Kinetics and Specificity of T_4 Polynucleotide Kinase†

Johan R. Lillehaug and Kjell Kleppe*

ABSTRACT: The kinetics of T_4 polynucleotide kinase has been investigated at pH 8.0 and 37°. Double reciprocal plots of initial rates vs. substrate concentrations as well as product inhibition studies have indicated that the enzyme reacts according to the ordered sequential mechanism shown in eq 2 in the text for phosphorylation of a DNA molecule. Based on this mechanism the rate equation for the overall reaction was deduced and the various kinetic constants estimated. Hill plots indicated little or no interaction between active sites in the enzyme. The apparent Michaelis constants and V_{max} were determined at a fixed ATP concentration, 66 μM, for a number of different substrates varying in chain length, base composition, and nature of the sugar, and a wide variation was found. For the nucleoside 3′-monophosphates tested both the apparent Michaelis constant and V_{max} values were from approximately 2 to 5 times larger than for the corresponding oligonucleotide. The following orders were obtained with regard to apparent Michaelis constants and V_{max} for the nucleoside 3′-monophosphates investigated: Michaelis constant, rGp > rUp > rCp > rAp > dTp; V_{max}, rGp > rCp > rAp > dTp > rUp. Somewhat similar results were also obtained with the deoxyoligonucleotides tested.

Phage-induced polynucleotide kinases (EC 2.7.1.78) were first discovered in 1965 (Novogrodsky and Hurwitz, 1966; Richardson, 1965). These enzymes catalyze the transfer of the γ-phosphate of ATP to the 5′-hydroxyl terminus of polynucleotides, oligonucleotides, 3′-mononucleotides (Richardson, 1965), and N-protected deoxyoligonucleotides (van de Sande and Bilsker, 1973). Recently it has been shown that the enzyme from T_4 infected *Escherichia coli* also will catalyze the reverse reaction (van de Sande et al., 1973). T_4 polynucleotide kinase has been used extensively in structural work on nucleic acids, in particular in sequence analysis (Weiss and Richardson, 1967; Jay et al., 1974), in fingerprinting of short oligonucleotides (Szekely and Sanger, 1969; Southern, 1970; Murray, 1973), and in the synthesis of oligonucleotides and nucleic acids (Khorana et al., 1972). Thus, T_4 polynucleotide kinase proved to be an indispensable tool in the synthesis of the genes corresponding to yeast alanine tRNA (Agarwal et al., 1970) and tyrosine tRNA precursor gene from *E. coli* (Caruthers et al., 1973).

T_4 polynucleotide kinase has recently been purified to homogeneity and shown to consist of four subunits, each having a molecular weight of approximately 35000 (Panet et al., 1973). Despite the wide use of the enzyme little is known about its mechanism of action. Such information would seem to be of importance with regard to future application of T_4 polynucleotide kinase. In the present work we have investigated the kinetics of this enzyme as well as some aspects of the substrate specificity. The effects of some activators and inhibitors are described in an accompanying paper (Lillehaug and Kleppe, 1975).

Experimental Section

Materials

Enzymes. T_4 polynucleotide kinase was isolated according to Panet et al. (1973). The purity and specific activity were as described in this paper. Micrococcal nuclease and bacterial alkaline phosphatase were products of the Worthington Biochemical Corporation.

Nucleic Acids and Nucleotides. Calf-thymus DNA and all unlabeled nucleotides were obtained from the Sigma Chemical Co. Oligonucleotides were purchased from P-L Biochemicals. T_7 DNA was isolated as described elsewhere (Lillehaug et al., 1973). [γ-^{32}P]ATP was prepared according to published procedure (Glynn and Chappell, 1964), the initial specific activity being approximately 5 Ci/mmol. The purity was checked by paper chromatography in solvent systems published elsewhere (Kleppe et al., 1971).

