Biochimica et Biophysica Acta, 660 (1981) 348–358
Elsevier/North-Holland Biomedical Press

BBA 69324

KINETIC PROPERTIES OF A NUCLEOSIDE PHOSPHOTRANSFERASE OF CHICK EMBRYO

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(Received September 25th, 1980)
(Revised manuscript received March 3rd, 1981)

Key words Nucleoside phosphotransferase Development Kinetic properties, (Chick embryo)

1 A nonspecific nucleoside phosphotransferase (nucleotide 3′-deoxynucleoside 5′-phosphotransferase, EC 2 7 1 77), purified from chick embryos, catalyzes the transfer of phosphate ester from a nucleotide donor to a nucleoside acceptor 2 The enzyme exhibits sigmoidal kinetics with respect to nucleoside monophosphate donors, but with respect to nucleoside di- or triphosphate donors and nucleoside acceptors hyperbolic kinetics were obtained 3 The nucleoside phosphotransferase of chick embryo is unstable to heat and is protected from inactivation by a large number of nucleotides 4 Nucleoside di- and triphosphates lower both the concentration of nucleoside monophosphates required for half-maximal velocity and the kinetic order of reaction measured with these phosphate donors On the contrary, nucleoside di- or triphosphates do not modify the kinetic parameters evaluated for nucleoside acceptors 5 We suggest that the nucleoside phosphotransferase contains both substrate and regulatory sites It seems that the free apoenzyme is converted, by means of cooperative interactions between regulatory sites, into an enzyme-nucleotide complex, which is particularly stable at 37°C

Introduction

Many authors [1–9] have described a group of enzymes, designated nucleoside phosphotransferases (nucleotide 3′-deoxynucleoside 5′-phosphotransferase, EC 2 7 1 77), which catalyze, in plants and in microorganisms, the transfer of organically esterified phosphoric acid to a nucleoside, it is suggested that these enzymes take part in the biosynthesis of nucleotides

A nucleoside phosphotransferase, which also exhibits hydrolase activity, was purified from carrots by Brunngraber and Chargaff [10–12] This enzyme has a pH optimum of about 5 0 and an M_r 44 000 in the active state, whereas its aggregates at a higher molecular weight are inactive

The presence of a nonspecific nucleoside phosphotransferase, which can transfer the ester phosphate from a nucleotide donor to a nucleoside acceptor, has also been reported in chick embryo and in adult chick liver [13] In previous papers [14–16], we described a purification and some properties of this enzyme and confirmed [13] that it is very different from the enzyme produced from carrots

Our present study has focused on the kinetic properties of nucleoside phosphotransferase from chick embryo It was observed that this enzyme is markedly affected by nucleotides which not only behave as phosphate donors but also as effectors, because they control the enzymic reaction and protect nucleoside phosphotransferase against thermal inactivation as well

We suggest that a nucleotide protector (N) combines reversibly with the enzyme (E) to form an enzyme-nucleotide complex (EN_n), which is less readily inactivated than the free enzyme

$$\begin{array}{ccc} & \overset{k_1}{\rightleftharpoons} & \\ E + N_n & \underset{k_2}{} & EN_n \\ \Big\downarrow k_0 & & \Big\downarrow k_\infty \\ F_1 & & F_2 \end{array} \qquad (1)$$

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In Eqn 1 F_1 and F_2 indicate low molecular weight (about 30000) enzymic forms which are almost inactive [16], whereas k_0 and k_∞ represent, according to Chuang and Bell [17], the first-order rate constants of inactivation of the free enzyme and of the enzyme-nucleotide complex, respectively

As postulated by Chuang and Bell [17], Eqn 2

$$v = k_0(E) + k_\infty(EN_n) = k[(E) + (EN_n)] \quad (2)$$

indicates that at a fixed nucleotide concentration, the total rate of inactivation is equal to an experimentally determined rate constant of inactivation (k), multiplied by the total concentration of the enzyme

The inactivation rate constant (k_∞) of an enzyme-protector complex and the protection constant (π) can be determined by means of one method of graphic analysis based, as reported by Chuang and Bell [17], on Eqn 3

$$\frac{1}{(k_0 - k)} = \frac{\pi}{(k_0 - k_\infty)} \frac{1}{(X)} + \frac{1}{(k_0 - k_\infty)} \quad (3)$$

π differs from K_m because it has the significance of a dissociation constant According to Burton [18], π value corresponds to the concentration of the protecting compound (X) which causes the half-maximal protection of the enzyme against thermal inactivation

Materials and Methods

Materials

Nucleotides and nucleosides were supplied by Boehringer, Biochemia (s r l Milan) Radioactive nucleosides were obtained from Sorin (Saluggia, Italy)

Methods

To evaluate the rate constants of inactivation, the standard reaction mixture contained, in 280 μl, 20 μmol Tris-HCl buffer (pH 8 8), 2 5 μmol $MgCl_2$ and variable concentrations of a nucleotide protector After 5 min at 37°C, an aliquot equivalent to 2 μl enzyme preparation (about 13 μg protein), diluted 1 10 immediately before use, was added and the samples were preincubated at 37°C for various time intervals The concentration of nucleotide in each sample was then rapidly adjusted to 5 mM and 10 nmol (0 5 μCi) [Me-^{3}H]thymidine were added The incubation was continued for 10 min at 37°C in a final volume of 500 μl

To evaluate the dependence of reaction rate upon phosphate donor concentration the standard reaction mixture contained 40 mM Tris-HCl buffer (pH 8 8), 5 mM $MgCl_2$, 20 μM (0 5 μCi) [Me-^{3}H]thymidine or other nucleoside and variable concentrations of a nucleotide After a 5 min at 37°C, 20 μl diluted enzyme were added and the samples were incubated at 37°C for 10 min, in a final volume of 500 μl

The reaction was stopped by adding 0 5 ml 10% trichloroacetic acid and the nucleoside phosphotransferase activity was measured [14]

