The enzyme was purified approximately 75-fold and, after electrophoresis on polyacrylamide gels, protein and activity stains showed it to be the principal protein band. The DEAE-cellulose fraction was stable for at least 6 months when stored at 4 °C and did not contain detectable levels of RNAase or DNAase activity as measured by the methods of Kunitz (1946, 1950). An absolute requirement for primer was not established during enzyme purification, although phosphorylase activity was stimulated 40 % by the addition of sheared poly(A). Neither extended storage at 4 °C nor treatment with RNAase [50 µg (mg protein)⁻¹ for 1 h at 34 °C] converted the enzyme to dependence on primer.

Substrate preference

ADP, UDP and CDP and poly(A), poly(U) and poly(C) were the most effective substrates for polynucleotide phosphorylase of *R. meliloti* F-28. In the polymerization reaction (Table 3), GDP was polymerized at less than 10 % of the rate of ADP polymerization. ATP and AMP, alone or in combination, were ineffective as substrates. ATP inhibited the rate of ADP polymerization by about 25 % while, surprisingly, AMP stimulated ADP polymerization by 30 %. GDP and dADP were effective inhibitors of ADP polymerization. More than 80 % inhibition was caused by 0.8 mM-GDP and 0.16 mM-dADP. In the phosphorolysis reactions (Table 4), poly(G) was degraded at less than 15 % of the rate of other homopolymers while the naturally occurring heteropolymers were phosphorolysed at 0.5 to 3 % of the rate of poly(A). The kinetics of the phosphorolysis of natural polymers are shown in Fig. 1. DNA

Table 1. *Polynucleotide phosphorylase activity in free-living and symbiotic R. meliloti, and in free-living R. japonicum*

Polynucleotide phosphorylase activity was determined in crude extracts by measuring the rate of ADP polymerization.

Organism	Phosphorylase activity (units mg ⁻¹)
Free-living <i>R. meliloti</i> F-28	
Growth (<i>E</i> ₈₈₀)	
0.10	4.10
0.45	4.11
0.70	3.00
1.10	2.23
1.50	2.00
Bacteroid form of <i>R. meliloti</i> F-28	
Age (week after infection)	
2	1.37
4	1.25
6	1.50
8	1.60
Free-living <i>R. meliloti</i> 3D0A1	
Growth (<i>E</i> ₈₈₀)	
0.68	<0.1
Free-living <i>R. japonicum</i> 705	
Growth (<i>E</i> ₈₈₀)	
0.68	1.21

Table 2. *Summary of the purification of polynucleotide phosphorylase of R. meliloti F-28*

Activity was determined by the polymerization assay.

Fraction	Volume (ml)	Protein concn (mg ml ⁻¹)	Total protein (mg)	Specific activity (units mg ⁻¹)	Total activity (units)
Crude extract	60.0	16.8	1008	3.0	3024
Heat-treated supernatant	47.0	8.8	413	7.1	2932
(NH ₄) ₂ SO ₄ fraction precipitated between 45 and 60 % saturation	4.0	18.5	74	20.3	1502
Sephadex G-200 combined fractions	40.0	0.86	34.4	34.5	1221
DEAE-cellulose combined fractions	25.0	0.049	1.23	230.0	282

Table 3. *Comparison of the effectiveness of various nucleotides in the polymerization reaction*

All substrates were tested at 1.6 mM, except in the last two mixed substrate experiments 0.16 mM-dADP and 0.8 mM-GDP, respectively, were used.

Substrate(s)	Specific activity (units mg ⁻¹)
ADP	256.0
CDP	207.4
UDP	134.7
GDP	23.0
AMP	5.1
ATP	2.6
ADP, ATP	189.4
ADP, AMP	332.8
ATP, AMP	7.7
ADP, dADP	17.9
ADP, GDP	41.0

Fig. 1. Phosphorolysis rates for various heteronucleotide polymers: ●, mRNA; ○, tRNA; ▲, sRNA; ■, rRNA; □, DNA.

Table 4. Comparison of the phosphorolysis rates for different nucleotide polymers

The concentration of each substrate was 1.0 mM calculated as AMP.

Substrate	Specific activity (units mg ⁻¹)
Poly(A)	208.1
Poly(C)	201.8
Poly(U)	218.5
Poly(G)	25.0
sRNA	2.12
tRNA	4.10
mRNA	6.24
rRNA	1.10
DNA	0

(RNAase-treated) was the only compound which was not degraded although the degradation of rRNA occurred very slowly.