Methods

Preparation of Substrates. Calf-thymus DNA was digested with micrococcal nuclease according to a published procedure (Richardson and Kornberg, 1964). The average length of the DNA used in phosphorylation experiments was estimated to be approximately 45 mononucleotide units based on the maximum incorporation of ^{32}P at the 5′ ends. Dephosphorylation of oligonucleotides was carried out using bacterial alkaline phosphatase as previously described (Novogrodsky et al., 1966). Bacterial alkaline phosphatase was inactivated by adjusting the pH to 13 and incubating the reaction mixture for 10 min at room temperature. After neutralization the oligonucleotides were separated from other components by gel filtration on a column of Sephadex G-50 equilibrated with 0.05 M triethylammonium bicarbonate. Native T_7 DNA and single-stranded T_7 DNA, L-strand, were sheared by sonication for 50 sec in a Branson sonicator, and then treated with bacterial alkaline phosphatase as described above. In this case the phosphatase was not removed, but inhibited by orthophosphate present in the reaction mixture.

Assay System. The standard assay for T_4 polynucleotide kinase contained 60 mM Tris (pH 8.0), 9 mM $MgCl_2$, 15 mM β-mercaptoethanol, 0.066 mM [γ-^{32}P]ATP, 0.23 mM calf-thymus DNA, expressed as phosphate, and approximately 1–1.7 units/ml of enzyme. The time of incubation was usually 15 min and the temperature 37°. Under these assay conditions the incorporation of labeled phosphate was

† From the Department of Biochemistry, University of Bergen, Bergen, Norway. *Received October 23, 1974.* This study was supported by the Norwegian Research Council for Science and Humanities and the Nansen Foundation.

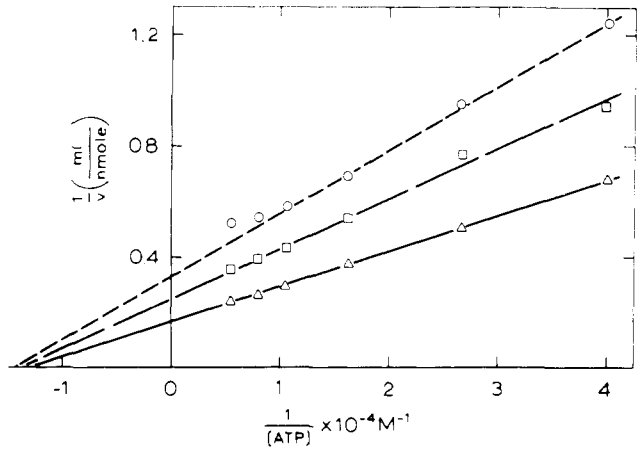


FIGURE 1: Double reciprocal plot of effect of ATP concentration on T_4 polynucleotide kinase activity at different calf-thymus DNA concentrations. Standard assay conditions were employed. Calf-thymus DNA concentrations (phosphate): (○) 95 μM; (□) 142 μM; (△) 237 μM.

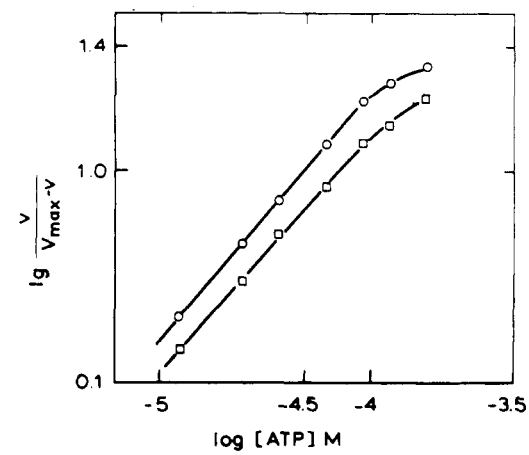


FIGURE 2: Hill plot of data from Figure 1. Calf-thymus DNA concentrations (phosphate): (○) 95 μM; (□) 142 μM.

linear for more than 60 min. The initial rate was proportional to the enzyme concentration up to 8.5 units/ml. The amount of radioactivity incorporated was determined by precipitating the DNA onto Whatman 3MM filter papers as previously described (Kleppe et al., 1971). When assays were carried out with oligonucleotides aliquots were spotted on DEAE paper strips. These were then developed in 0.3 M ammonium formate. After drying, the strips were scanned for radioactivity and the radioactive bands were cut out and counted (Kleppe et al., 1971).