1 enzyme unit is taken as the amount of enzyme which converts 1 nmol deoxy-thymidine (or other nucleoside) to the relating nucleoside monophosphate, in 10 min

Although many nucleotides behave as phosphate donors or effectors, only data concerning UMP and UDP are shown in the figures

We used an $(NH_4)_2SO_4$ preparation, obtained following an earlier procedure [16], performed in the absence of a nucleotide protector, which consists of successive precipitations by means of protamine sulfate and $(NH_4)_2SO_4$ It was possible to achieve higher degrees of purification (about 900-fold) by submitting the $(NH_4)_2SO_4$ preparation to two successive DEAE-cellulose steps [16], but in this case it was necessary to protect the enzyme with nucleotides (50 μM dTTP or UDP) After DEAE-cellulose chromatography, the enzyme was precipitated by means of $(NH_4)_2SO_4$ and then dialyzed for 2 h against 10 vol 5 mM Tris-HCl buffer (pH 8 0) With this preparation, we have confirmed the most important experimental observations reported in this paper, but the results of these experiments were affected by the presence of the nucleotide protector, even if in small aliquots, in the dialyzate On the other hand, dialysis performed against a greater volume of Tris-HCl or for a longer period of time caused a marked inactivation of the enzyme For these reasons, the results obtained with the enzyme at high degrees of purity are not reported in this paper We preferred to report the results with the less purified preparation because, in this case, the nucleoside phosphotransferase was much more stable during dialysis In particular, an $(NH_4)_2SO_4$ preparation,

purified 120-fold, with a specific activity of 150 U/mg protein was used

Results

Protection of nucleoside phosphotransferase against thermal inactivation

Inactivation and protection constants Our experiments have demonstrated that a large number of nucleotides protect nucleoside phosphotransferase against inactivation at 37°C These include the deoxyribonucleotides and the pyrimidine ribonucleotides, whereas purine ribonucleotides showed little effect

The inactivation of the nucleoside phosphotransferase follows, both in the absence and in the presence of nucleotide protectors, a first-order reaction (Fig 1), expressed by Eqn 4

$$\log(\%\text{ activity remaining}) = -(k/2\,303)\,t + 2 \qquad (4)$$

where $-(k/2\,303)$ is the slope

Double-reciprocal plots of $1/(k_0 - k)$ vs 1/(nucleotide), based on Eqn 3 (Fig 2), indicate a non-linear relationship, whatever nucleotide protector is employed Consequently, we have indicated the concentration of the protecting nucleotide which causes half-maximal protection with the term $P_{0\,5}$

In Tables I and II, $P_{0\,5}$ values indicate that within each class of nucleotides, diphosphate forms are the most effective protectors, followed by triphosphates, whereas the respective monophosphates are much less effective Among all the nucleotides tested, the highest degree of protection was exhibited by pyrimidine deoxyribonucleoside diphosphates (dUDP and dTDP) Low values of $P_{0\,5}$ were also measured for the corresponding triphosphate forms (dUTP, dTTP and dCTP) and for the pyrimidine ribonucleoside diphosphates (UDP and CDP) The protective effect seems to be specific for the nucleotides, because neither nucleosides nor a large number of phosphate esters were able to reduce the inactivation of nucleoside phosphotransferase at 37°C

Another interesting observation suggested by Fig 2 is that, in our experimental conditions, the inactivation of the enzyme-nucleotide complex is negligible

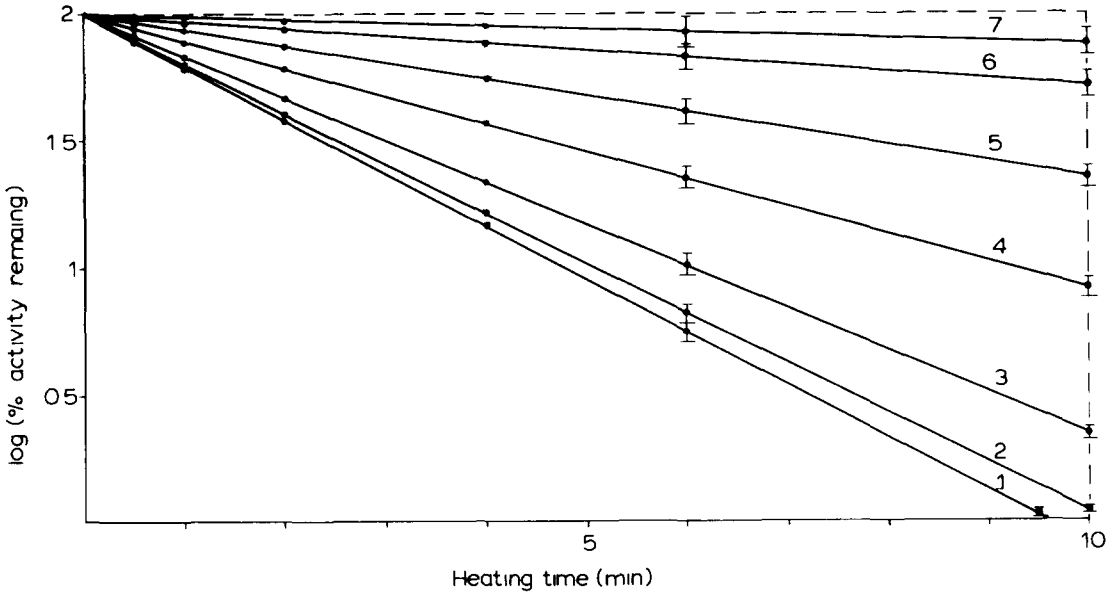

Fig 1 Time course of inactivation at 37°C of nucleoside phosphotransferase Each sample was preincubated, as described in Materials and Methods, in the absence or presence of varying UMP concentrations At the indicated times, UMP was adjusted to 5 mM and 10 nmol deoxy-thymidine were added The incubation was protracted for 10 min The values of the inactivation constants were obtained from the slopes of the lines 1, preincubation without UMP, 2, 3, 4, 5, 6, 7 preincubated with 0 05, 0 1, 0 2, 0 3, 0 5, 1 0 mM UMP, respectively Values are the mean ± S E of ten separate experiments