Partially-purified extracts of *R. meliloti* F-28 also catalysed the β -phosphate exchange reaction. The exchange rates for ADP, CDP, UDP and GDP were 244, 217, 261 and 50 units (mg protein)⁻¹, respectively.

Cation requirement

Polynucleotide phosphorylase activity was dependent on Mg²⁺ concentration (Table 5). The optimum concentration for both the polymerization and phosphorolysis reactions was 0.4 mM at standard substrate concentrations. However, the optimum Mg²⁺ concentration was related to nucleotide or polymer concentrations and to the specific substrate, as reported in other phosphorylase systems (Grunberg-Manago, 1963). The optimum nucleotide:Mg²⁺ ratios for ADP, CDP and UDP were 4:1, 4:1 and 5:1, respectively. The optimum polymer:Mg²⁺ ratios for the phosphorolysis reaction were 1:10 for poly(A) and 1:50 for both poly(U) and poly(C). The substitution of Mn²⁺ for Mg²⁺ resulted in only 20 to 30 % of the polynucleotide phosphorylase activity obtained with Mg²⁺. In the phosphorolysis reaction the effective polymer:Mg²⁺ ratio was 1:1.

Table 5. *Effect of magnesium concentration on polynucleotide phosphorylase activity*

Mg ²⁺ concn (mM)	Phosphorylase activity (units mg ⁻¹)	
	Polymerization [ADP]	Phosphorolysis [Poly(A)]
0	< 5	< 5
0.04	148	ND
0.1	200	40
0.4	258	92
1	220	154
2	170	186
4	ND	210
5	70	190
10	55	124
20	48	75
40	48	25

ND, Not determined.

Table 6. *Effect of pH on polynucleotide phosphorylase activity*

pH	Phosphorylase activity (units mg ⁻¹)	
	Polymerization [ADP]	Phosphorolysis [Poly(A)]
7.0	182	133
7.5	232	154
7.75	252	195
8.0	273	229
8.5	272	189
9.0	274	142

pH optimum

The optimum pH for the phosphorolysis reaction was 8.0 (Table 6). The optimum pH for the polymerization reaction, however, was less well defined. Maximum or near maximum activity occurred at pH 8.0 which was the pH used in the investigation.

Enzyme kinetics

Polynucleotide polymerization responded to increasing concentrations of ADP over a range from 0.1 μ M to 64 mM (Fig. 2). At very low substrate concentrations (0.1 to 1.6 μ M-ADP), a definite upward curvature was noted (Fig. 2*b*). The initial curvature could have resulted from second-order kinetics or from substrate cooperativity. A Hill plot indicated positive cooperativity at low and high substrate concentrations, with *c* values of 1.38 and approximately 1.4, respectively, but negative cooperativity (*c* = 0.75) at intermediate ADP concentrations (Fig. 3).

The effect of poly(A) and P_i concentrations on polynucleotide phosphorolysis activity was investigated. Increasing concentrations of poly(A) at a constant P_i concentration gave Michaelis-Menten kinetics with an apparent *K_m* of 0.32 mM (Fig. 4*a*). However, the response to increasing concentrations of phosphate at a constant poly(A) concentration did not follow normal Michaelis-Menten kinetics (Fig. 4*b*). A Hill coefficient of 0.83 was determined from the data, indicating negative cooperativity for phosphate binding (Fig. 5).

Fig. 2. Effect of ADP concentration on polynucleotide phosphorylase polymerization. The ADP: Mg^{2+} ratio was maintained at 4:1. All points are averages of at least three separate experiments. Polymerization activity was determined as described in Methods except that $[U-^{14}C]ADP$ was used at $0.10 \mu Ci$ per reaction.

Fig. 3. Hill plot of polynucleotide phosphorylase activity at different ADP concentrations based on the data shown in Fig. 2.

Fig. 4

Fig. 5

Fig. 4. Lineweaver-Burk plots of polynucleotide phosphorylase activity at different poly(A) and P_i concentrations. (a) P_i concentration held at 10 mM and poly(A) concentration varied; (b) poly(A) concentration held at 10 mM and P_i concentration varied. The Mg^{2+} :polymer ratio was maintained at 10:1.

Fig. 5. Hill plot of polynucleotide phosphorylase activity at different concentrations of inorganic phosphate, based on the data shown in Fig. 4(b).