Phosphorylated mononucleotides were separated from [γ-^{32}P]ATP on Whatman 40 paper in two different solvent systems: solvent system I for dTp, rUp, rGp, and rCp: 60 g of ammonium sulfate, 100 ml of 0.1 M sodium phosphate (pH 6.8), and 2 ml of 1-propanol; solvent system II for rAp: isobutyric acid–concentrated ammonia–water (66:1:33, v/v).

Results

Effect of ATP Concentration. The influence of ATP concentration on the rate of reaction at a fixed concentration of DNA was investigated for several types of DNAs. The data in Figure 1 show the results obtained with three different concentrations of calf-thymus DNA. In all cases the double reciprocal plots (Lineweaver and Burk, 1934) yielded straight nonparallel lines having a common crossover point below the 1/[ATP] axis. These results strongly suggest that T_4 polynucleotide kinase reacts according to a sequential mechanism (Cleland, 1970). By replotting the intercepts at the $1/v$ axis vs. 1/[DNA] the true Michaelis constant for calf-thymus DNA, $K_{DNA_{CT}}$, was found to be 8.2×10^{-4} M (phosphate) and the true V_{max} was 30 nmol/ml. Based on the concentration of 5′-hydroxyl ends, the true Michaelis constant was then estimated to be 1.8×10^{-5} M. Using a molecular weight of 140,000 for the enzyme the turnover number can be calculated to be 25,000 min^{-1}. In analogous experiments, the Michaelis constant for 3′-dTp (K_{dTp}) was determined to be 50 μM.

The apparent Michaelis constant for ATP varied somewhat with the DNA substrate used. At the same concentration of DNA substrate, 2×10^{-4} M, the following values were obtained for calf-thymus DNA, T_7 DNA, and $dT(pT)_9$, respectively: 1.4×10^{-4}, 2.6×10^{-4}, and 1.3×10^{-5} M. To obtain the true Michaelis constant for ATP when 3′-dTp was the other substrate, the concentration of 3′-dTp was varied from 4 to 50 μM at three different ATP concentrations. The inverse plots gave straight lines with a common crossover point below the x axis as expected. The intercepts were then replotted vs. 1/[ATP] and the true Michaelis constant for ATP was found to be 4×10^{-5} M from the intercept on the 1/[ATP] axis. When heat-denatured calf-thymus DNA was used the same kinetic constants were obtained as for the native DNA.

Physical studies (Panet et al., 1973) on T_4 polynucleotide kinase have revealed that the active form of the enzyme consists of four subunits and consequently the enzyme may have several active sites. The possibility of allosteric interactions accordingly cannot be ruled out. The data in Figure 1 were therefore replotted according to Hill (1910), Figure 2. The Hill coefficients, n_H, for the three DNA concentrations used were calculated to be 1.16, 1.08, and 1.00. Since all these coefficients are close to 1 it was concluded that allosteric interactions, if present, are of rather little significance.

Influence of DNA Concentrations. The results above suggested that the reaction mechanism was sequential. Further evidence for this view was obtained by determining initial velocities at different DNA concentrations and fixed concentrations of ATP. Again straight nonparallel lines were obtained, having a crossover point below the 1/[DNA] axis. At 62, 124, and 308 μM of ATP the slopes were 0.046, 0.042, and 0.039 mM/nmol per ml, respectively, and the intercepts were determined to be 0.120, 0.083, and 0.056 ml/nmol. At very low concentrations of ATP, i.e. below 0.6×10^{-4} M, some substrate inhibition was observed at high concentrations of DNA. This effect was not observed at high concentrations of ATP. It could also be abolished by addition of salts or polyamines as described in an accompanying paper (Lillehaug and Kleppe, 1975). By plotting the intercepts at the $1/v$ axis against 1/[ATP] the true Michaelis constant for ATP, K_{ATP}, was estimated to be 1.5×10^{-4} M. The data given above were also plotted according to Hill (1910) (not shown). The Hill coefficients were all close to 1.0 thus offering further evidence for noninteracting active sites in the enzyme.