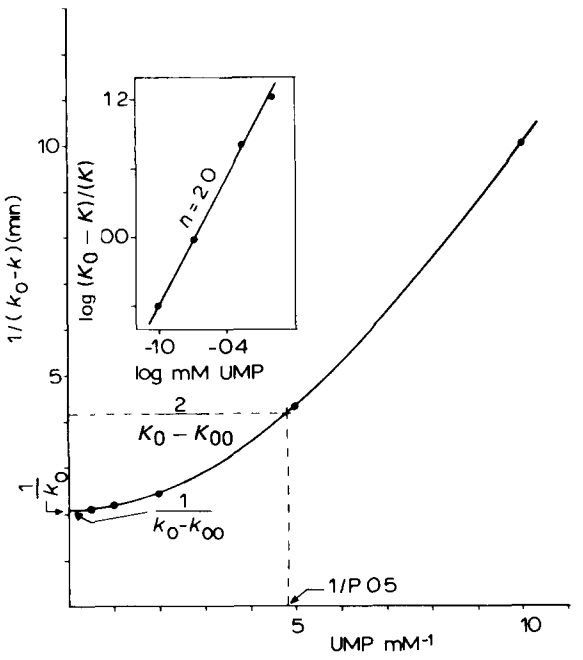

Fig 2 Double-reciprocal plot, based on Eqn 3, of $1/(k_0 - k)$ vs 1/(UMP) k values indicate the first-order rate constants of inactivation measured in the presence of variable UMP concentrations k_0 and k_∞ correspond to the first-order rate constant of inactivation in the absence of UMP and the presence of saturating UMP concentrations, respectively $P_{0.5}$ is the concentration of UMP that affords half-maximal protection of the enzyme against inactivation at 37°C Inset shows logarithmic plot of $(k_0 - k)/k$ vs (UMP) based on Eqn 7

In fact, we cannot distinguish the value of $1/(k_0 - k_\infty)$, indicated in the figure by the intercept on the ordinate, from $1/k_0$ (2 08 ± 0 24 min) Therefore, k_∞ assumes a value near zero Data reported in Fig 2 were obtained by using UMP as the protector, but the same observations were made with all the nucleotide protectors employed

One method to evaluate cooperative interactions between different sites by means of the rate constants of inactivation As reported above, the relationship between $1/(k_0 - k)$ and 1/(nucleotide) is not linear To determine if the protective effect against thermal inactivation depended upon cooperative interactions, we have developed a procedure which makes use of the rate constants of inactivation In Eqn 1 the enzyme-nucleotide complex is represented by EN_n, where n indicates the minimal number of nucleotide molecules that must be assumed to interact with each enzyme molecule to explain the observed protective response Eqn 2 can be rewritten as

$$\frac{(EN_n)}{(E)} = \frac{(k_0 - k)}{(k - k_\infty)} \tag{5}$$

Besides, for an enzyme with n interacting sites

$$\frac{(EN_n)}{(E)} = \frac{(N)^n}{K} \tag{6}$$

where K is the product of n dissociation constants [19] (K corresponds to π value only when $n = 1$)

TABLE I

KINETIC PARAMETERS OF NUCLEOSIDE PHOSPHOTRANSFERASE WITH RESPECT TO NUCLEOSIDE DI- AND TRIPHOSPHATES

20 μM (0 5 μCi) thymidine were employed as the acceptor V values are expressed as nmol dTMP formed/10 min per 13 μg protein (2 μl enzyme preparation) n_1 and n_2 represent, respectively, the interaction coefficients measured by means of Eqn 7 and by Hill equations Values are the mean ± S E of six separate experiments

Nucleotides	$S_{0.5}$ (μM)	V	$P_{0.5}$ (μM)	n_1	n_2
CDP	2 500 ± 215	0 65 ± 0 05	15 0 ± 1 22	2 05 ± 0 18	1 18 ± 0 09
UDP	800 ± 71	0 69 ± 0 06	4 5 ± 0 38	2 04 ± 0 20	1 06 ± 0 10
dTDP	360 ± 30	1 80 ± 0 16	1 5 ± 0 11	2 01 ± 0 19	1 19 ± 0 11
dUDP	550 ± 48	1 72 ± 0 16	0 7 ± 0 06	1 97 ± 0 17	1 17 ± 0 09
UTP	2 050 ± 192	0 37 ± 0 03	38 1 ± 3 24	2 00 ± 0 18	1 09 ± 0 08
dATP	2 400 ± 221	0 45 ± 0 04	22 2 ± 1 98	2 02 ± 0 20	1 12 ± 0 10
dGTP	3 070 ± 279	0 25 ± 0 02	60 0 ± 5 45	1 98 ± 0 16	1 10 ± 0 09
dCTP	360 ± 31	1 66 ± 0 12	7 0 ± 0 61	2 02 ± 0 18	1 02 ± 0 09
dTTP	220 ± 19	1 14 ± 0 09	4 0 ± 0 31	1 91 ± 0 17	1 00 ± 0 08
dUTP	830 ± 71	1 00 ± 0 08	1 7 ± 0 13	1 90 ± 0 16	1 05 ± 0 09

TABLE II

KINETIC PARAMETERS OF NUCLEOSIDE PHOSPHOTRANSFERASE WITH RESPECT TO NUCLEOSIDE MONOPHOSPHATES IN THE ABSENCE OR PRESENCE OF 20 μM UDP

20 μM (0 5 μCi) thymidine were employed as the acceptor Values are the mean ± S E of six separate experiments