DISCUSSION

The properties of polynucleotide phosphorylase of free-living *R. meliloti* F-28 were similar to those reported for this enzyme from other micro-organisms (Godefroy-Colburn & Grunberg-Manago, 1972). Certain differences, however, were noted and are worth further consideration. One was that polynucleotide phosphorylase of *R. meliloti* F-28 was not absolutely primer-dependent, which was unlike the phosphorylase of *Escherichia coli* and a *Micrococcus* sp. (Godefroy-Colburn & Grunberg-Manago, 1972). The lack of primer dependence could be similar to that encountered in the *Azotobacter* system (Ochoa, Krakow & Basilio, 1965). A second difference was the relative ineffectiveness of GDP and poly(G) as substrates for the *R. meliloti* phosphorylase system. In *E. coli*, for example, the rates of diphosphate- P_i exchange for ADP and GDP were similar (Kimhi & Littauer, 1968). Methods used in other systems to stimulate GDP polymerization (Fresco & Su, 1962), e.g. the addition of Mn^{2+} , the use of a high enzyme:low GDP ratio, or incubation of 50 °C, were ineffective in increasing GDP polymerization by the *R. meliloti* system.

One of the most interesting aspects of the *R. meliloti* F-28 polynucleotide phosphorylase system was the positive:negative:positive cooperativity for the polymerization reaction. This can represent an enzyme with four binding sites (Segel, 1975). The very long, low slope of the saturation curve at the intermediate ADP concentrations indicates the enzyme was in the ES_2 form, a characteristic of a negatively cooperative binding step. High substrate concentrations were required to overcome this aversion to binding. The kinetic data of the phosphorolysis reaction appeared similar to those reported for a *Micrococcus* sp. (Chou, Singer & McPhie, 1975). The negatively cooperative binding obtained with the phosphorolysis reaction at varying concentrations of phosphate may also be associated with the *Micrococcus* system.

Rhizobium meliloti F-28 from a culture with E_{660} of 0.7 appeared to be a good source of polynucleotide phosphorylase. The specific activity (polymerization assay) of crude extracts of *R. meliloti* was 70 to 80 % of that of *Achromobacter* sp. KR 170-4, a superior source of enzyme (Rokugawa *et al.*, 1975), and 7 and 17 times greater than the activities reported for *E. coli* and *Clostridium perfringens*, respectively (Grunberg-Manago, 1963). In addition, the phosphorolysis activity of crude extracts of *R. meliloti* [$1.85 \text{ units (mg protein)}^{-1}$] was three times greater than that reported in crude extracts of *E. coli* (Kimhi & Littauer, 1968).

The lack of a substantial decrease in polynucleotide phosphorylase activity in bacteroids of alfalfa nodules relative to free-living *R. meliloti* F-28 was unlike the activity of two other enzymes compared in these systems. Both DNA polymerase and ribonucleotide reductase activities were approximately 10 times lower in bacteroids of alfalfa nodules than in free-living *R. meliloti* F-28 (Cowles *et al.*, 1969; Pau & Cowles, 1975), whereas there was only a two-fold average difference in polynucleotide phosphorylase activity between the cultures and symbiotic systems. The almost constant level of polynucleotide phosphorylase activity in bacteroids of nodules from alfalfa plants was different from the varying levels of DNA polymerase activity found in developing nodules (A. Paau & J. R. Cowles, unpublished work). Whether polynucleotide phosphorylase has a significant role in reducing the RNA content in developing alfalfa nodules remains to be determined but the relatively high and constant level of activity in the bacteroids certainly indicates this potential.