Effects of Products. To understand the reaction mechanism in more detail the inhibition patterns caused by ADP

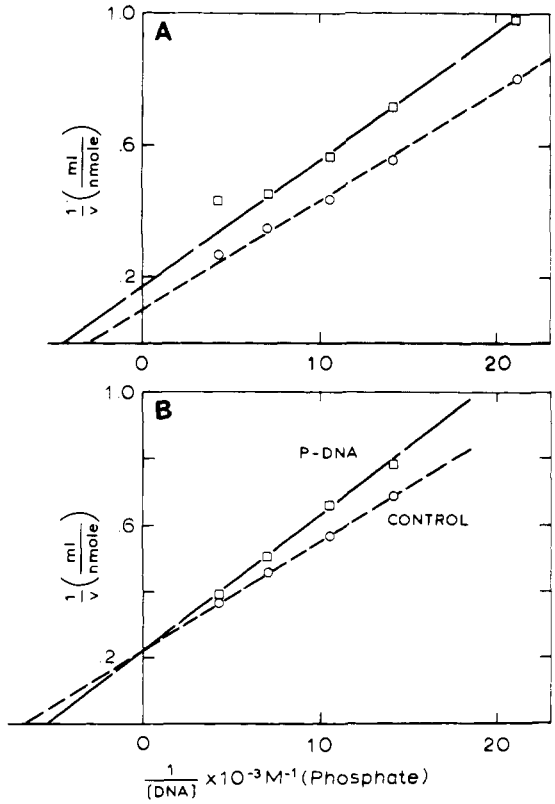


FIGURE 3: Double reciprocal plots of effect of calf-thymus DNA concentration on T_4 polynucleotide kinase activity in the presence of reaction products. Standard assay conditions: (A) (○) control, (□) 9.25×10^{-4} *M* ADP added; (B) (○) control, (□) 1.3×10^{-4} *M* (phosphate) calf-thymus DNA containing 5′-phosphate added.

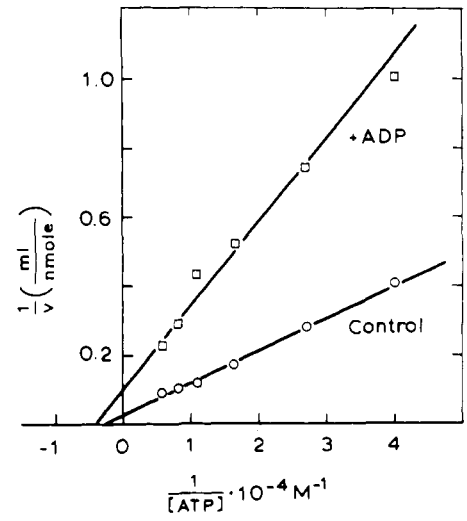


FIGURE 4: Double reciprocal plots of effect of ATP concentration on T_4 polynucleotide kinase activity in the presence of ADP: (○) control, (□) 9.25×10^{-4} *M* ADP added; otherwise standard assay conditions.

and P-DNA,[1] i.e. the two products formed in the forward reaction, were examined. As indicated in Figure 3, P-DNA is a competitive inhibitor of HO-DNA whereas ADP is a noncompetitive inhibitor. Similar findings for P-DNA have been reported for T_2 polynucleotide kinase (Novogrodsky et al., 1966). The apparent inhibitor constant, K_{iADP}, was estimated to be 1.3×10^{-3} *M*. In a separate experiment the effect of ATP on inhibition by ADP was investigated. As shown in Figure 4 the inhibition again appears to be noncompetitive.