Nucleotides	Without UDP					With 20 μM UDP		
	$S_{0\,5}$ (μM)	V	$P_{0\,5}$ (μM)	n_1	n_2	$S_{0\,5}$ (μM)	V	n_2
CMP	950 ± 81	1 87 ± 0 14	500 ± 42	2 11 ± 0 18	2 04 ± 0 16	200 ± 17	1 85 ± 0 14	1 02 ± 0 08
UMP	370 ± 29	1 92 ± 0 16	210 ± 18	2 00 ± 0 16	2 22 ± 0 19	170 ± 13	1 90 ± 0 15	1 08 ± 0 08
dAMP	520 ± 44	1 80 ± 0 13	220 ± 19	2 07 ± 0 16	1 95 ± 0 15	280 ± 21	1 80 ± 0 13	1 01 ± 0 07
dGMP	810 ± 72	2 00 ± 0 19	420 ± 34	2 10 ± 0 17	2 12 ± 0 18	350 ± 29	1 95 ± 0 16	0 98 ± 0 06
dTMP	50 ± 4	1 96 ± 0 16	26 ± 2	1 98 ± 0 15	2 00 ± 0 16	30 ± 3	1 90 ± 0 14	1 10 ± 0 09
dUMP	36 ± 3	1 96 ± 0 15	17 ± 2	2 02 ± 0 17	2 00 ± 0 15	20 ± 2	1 95 ± 0 15	1 20 ± 0 10

From Eqn 5 and 6 we obtain

$$\log \frac{(k_0 - k)}{(k - k_\infty)} = n \log(\mathrm{N}) - \log K \qquad (7)$$

Thus, a plot of $\log[(k_0 - k)/(k - k_\infty)]$ as a function of log (nucleotide) should give a straight line of slope n

Logarithmic plots of $(k_0 - k)/k$ vs (nucleotide) (inset in Fig 2) (k_∞ is not considered because its value is near zero) yielded, for all the nucleotides tested, interaction coefficients of about 2 (n_1 in Tables I and II) Thus, the protection of the nucleoside phosphotransferase against thermal inactivation should be a function of cooperative interactions between different sites

We suggest that the above-reported procedure might be a useful method for investigating other enzymes, in order to determine if a mechanism of protecting against thermal inactivation depends upon cooperativity induced by effectors

Dependence of reaction rate upon phosphate donor concentration

Since, as reported above, nucleoside phosphotransferase is unstable at 37°C and nucleotides also behave as protectors, our data were distorted owing to the enzyme inactivation, which was variable in relation to the level of the nucleotide phosphate donor It was possible to correct this distortion by means of Eqn 8

$$v = \frac{V_{\mathrm{meas}} \times 20}{\mathrm{A}} \qquad (8)$$

where A is a geometrical area, which varies according to the level employed This corresponds to a triangle, when the enzyme is completely inactivated within 10 min (line 1 in Fig 1), to a trapezium (lines 2–7) when the enzyme is partially inactivated within 10 min and to a rectangle (with an area equal to 20, broken line) if the nucleoside phosphotransferase is not inactivated under our experimental conditions V_{meas} corresponds to the value of phosphotransferase activity experimentally measured in the presence of an established concentration of nucleotide, whereas v indicates the value which might be obtained if the enzyme were not inactivated by heat

For a correct application of the above-described procedure, the concentration of the nucleotide employed to measure the enzyme activity must remain almost constant during incubation With this in mind, our experimental condition was chosen so that only a small percentage of the nucleotide employed, especially at low substrate donor levels, was consumed in 10 min (about 0 1–0 4%) After the correction of the data, the reaction rate was linear with incubation time for at least 10 min This was also true with low levels of a nucleotide donor

In this paper only data related to an incubation time of 10 min were employed after correction by means of Eqn 8, also to measure $S_{0\,5}$ and V With a lower incubation time, values were often very low and of a minor statistical value, particularly at low levels of some nucleoside di- and triphosphates

Our results have shown that the enzyme can

employ a large number of nucleotides as phosphate donors, which include all the deoxyribonucleotides and the pyrimidine ribonucleotides tested Among the purine ribonucleotides, AMP has shown only slight activity, whereas ADP and ATP were inactive Within each class of nucleotides, the highest V and the lowest value of $S_{0.5}$ (Table I and II) were observed with the monophosphate forms which, therefore, appear to be the preferred phosphate donors

With respect to nucleoside monophosphates, the substrate-velocity plots were sigmoidal and the reciprocal plots were not linear (UMP, Fig 3) In these cases, Hill plots of the $\log[v/(V-v)]$ vs log (nucleotide) yielded interaction coefficients of about 2 (n_2 in Table II, UMP in Fig 4) On the contrary, as far as nucleoside and di- and triphosphates are concerned, the kinetic responses were hyperbolic and the reciprocal plots linear (UDP, Fig 3) In these cases, the interaction coefficients were about 1 (n_2 in Table I, UDP in Fig 4)

Among the nucleotides employed as phosphate donors the lowest values of $S_{0.5}$ were observed for

