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Influence of nucleic acid base aromaticity on substrate reactivity with enzymes acting on single-stranded DNA

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ABSTRACT

Stacking between aromatic amino acids and nucleic acid bases may play an important role in the interaction of enzymes with nucleic acid substrates. In such circumstances, disruption of base aromaticity would be expected to decrease enzyme activity on the modified substrates. We have examined the requirement for DNA base aromaticity of five enzymes that act on single-stranded DNA, T4 polynucleotide kinase, nucleases P1 and S1, and snake venom and calf spleen phosphodiesterases, by comparing their kinetics of reaction with a series of dinucleoside monophosphates containing thymidine or a ring-saturated derivative. The modified substrates contained either *cis*-5*R*,6*S*-dihydro-5,6-dihydroxythymidine (thymidine glycol) or a mixture of the 5*R* and 5*S* isomers of 5,6-dihydrothymidine. It was observed that for all the enzymes, except snake venom phosphodiesterase, the parent molecules were better substrates than the dihydrothymidine derivatives, while the thymidine glycol compounds were significantly poorer substrates. Snake venom phosphodiesterase acted on the unmodified and dihydrothymidine molecules at almost the same rate. These results imply that for all the remaining enzymes base aromaticity is a factor in enzyme-substrate interaction, but that additional factors must contribute to the poorer substrate capacity of the thymidine glycol compounds. The influence of the stereochemistry of the dihydrothymidine derivatives was also investigated. We observed that nuclease P1 and S1 hydrolysed the molecules containing 5*R*-dihydrothymidine approximately 50-times faster than those containing the *S*-isomer. The other enzymes displayed no measurable stereospecificity.

INTRODUCTION

Protein recognition of DNA involves a combination of electrostatic, van der Waals, hydrophobic and hydrogen-bonding interactions. Several studies have shown that the presence of aromatic amino acids in both small and large peptide chains enhance binding to single-stranded DNA by interaction with the nucleic acid bases in the form of either stacking or other hydrophobic interactions (1–4). Clearly, where stacking is involved, aromaticity of the bases must play an important role.

In previous studies we have used model compounds to begin to elucidate the substrate elements required for full activity of a series of enzymes which act on single-stranded DNA (5–9). The enzymes include T4 polynucleotide kinase, which catalyses the phosphorylation of terminal 5′-hydroxyl groups of DNA and RNA polynucleotides, oligonucleotides and 3′-mononucleotides; nucleases P1 and S1, which are single strand specific endonucleases and also possess 3′-phosphatase activity; and snake venom and calf spleen phosphodiesterases, which are exonucleases that cleave DNA in a 3′ to 5′ and 5′ to 3′ direction, respectively. An important reason for examining these particular enzymes is their widespread use in assays for DNA damage (10,11). Studies with 'dinucleotides' lacking either a 3′ or 5′ base (7,9) indicated that under usual conditions of reaction, (i) polynucleotide kinase requires a base attached to the sugar residue that is to be phosphorylated, (ii) nucleases P1 and S1 interact with the base 5′ to the internucleotide phosphate group, (iii) snake venom phosphodiesterase interacts with the base 3′ to the phosphodiester linkage, and (iv) calf spleen phosphodiesterase interacts with both bases flanking the phosphodiester linkage, but has an absolute requirement for the 5′ base. In the experiments reported here, we have addressed the question of the importance of aromaticity of the bases in the enzyme-substrate interactions.

Previously, we and others (6,8,12,13) have observed that several base modifications that result in loss of aromaticity, including cyclobutane and 6–4 photoproducts of thymidylyl(3′-5′)thymidine and thymine glycols, can severely inhibit the action of these and other enzymes, such as DNA polymerases (14). However, these particular base modifications also introduce relatively large steric distortions to the substrate (15–18). Reduction of thymine to dihydrothymine, on the other hand, causes less distortion (17), while still resulting in loss of aromaticity. This study presents a comparison of the reactivity of the enzymes towards dinucleoside monophosphates and 3′-mononucleotides containing thymidine, *cis*-5*R*,6*S*-dihydro-5,6-dihydroxythymidine (thymidine glycol, T^g) and a mixture of the 5*R* and 5*S* isomers of 5,6-dihydrothymidine (T^h) (Figure 1). In addition to the question of base aromaticity, we have also examined differences in substrate reactivity related to the stereochemistry of dihydrothymidine.