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REFERENCES

- ASHWELL, G. (1957). Colorimetric analysis of sugars. *Methods in Enzymology* **3**, 73-105.
- BRISHAMMAR, S. & JUNTITI, N. (1974). RNA-synthesizing enzymes in healthy and TMV-infected tobacco leaves—partial purification and characterization of tobacco polynucleotide phosphorylase. *Archives of Biochemistry and Biophysics* **164**, 224-232.
- CHOU, J. Y., SINGER, M. F. & MCPHIE, P. (1975). Kinetic studies on the phosphorolysis of polynucleotides by polynucleotide phosphorylase. *Journal of Biological Chemistry* **250**, 508-514.
- COWLES, J. R. & EVANS, H. J. (1968). Some properties of the ribonucleotide reductase from *Rhizobium meliloti*. *Archives of Biochemistry and Biophysics* **127**, 770-779.
- COWLES, J. R., EVANS, H. J. & RUSSELL, S. A. (1969). B₁₂ Coenzyme-dependent ribonucleotide reductase in rhizobium species and the effects of cobalt deficiency on the activity of the enzyme. *Journal of Bacteriology* **97**, 1460-1465.
- DAVIS, B. J. (1964). Disc electrophoresis II. Method and application of human serum proteins. *Annals of the New York Academy of Sciences* **121**, 404-427.
- DILWORTH, M. J. & WILLIAMS, D. C. (1967). Nucleic acid changes in bacteroids of *Rhizobium lupini* during nodule development. *Journal of General Microbiology* **48**, 31-36.
- FRESCO, J. R. & SU, D. (1962). Polynucleotides IV. Synthesis of polyriboguanilic acid catalyzed by polynucleotide phosphorylase. *Journal of Biological Chemistry* **237**, 3305-3306.
- GODEFROY-COLBURN, T. & GRUNBERG-MANAGO, M. (1972). Polynucleotides phosphorylase. In *The Enzymes*, 3rd edn, vol. 7, pp. 533-574. Edited by P. D. Boyer. New York and London: Academic Press.
- GRUNBERG-MANAGO, M. (1963). Polynucleotide phosphorylase. *Progress in Nucleic Acid Research* **1**, 93-133.
- GRUNBERG-MANAGO, M., ORTIZ, P. J. & OCHOA, S. (1956). Enzymic synthesis of polynucleotides I. Polynucleotide phosphorylase of *Azotobacter vinelandii*. *Biochimica et biophysica acta* **20**, 269-285.
- KAPLAN, R. & APIRION, D. (1975). The fate of ribosomes in *Escherichia coli* cells starved for a carbon source. *Journal of Biological Chemistry* **250**, 1854-1863.
- KIMHI, Y. & LITTAUER, U. Z. (1968). Purification and properties of polynucleotide phosphorylase from *Escherichia coli*. *Journal of Biological Chemistry* **243**, 231-240.
- KINSCHERF, T. B. & APIRION, D. (1975). Polynucleotide phosphorylase can participate in decay of mRNA in *Escherichia coli* in the absence of ribonuclease II. *Molecular and General Genetics* **139**, 357-362.
- KIRBY, K. (1965). Isolation and characterization of ribosomal ribonucleic acid. *Biochemical Journal* **96**, 266-269.
- KUNITZ, M. (1946). A spectrophotometric method for the measurement of ribonuclease activity. *Journal of Biological Chemistry* **164**, 563-568.
- KUNITZ, M. (1950). Crystalline desoxyribonuclease I. Isolation and general properties: spectrophotometric method for the measurement of desoxyribonuclease activity. *Journal of General Physiology* **33**, 349-362.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- OCHOA, S., KRAKOW, J. S. & BASILIO, C. (1965). Polynucleotide phosphorylase from *Azobacter vinelandii*. *Methods in Enzymology* **6**, 3-11.
- ORNSTEIN, L. (1964). Disc electrophoresis I. Background and theory. *Annals of the New York Academy of Sciences* **121**, 321-349.
- PAAU, A. & COWLES, J. R. (1975). Comparison of DNA polymerase of *Rhizobium meliloti* and alfalfa bacteroids. *Plant Physiology* **56**, 526-528.
- ROKUGAWA, K., KATOH, Y., KUNINAKA, A. & YOSHINO, H. (1975). Polynucleotide phosphorylase from *Achromobacter* sp. KR 170-4. *Agricultural and Biological Chemistry* **39**, 1455-1460.
- SEE, Y. P. & FITT, P. S. (1972). Partial purification and properties of rat liver mitochondrial polynucleotide phosphorylase. *Biochemical Journal* **130**, 343-353.
- SEGEL, I. H. (1975). *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems*, pp. 380-381. New York: John Wiley.
- SUGINO, Y. & MIYOSHI, Y. (1967). The specific precipitation of orthophosphate and some biochemical applications. *Journal of Biological Chemistry* **239**, 2360-2364.
- THANG, M. N., THANG, D. C. & LEAUTEY, J. (1967). Physicochimie biologique - séparation et identification de polynucleotide phosphorylase per électrophorèse sur gel polyacrylamide. *Comptes rendus hebdomadaire des séances de l'Académie des sciences* **265**, 1823-1826.
- WEBER, K. & OSBORN, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate - polyacrylamide gel electrophoresis. *Journal of Biological Chemistry* **244**, 4406-4412.