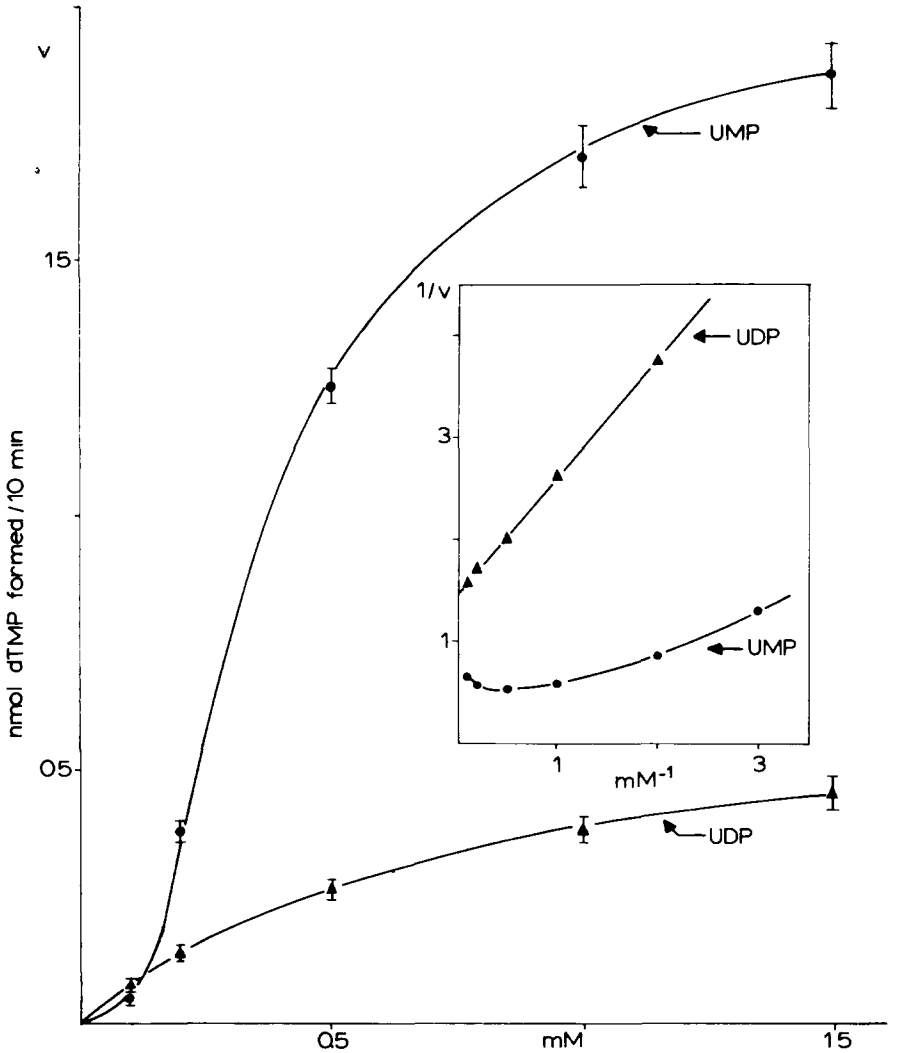

Fig 3 Substrate-velocity plots with respect to nucleotide phosphate donors (●——●, UMP, ▲——▲, UDP) by using 20 μM deoxy-thymidine as acceptor. Experimental conditions were as described in Materials and Methods Values are the mean ± S E of ten separate experiments Inset shows double-reciprocal plots of the same data

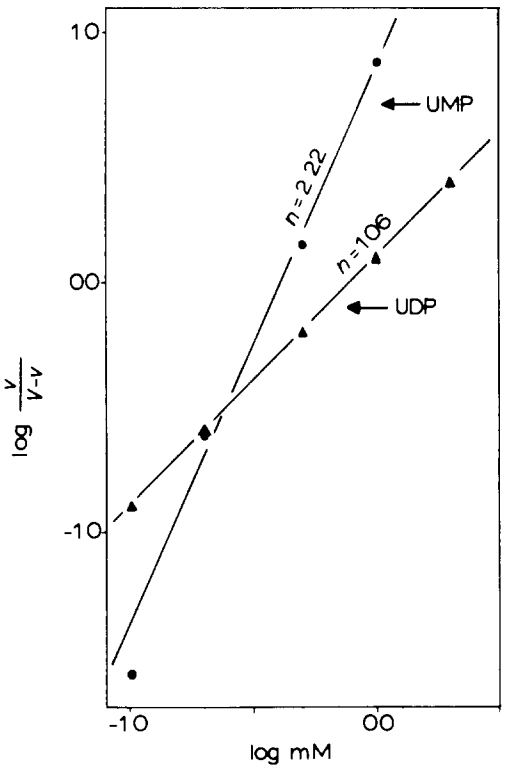

Fig 4 Hill plots with UMP (●——●) and UDP (▲——▲) varied

pyrimidine deoxyribonucleoside monophosphates (dTMP and dUMP) (Table II), although the kinetic response obtained with these compounds shows a particular characteristic As described in Fig 5, v increases, at first, in relation to mM concentration of dTMP, reaches a maximum when dTMP is about 0 5 mM and then decreases The response with dUMP was similar This phenomenon was also observed with UMP (Fig 5) and CMP, although V corresponds, in these cases, to a greater concentration of nucleotide (about 2 mM)

The results reported in this paper were generally obtained by using deoxy-thymidine as the nucleoside acceptor When other nucleosides were employed as acceptors (uridine, Table III) a reduction of V was found with respect to various nucleotide donors, although no consistent variation was observed for either $S_{0\ 5}$ or the kinetic order of reaction

Effects of nucleoside diphosphates and triphosphates on the nucleoside phosphotransferase reaction

Nucleoside di- and triphosphates, except for the purine ribonucleotides, also exert a clear effect on the enzymic reaction The addition of small aliquots of one of these nucleotides diminishes the sigmoidicity