EXPERIMENTAL PROCEDURES

Chemicals

Thymidine, dihydrothymidine, thymidine 3′-monophosphate and the oligodeoxyribonucleotides d-ApT and d-TpA were purchased from Sigma Chemical Co. (St. Louis, MO). Potassium

permanganate and rhodium on alumina powder were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Enzymes

Snake venom phosphodiesterase (*Crotalus adamanteus*, type II), adenosine deaminase, and prostatic acid phosphatase were supplied by Sigma Chemical Co. Nuclease P1, nuclease S1, and calf intestinal phosphatase were purchased from Boehringer Mannheim Canada (Dorval, PQ) and calf spleen phosphodiesterase and T4 polynucleotide kinase from Pharmacia Canada (Baie d'Urfé, PQ). Unit definitions of the enzymes are as follows: T4 polynucleotide kinase—1 unit catalyzes the transfer of 1 nmole of phosphate from ATP to duplex DNA partially digested with micrococcal nuclease in 30 min at 37°C; nuclease P1—1 unit hydrolyzes 1.0 μmole equivalent of RNA phosphodiester linkages per min at pH 5.3 at 37°C; nuclease S1—1 unit causes 1.0 μg of heat denatured DNA to become acid soluble per minute at 37°C at pH 4.5; snake venom phosphodiesterase—1 unit hydrolyzes 1.0 μg of bis(p-nitrophenyl) phosphate per min at pH 8.8 at 37°C; calf spleen phosphodiesterase—1 unit produces 16 AU_{260nm} of nucleotides from RNA in 30 min at 37°C at pH 6.5 in a 2 ml reaction mixture (19).

Preparation of substrates

The synthesis and NMR characterization of these molecules will be more fully described elsewhere (20). Dinucleoside monophosphates containing a T^g were prepared by oxidation of d-ApT and d-TpA with KMnO4 (21), and Th-containing compounds by rhodium-catalyzed hydrogenation of the parent molecules (22). NMR analysis indicated that the T^g-compounds isolated contained only the 5*R*,6*S* isomer of cis-thymine glycol. d-ApT^h was shown to contain a mixture of the 5*S* and 5*R* isomers of dihydrothymine in a ratio of ~2:1, d-T^hpA contained the isomers in a ratio of ~1:1, and 3'-T^hMP had an isomeric ratio of ~2:1.

HPLC conditions and retention times

Enzyme reactions were monitored by reverse phase HPLC on a Waters μBondapak C_{18} RCM 8×10 Radial-Pak cartridge. Gradient conditions for most of analyses were as follows: 100% buffer A [50 mM NaH_2PO_4, pH 4.5] and 0% buffer B [100 mM NaH_2PO_4, pH 4.5/methanol (1:1, v/v)] for 1 min followed by a linear gradient to 20% buffer A /80% buffer B over 30 min at a flow rate of 1 ml/min. The dinucleosides, d-ApT and d-TpA, and their saturated pyrimidine derivatives could be detected at 260 nm, monomers of dihydrothymidine and thymidine glycol at 230 nm. Retention times in this system were as follows: thymidine—22.8 min; T^g—13.5 min; 5*S*-T^h—17.7 min; 5*R*-T^h—18.6 min; 3'-TMP—16.5 min; 5*S*-3'-T^hMP—11.3; 5*R*-3'-T^hMP—12.0 min; d-ApT—30.9 min; d-ApT^g—30.0 min; d-ApT^h—29.7 min; d-TpA—29.5 min; d-T^gpA—25.8 min; and d-T^hpA—27.4 min.

Better separation of the isomers of dihydrothymidine was achieved with an isocratic system of 90% buffer A /10% buffer B; 5*S*-T^h—12.8 min, 5*R*-T^h—15.0 min.