Kinetic Parameters for Mono- and Oligonucleotides. Previous studies (Richardson, 1965; van de Sande and Bilsker, 1973) have indicated that T_4 polynucleotide kinase exhibits a rather low specificity with regard to the nucleotide at the 5′-hydroxyl end. However, these studies were all carried out using fixed substrate concentrations. It was therefore of some interest to compare different kinetic parameters of substrates having a defined structure. The apparent Michaelis constant and apparent V_{max} were determined for a number of mono- and oligonucleotides varying both in length and nucleotide composition. The concentration of ATP used was fixed, 66 μM. With some oligonucleotides some substrate inhibition was observed similar to that observed for calf-thymus DNA. At high concentration of ATP no inhibition was detected suggesting that oligonucleotides possibly can bind also to the ATP binding site. Double reciprocal plots at different concentrations of the DNA substrates gave a similar picture to that obtained for calf-thymus DNA.

[1] Abbreviations used are: HO-DNA, a DNA carrying a free 5′-hydroxyl group; P-DNA, a DNA carrying a 5′-phosphoryl group.

Table I: Apparent Michaelis Constants and V_{max} Values for Some Nucleoside 3′-Monophosphates and Oligonucleotides.[a]

Substrate	Apparent Michaelis Constant (μM)	Apparent V_{max}[b] Rel
3′-dTp	22.2	10.5
3′-rUp	53.9	5.3
3′-rCp	43.5	17.0
3′-rAp	41.7	15.0
3′-rGp	143.0	34.3
$dT(pT)_4$	5.5	1.0
$dT(pT)_9$	1.8	2.8
$dT(pT)_{14}$	3.5	2.5
$dC(pC)_9$	13.3	1.4
$dA(pA)_9$	18.8	3.7
$dG(pG)_9$	29.6	9.8
Micrococcal nuclease treated calf-thymus DNA	7.6	16.7

[a] The concentration of ATP was 66 μM; other conditions are as described in the Experimental Section. The concentrations of nucleotides were estimated spectrophotometrically using published extinction coefficients (Cassani and Bollum, 1969; Ts'o et al., 1966; Lefler and Bollum, 1969). [b] The apparent V_{max} for $dT(pT)_4$ was taken as unity.

The kinetic data listed in Table I showed a variation up to 80-fold in the apparent Michaelis constant and 34-fold for the apparent V_{max} values. The apparent Michaelis constant and V_{max} values for the nucleoside 3′-monophosphates were from 2 to 5 times higher than the corresponding oligonucleotides. It is unlikely that this difference can be ascribed to the fact that for all the nucleoside 3′-monophosphates, except Tp, the ribose compounds were employed whereas the oligonucleotides were all of the deoxy form. A more reasonable explanation is that the chain length as well as an extra charge on the 3′-phosphate group has an effect on the binding of the substrate. The former is clearly evident in the case of the thymidylic acid oligomers. The highest values for both the apparent Michaelis constant and V_{max} were obtained with substrates having a 5′-guanine residue. Substrates possessing a 5′-thymidylate terminal nucleotide gave the lowest value. For comparison micrococcal

nuclease treated calf-thymus DNA was also included in the table. Such a DNA contains only adenylate and thymidylate as 5′-terminal nucleotides (Richardson, 1965; Sulkowski ad Laskowski, 1962). In agreement with this observation the apparent Michaelis constant for this DNA was found to have a value between that of the thymidylic acid oligomers and $dA(pA)_9$. The apparent V_{max} for this DNA was, however, surprisingly high. Nucleoside 2′-monophosphates were not phosphorylated to any extent in agreement with earlier findings of Richardson (1965).