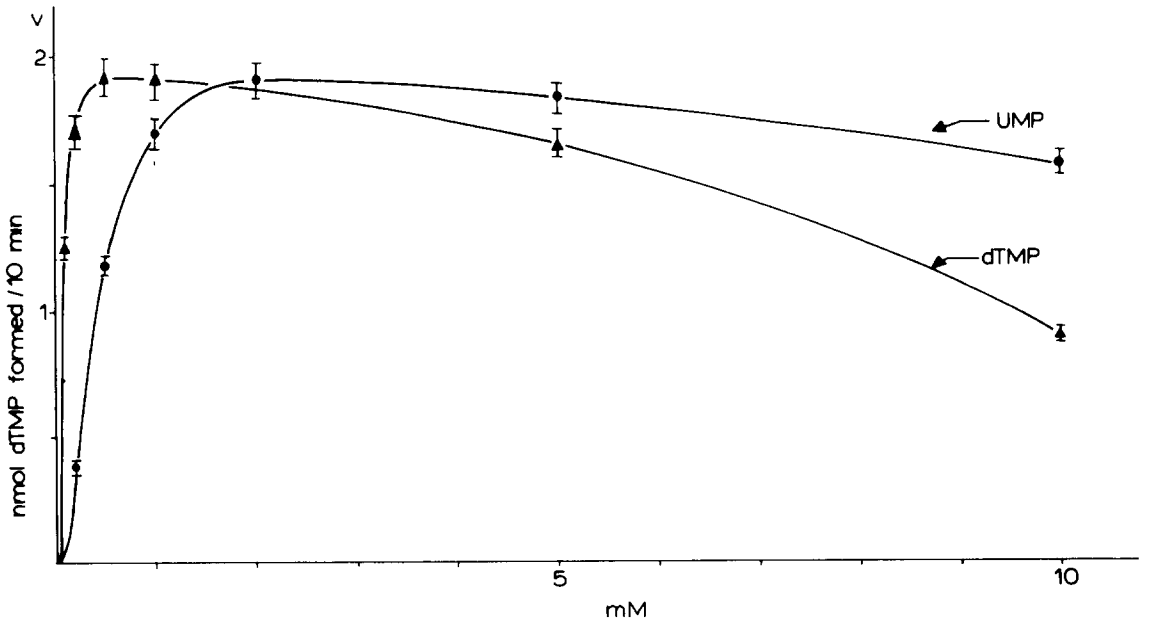

Fig 5 Dependence of the reaction rate upon UMP (●——●) and dTMP (▲——▲) concentrations 20 μM deoxy-thymidine were used as acceptor Values are the mean ± S E of ten separate experiments

TABLE III

$S_{0.5}$ AND V VALUES WITH RESPECT TO VARIOUS NUCLEOTIDES BY USING 20 μM (0 5 μCi) URIDINE AS ACCEPTOR

Values are the mean ± S E of six separate experiments

Nucleotides	$S_{0.5}$ (μM)	V
CMP	1 440 ± 119	1 29 ± 0 11
UMP	380 ± 31	1 08 ± 0 09
dGMP	1 150 ± 103	1 22 ± 0 10
dTMP	55 ± 5	1 15 ± 0 09
UDP	810 ± 72	0 35 ± 0 03
dTTP	300 ± 26	0 95 ± 0 08

of the rate curve obtained with respect to nucleoside monophosphates In the presence of 20 μM UDP, the substrate-velocity plots become hyperbolic, the reciprocal plots linear (Fig 6) and the kinetic orders of the reaction are about 1 (Fig 7) Furthermore, 20 μM UDP lowers the values of $S_{0.5}$ for the monophosphate forms (Table II) about 2-fold, whereas it does not modify the values obtained for di- and triphosphates

The above-reported effects are exerted by all the nucleoside di- and triphosphates listed in Table I, independent of the nucleoside monophosphate employed as the phosphate donor and the nucleoside employed as the acceptor