Enzyme reactions

Phosphorylation by T4 polynucleotide kinase. Dinucleoside monophosphates (50 nmol) were incubated at 37°C with 250 nmol ATP, 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine, 0.1 mM EDTA, and 25 units of PNK in a total volume of 200 μl. At the times indicated in Figure 2a, 20 μl samples were taken and added to 10 μl 0.5 M EDTA and then stored on dry ice until injected onto the HPLC.

For the Lineweaver–Burk plot, between 0.8 and 5 nmol of d-TpA and d-T^hpA was incubated with 0.3 units of enzyme in the same conditions as above. Aliquots were removed at various time intervals up to 15 min, the reaction stopped by addition of 10 μl of 0.5 M EDTA and the samples held on dry ice until analysed by HPLC.

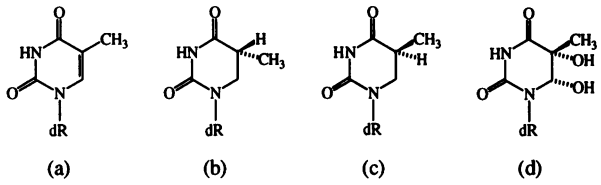
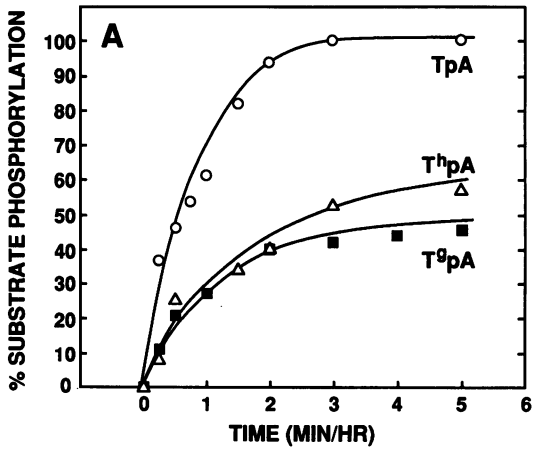


Figure 1. Chemical structures of thymidine (a) and ring-saturated derivatives, 5*S*-5,6-dihydrothymidine (b), 5*R*-5,6-dihydrothymidine (c) and *cis*-5*R*,6*S*-dihydro-5,6-dihydroxythymidine (thymine glycol, d).



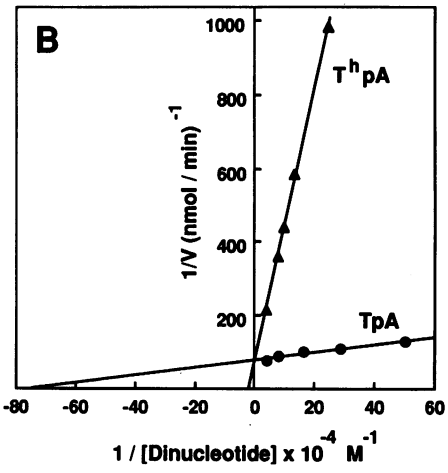


Figure 2. Kinetics of phosphorylation by T4 polynucleotide kinase. (A) Comparison of d-TpA with its ring-saturated thymine derivatives. Time units for the rates shown with open symbols are minutes and closed symbols are hours. (B) Lineweaver-Burk plot showing that the enzymatic phosphorylation reactions of d-TpA and d-T^hpA have similar V_{max} values but an approximately 40-fold difference in K_m values.

Digestion by nuclease P1. Dinucleoside monophosphates (25 nmol) were incubated at 37°C with 0.3 unit of nuclease P1 (and 0.25 unit of prostastic acid phosphatase for the reaction of d-T^{h}pA) in 250 μl of buffer (10 mM sodium acetate, pH 5.3, 1 mM $ZnSO_4$). Aliquots (100 μl) were withdrawn at various time intervals and the reaction in each was quenched by addition of 2.5 μl of 0.5 M EDTA. The samples were held on dry ice until analysis by HPLC for the disappearance of substrate and appearance of reaction products.

The nuclease P1 concentration in the reaction mixture was increased to 12 units/ml and the prostatic acid phosphatase was omitted in the reactions with 3'-mononucleotides.