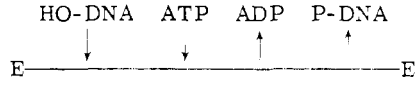
Discussion

In the present work we have investigated the initial reaction rates and determined the kinetic parameters for some of the substrates of T_4 polynucleotide kinase. All experiments were carried out at pH 8.0 and 37°. The overall reaction can be listed as follows for the phosphorylation of a DNA substrate:

$$\text{ATP} + \text{HO-DNA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ADP} + \text{P-DNA} \qquad (1)$$

It has previously been reported (van de Sande et al., 1973) that the overall equilibrium constant $K_{eq} = k_1/k_{-1}$ has the value of approximately 50 at pH 8.0. Furthermore, it has also been proposed that an enzyme-phosphate intermediate is involved in the reaction.

With regard to the overall reaction mechanism two possibilities seem to exist: (1) a ping-pong mechanism and (2) a sequential reaction mechanism. A ping-pong mechanism can be ruled out from the intersecting patterns shown in Figure 1 and the data obtained at different DNA concentrations and, furthermore, the high reversibility of the reaction (van de Sande et al., 1973) excludes any irreversible steps in the mechanism. Such irreversibility is (Cleland, 1963a) a necessary requirement for a ping-pong mechanism. Our data suggest that the enzyme reacts according to a sequential mechanism where both substrates react with the enzyme before either product dissociates. The mechanism could be either of the ordered, Theorell-Chance, or the rapid-equilibrium random type (Cleland, 1963a). It is possible to distinguish between these mechanisms by determining the mode of inhibition caused by the two products formed. The data in Figure 3 clearly indicated that P-DNA was a competitive inhibitor of HO-DNA. The only mechanism which will explain the inhibition data of T_4 polynucleotide kinase by P-DNA is:



(2)

The inhibition data of ADP also support this conclusion. Furthermore, such a reaction scheme is also in complete agreement with data from the reversal experiments where it was shown that reversal only took place in the presence of P-DNA (van de Sande et al., 1973). In the presence of salt or polyamines (Lillehaug and Kleppe, 1975) the mechanism is still sequential but probably of the rapid-equilibrium random type.

Neglecting the reverse reaction the rate equation for phosphorylation of a DNA substrate (Cleland, 1963a) by T_4 polynucleotide kinase can be written as follows:

$$v = \frac{V_1[\text{HO-DNA}][\text{ATP}]}{K_{i\text{HO-DNA}}K_{\text{ATP}} + K_{\text{ATP}}[\text{HO-DNA}] + K_{\text{HO-DNA}}[\text{ATP}] + [\text{HO-DNA}][\text{ATP}]} \qquad (3)$$

$K_{i\text{HO-DNA}}$ can be determined from the point of interception obtained from the kinetic data when calf-thymus DNA is the substrate; $K_{i\text{HO-DNA}} = 11.9 \times 10^{-5}$ M (phosphate) or 2.6×10^{-6} M based on molarity of 5′-hydroxyl ends. It is possible to estimate three of the rate constants from the relationship between the measured kinetic constants (Cleland, 1963a):

$$k_1 = \frac{V_1}{[\text{E}]_t K_{\text{HO-DNA}}} = 4 \times 10^7\ M^{-1}\ \text{sec}^{-1} \qquad (4)$$

$$k_2 = \frac{V_1 K_{i\text{HO-DNA}}}{[\text{E}]_t K_{\text{HO-DNA}}} = 104\ \text{sec}^{-1} \qquad (5)$$

$$\frac{1}{k_4} = \frac{[\text{E}]_t}{V_2} - \frac{1}{k_2} \qquad k_4 = 12\ \text{sec}^{-1} \qquad (6)$$

where V_2 was estimated from the data published by van de Sande et al. (1973). When calculating $[E]_t$ we have used a mol wt of 140,000 for the enzyme. However, since the enzyme molecule consists of four subunits the possibility exists that there are several active sites on the enzyme. Experiments are currently in progress to determine the number of active sites. The rate constants may therefore have to be revised when such data become available.