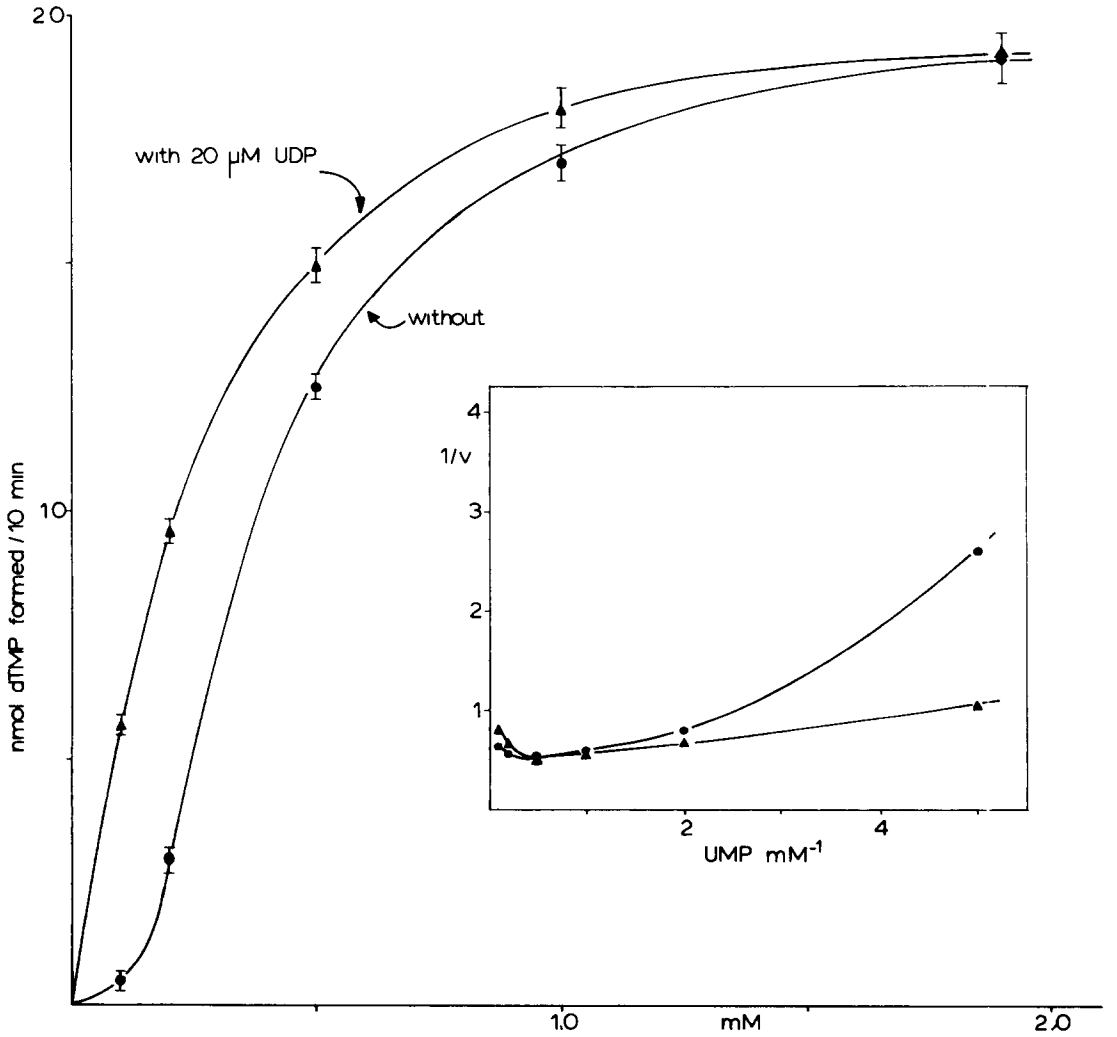

Fig 6 Substrate-velocity plots, with UMP varied, in the absence (●——●) and presence of 20 μM UDP (▲——▲) 20 μM deoxy-thymidine were employed as acceptor Values are the mean ± S E of ten separate experiments Inset shows double-reciprocal plots of the same data

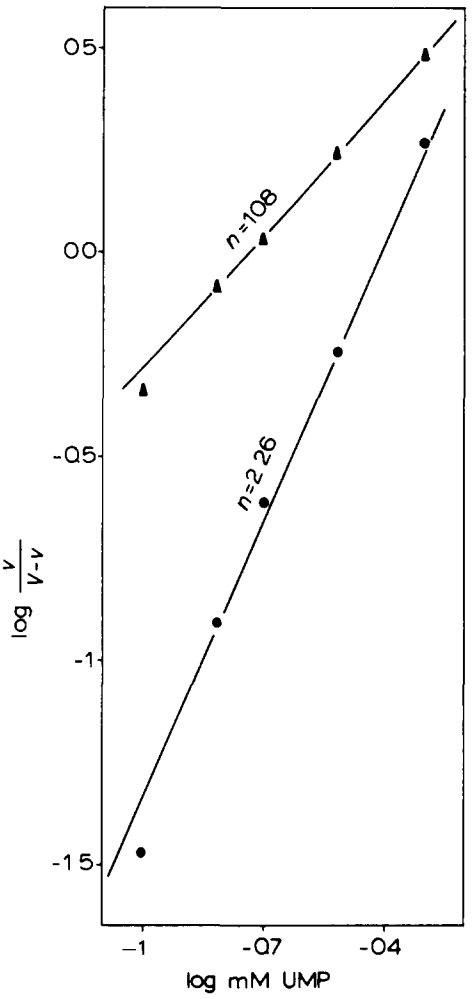

Fig 7 Hill plots, with UMP varied, without (●——●) and with 20 μM UDP (▲——▲)

At this point it seems possible to conclude that nucleoside di- and triphosphates cause two distinct effects, one concerns the enzymic reaction and the other is related to the protection of the enzyme These effects are indicated in Fig 8 which permits us to distinguish three successive stages (1) At an increasing UDP concentration, at first, an increase in the reaction rate is observed, expressed by sigmoidal behaviour, whereas inactivation of the enzyme has not yet occurred (2) Successively an increase in UDP concentration reduces the thermal inactivation of the enzyme (3) Finally, the increment of the effector above 5 μM causes a decrease in the reaction rate It

TABLE IV

$S_{0.5}$ AND V VALUES WITH RESPECT TO VARIOUS NUCLEOSIDES

5 0 mM UMP were employed as the phosphate donor For these experiments the specific radioactivity of the nucleoside acceptors was 2 μCi/μmol Values are the mean ± S E of six separate experiments

Nucleosides	$S_{0.5}$ (μM)	V
Cytidine	850 ± 72	8 60 ± 0 70
Uridine	750 ± 68	9 10 ± 0 81
d-Thymidine	270 ± 20	43 50 ± 3 86
d-Uridine	320 ± 24	39 80 ± 3 51

is interesting to note that the sigmoidal response was exhibited at such a low concentration of UDP (2–3 μM) that the enzyme stability is not consistently modified, whereas the decrease of the enzymic reaction was observed at a concentration of UDP sufficient to protect the enzyme almost entirely against its inactivation These phenomena were also demonstrated with the other effectors employed

Dependence of the reaction upon nucleoside acceptor concentration

With respect to nucleoside acceptors, hyperbolic substrate-velocity plots and linear double-reciprocal plots were observed Therefore, we exclude cooperative phenomena dependent upon nucleosides

Among the nucleoside acceptors deoxy-thymidine and deoxy-uridine show the lowest $S_{0.5}$ values and the highest V (Table IV) Furthermore, the same value of $S_{0.5}$ was determined for a nucleoside independent of the nucleotide donor employed Finally, the addition of a nucleoside di- or triphosphate does not modify the $S_{0.5}$ value measured for a nucleoside acceptor

Discussion

Nucleoside di- and triphosphates which behave as powerful protectors are, by contrast, not very effective phosphate donors This observation suggests that the protective effect cannot be considered as a consequence of the nucleotide binding to substrate sites

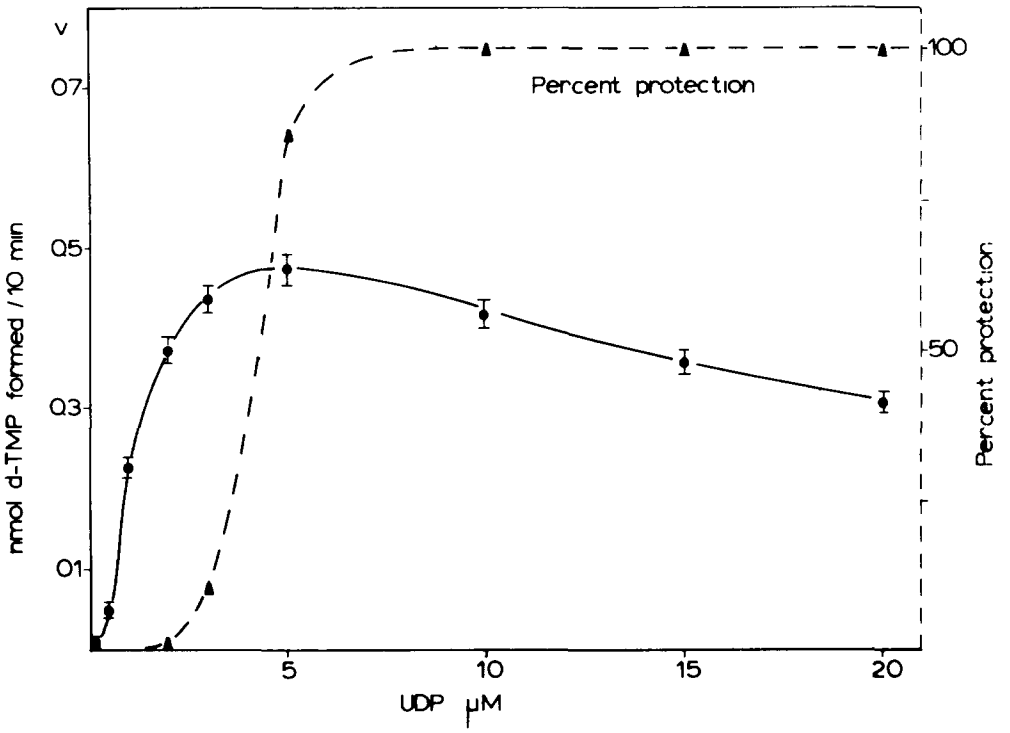

Fig 8 Effect of UDP (●——●) on the reaction rate evaluated with 100 μM as the phosphate donor and 20 μM deoxy-thymidine as the acceptor The incubation was carried out for 10 min at 37°C Values are the mean ± S E of six separate experiments Protective effect of UDP (▲ - - - - - - ▲) against the inactivation of the enzyme at 37°C In this respect samples were preincubated at 37°C for 4 min in the absence or presence of variable concentrations of UDP After the preincubation, 20 μM deoxy-thymidine and 5 mM UDP were added and the incubation was protracted for 10 min As 100% of protection we have considered the value obtained for a sample not preincubated and as zero protection the value obtained for a sample preincubated at 37°C, for 4 min, in the absence of UDP Values of intermediate percentage of protection were obtained with the indicated UDP concentrations Values are the mean of six separate experiments

Therefore it seems likely that the enzyme is also provided with regulatory sites and the binding of a nucleoside di- or triphosphate at these sites induces the conversion of the apoenzyme into a stable complex This conversion seems to be dependent upon cooperative interactions between the regulatory sites, as demonstrated by the results of the analysis performed by means of Eqn 7

Nucleoside di- and triphosphates not only stabilize the enzyme, but also control the enzymic reaction because they lower the coefficient n of the Hill equation, when measured for nucleoside monophosphates As this effect is accompanied by a decrease in $S_{0.5}$ values, it seems that the binding of the effector may alter the conformation of the protein so that the affinity for the substrate is increased At the same time the nucleotide effector favours its binding to other regulatory sites (see in Fig 8 the sigmoidal increment of reaction rate, stage 1 in Results) This observation allows us to propose that the conformation change might bring about a change in the strength of the intersubunit bonds resulting in a more stable form of the enzyme (stage 2) Finally, when the enzyme is in this stable state, it appears that the nucleotide effectors may cause a different conformation change of the subunits as indicated by the decrease in the reaction rate (stage 3)

One interesting observation reported in Results is that the kinetic response shown in the substrate-velocity plots was hyperbolic for all the nucleoside di- and triphosphates, but sigmoidal for the monophosphate forms To explain this difference we

note that all the nucleoside and di- and triphosphates are very good effectors at such a low level that their utilization as phosphate donors is not significant On the contrary, the sigmoidal responses observed for the monophosphate forms can be interpreted by the observation that these nucleotides can act as effectors only at a level where they are used as phosphate donors

As reported in Results, the nucleotide effectors do not modify the enzyme affinity for the nucleoside acceptors This permits us to hypothesize that the enzyme is provided with distinct substrate sites (1) donor sites with higher affinity for nucleotides and responding to effectors with an enhancement of the affinity for phosphate donors and (2) acceptor sites which show higher affinity for nucleosides and are not dependent upon the effectors This hypothesis is also confirmed by the observation that the same $S_{0.5}$ value was obtained for a nucleotide, independent of the nucleoside employed and vice versa

It is possible that some nucleotides (dTMP and dUMP) can compete with the nucleoside for binding to the acceptor site, as indicated by the decrease in the reaction rate, observed with these nucleotides at a concentration above 0 5 mM (Fig 5)

Since numerous effectors show very low $P_{0.5}$ values, it seems likely that nucleoside phosphotransferase is present in the cells in the form of a stable enzyme-nucleotide complex, which can employ as effective substrates all of the nucleoside monophosphates, except for the purine ribonucleotides

Nucleoside phosphotransferase is not only associated with embryos, but we have confirmed (unpublished data) that it is present in numerous tissues of the adult chicken [13] We believe that the enzyme takes part in controlling the pools of both purine and pyrimidine nucleotides, and nucleosides Aside from this general role, the observation that, among all the substrates employed, dTMP and dUMP are the preferred phosphate donors, just as deoxy-thymidine and deoxy-uridine are the preferred acceptors, leads us to the conclusion that the enzyme may play a role in the metabolism of pyrimidine deoxyribonucleotides In this connection, it is interesting to observe that, due to the presence of an enzyme such as nucleoside phosphotransferase, dUMP and dTMP can be produced by means of a simple mechanism which is not dependent upon ATP and is not inhibited by dTTP, a compound which reduces the activity of thymidine kinase [20] as well as that of deoxycytidylate deaminase [21]

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