It may be argued that the rate constants obtained using micrococcal treated calf-thymus DNA as substrate are not well defined due to the presence of both adenylate and thymidylate as 5′-terminal nucleotides. We have therefore determined k_1, k_2, and k_4 when 3′-dT_p is the substrate. The values obtained were $0.8 \times 10^7\ M^{-1}\ \text{sec}^{-1}$, $45\ \text{sec}^{-1}$, and $9\ \text{sec}^{-1}$, respectively. As can be seen, only k_1 differs significantly.

In the case of T_2 polynucleotide kinase certain kinetic parameters have been reported (Novogrodsky et al., 1966). Direct comparison with the present results is difficult since different conditions were employed in the assays and furthermore only apparent V_{max} and Michaelis data were given. In general, though, it would appear that the kinetic parameters for the two enzymes are similar.

The data obtained with mono- and oligonucleotides may suggest that T_4 polynucleotide kinase possesses a certain specificity with regard to 5′-terminal nucleotide. This finding could be of importance with regard to practical application of the enzyme. The question may also be raised whether or not there are differences in kinetic parameters with regard to phosphorylation of single- and double-stranded DNAs or RNAs, in particular double-stranded molecules containing protruding 3′-hydroxyl ends. Some experiments with double-stranded DNAs of defined sequence (K. Kleppe and H. G. Khorana, unpublished) have shown that these differ vastly with regard to rate of phosphorylation. The micrococcal nuclease treated calf-thymus DNA used in the present work probably is more or less completely single stranded since no differences were obtained between native and heat denatured substrate. Further experiments with single- and double-stranded DNAs of defined sequence should provide more information concerning the specificity and in vivo role of T_4 polynucleotide kinase.

Acknowledgment

We are indebted to Kristin Kinstad for excellent technical assistance.

References

Agarwal, K. L., Büchi, H., Caruthers, M. H., Gupta, N., Kleppe, K., Kumar, A., Ohtsuka, E., Raj Bhandary, U.

L., van de Sande, J. H., Sgaramella, V., Weber, H., Yamada, T., and Khorana, H. G. (1970), *Nature (London) 227,* 27.
Caruthers, M. H., Agarwal, K., Besmer, P., Cashion, P., Fridkin, M., Jay, E., Kumar, A., Loewen, P., Miller, R., Minamoto, K., Rama Moorty, B., Panet, A., van de Sande, J. H., Sekia, T., Siderova, N., Khorana, H. G. (1973), Ninth International Congress of Biochemistry, Stockholm, Abstract 3010, p 194.
Cassani, G. R., and Bollum, F. J. (1969), *Biochemistry 8,* 3928.
Cleland, W. W. (1963a), *Biochim. Biophys. Acta 67,* 104.
Cleland, W. W. (1963b), *Biochim. Biophys. Acta 67,* 173.
Cleland, W. W. (1970), *Enzymes, 3rd Ed., 2,* 1.
Glynn, I. M., and Chappell, J. B. (1964), *Biochem. J. 90,* 147.
Hill, A. V. (1910), *J. Physiol. (London) 40,* iv–viii.
Jay, E., Bambana, R., Padmanabhan, R., and Wu, R. (1974), *Nucleic Acids Res. 1,* 331.
Khorana, M. G., Agarwal, K. L., Büchi, H., Caruthers, M. H., Gupta, N. K., Kleppe, K., Kumar, A., Ohtsuka, E., Raj Bhandary, U. L., van de Sande, J. H., Sgaramella, V., Terao, T., Weber, H., and Yamada, T. (1972), *J. Mol. Biol. 72,* 209.
Kleppe, K., Ohtsuka, E., Kleppe, R., Molineux, I. J., and Khorana, H. G. (1971), *J. Mol. Biol. 56,* 341.
Lefler, C. F., and Bollum, F. J. (1969), *J. Biol. Chem. 224,* 594.
Lillehaug, J. R., Helland, D., and Sjoberg, N. O. (1973), *FEBS Lett. 33,* 335.
Lillehaug, J. R., and Kleppe, K. (1975), *Biochemistry,* following paper.
Lineweaver, H., and Burk, D. (1934), *J. Am. Chem. Soc. 56,* 658.
Murray, K. (1973), *Biochem. J. 131,* 569.
Novogrodsky, A., and Hurwitz, J. (1966), *J. Biol. Chem. 241,* 2923.
Novogrodsky, A., Tal, M., Traub, A., and Hurwitz, J. (1966), *J. Biol. Chem. 241,* 2933.
Panet, A., van de Sande, J. H., Lowen, P. C., Khorana, H. G., Raae, A. J., Lillehaug, J. R., and Kleppe, K. (1973), *Biochemistry 12,* 5045.
Richardson, C. C. (1965), *Proc. Natl. Acad. Sci. U.S.A. 54,* 158.
Richardson, C. C., and Kornberg, A. (1964), *J. Biol. Chem. 239,* 242.
Southern, E. M. (1970), *Nature (London) 227,* 794.
Sulkowski, E., and Laskowski, M. (1962), *J. Biol. Chem. 237,* 2620.
Szekely, M., and Sanger, F. (1969), *J. Mol. Biol. 43,* 607.
Ts'o, P., Rapaport, S. A., and Bollum, F. J. (1966), *Biochemistry 5,* 4153.
van de Sande, J. H., and Bilsker, M. (1973), *Biochemistry 12,* 5056.
van de Sande, J. H., Kleppe, K., and Khorana, H. G. (1973), *Biochemistry 12,* 5050.
Weiss, B., and Richardson, C. C. (1967), *J. Mol. Biol. 23,* 405.

Effect of Salts and Polyamines on T_4 Polynucleotide Kinase†

Johan R. Lillehaug and Kjell Kleppe*

ABSTRACT: The activity of T_4 polynucleotide kinase (EC 2.7.1.78) was found to be greatly stimulated by salts, such as NaCl and KCl, and polyamines such as spermine and spermidine. Up to a sixfold increase in initial rates was observed with a variety of different single-stranded DNAs and mono- and oligonucleotides. The optimal concentrations of salts were 0.125 *M*, corresponding to a total ionic strength of $\mu = 0.19$. For polyamines the optimal concentrations were found to be at approximately 2 m*M*. With low enzyme concentration and in the absence of activators complete phosphorylation was not achieved for a number of substrates. In the presence of salts or polyamines or high concentration of enzyme the phosphorylation proceeded to completion. Addition of salt led to an increase in both the apparent V_{max} and the Michaelis constant for the DNA substrate whereas the Michaelis constant of ATP remained unchanged. Polyamines had a similar influence on the kinetic constants for the DNA substrate whereas a decrease was found for the apparent Michaelis constant for ATP. The overall mechanism in the presence of activators was found to be sequential but probably of a rapid equilibrium random type. Of the inorganic anions tested both P_i and PP_i inhibited the enzyme in a competitive manner with both substrates.

Polynucleotide kinase (T_4) (EC 2.7.1.78) catalyzes the phosphorylation of 5′-hydroxyl termini of nucleic acids and oligo- and mononucleotides using ATP as a phosphate donor (Richardson, 1965). The enzyme has recently been purified to homogeneity and some of its properties studied (Panet et al., 1973). Despite its wide use today in structural work on nucleic acids little is known about the function of this enzyme in vivo.

A number of factors are likely to influence the activity of T_4 polynucleotide kinase. With regard to practical application, function, and regulation in vivo, information about these aspects would seem to be of importance. In the present work we describe the effect of some activators and inhibitors of T_4 polynucleotide kinase employing mono- and

† From the Department of Biochemistry, University of Bergen, Bergen, Norway. *Received October 23, 1974.* This study was supported by the Norwegian Research Council for Science and Humanities and the Nansen Foundation.