Digestion by nuclease S1. For each time point, 5 nmol of dinucleoside monophosphate was incubated at 37°C with 250 units of nuclease S1 in 100 μl of buffer (10 mM sodium acetate, pH 4.3, 50 mM NaCl, 1 mM $ZnCl_2$). Reactions were stopped by addition of 2.5 μl of 0.5 M EDTA and held on dry ice until analysed by HPLC.

Digestion by snake venom phosphodiesterase. Dinucleoside monophosphates (50 nmol) were incubated at 37°C with 0.001 units of the phosphodiesterase, 50 mM Tris−HCl (pH 7.6), and 10 mM $MgCl_2$ in a total volume of 0.5 ml. At the times indicated in Figure 6, 50 μl aliquots were removed, added to 5 μl 0.5 M EDTA and stored frozen until analysed.

Digestion by calf spleen phosphodiesterase. For each time point, 5 nmol of dinucleoside monophosphate was incubated in 100 mM Tris−HCl (pH 7.6) at 37°C with 0.02 unit of phosphodiesterase. Reactions were stopped by addition of 1 μl 0.5 M EDTA and stored frozen until analysed. Because deoxyadenosine comigrated with d-T^{g}pA, adenosine deaminase (0.4 units) was included in the reaction to convert deoxyadenosine to deoxyinosine.

RESULTS

Phosphorylation by T4 polynucleotide kinase

The kinetics of phosphorylation of d-TpA and its two congeners, shown in Figure 2A, reveal that replacement of thymine with dihydrothymine reduced the initial rate of reaction ~3-fold, but replacement with thymine glycol reduced the rate almost 200-fold (note the time units for d-T^{g}pA are hours).

The reaction of d-T^{h}pA was further examined to compare the phosphorylation of both isomers. Two reactions were allowed to proceed to 33 and 71% phosphorylation, respectively, and the phosphorylated products purified from each. They were then digested with snake venom phosphodiesterase and calf alkaline phosphatase, and the ratio of 5*R* to 5*S* dihydrothymidine compared by HPLC analysis. Figure 3 shows the outcome after 33% reaction, and indicates that both isomers are equally good substrates. Similar results (not shown) were obtained for the 71% phosphorylation reaction.

Since the d-T^{h}pA we had purified was composed of an almost exactly 1:1 mixture of the *R* and *S* dihydrothymine isomers and the isomeric d-T^{h}pA molecules were phosphorylated at identical rates, we were able to derive K_m and V_{max} values for d-T^{h}pA from a double reciprocal plot (Figure 2B) of reaction velocity vs substrate concentration (23). The V_{max} value for d-TpA was virtually the same as that for the parent compound, 0.014 nmol/min and 0.013 nmol/min, respectively. However, there was an almost 40-fold difference in K_m values, 51.9 μM for d-T^{h}pA vs 1.4 μM for d-TpA. Using a value of 30,000 units/mg protein (provided by the supplier) and a molecular weight for the enzyme of 136,000 (24), we obtained a turn over number for the enzyme reaction with d-TpA and d-T^{h}pA of ~3 per second.

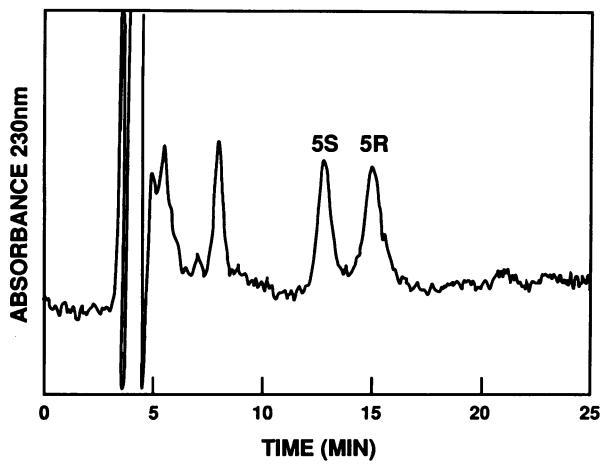


Figure 3. HPLC chromatogram showing the ratio of *R* and *S*-isomers of dihydrothymidine in d-pT^{h}pA recovered after 33% phosphorylation. The two diastereoisomers of d-pT^{h}pA could not be separated by HPLC, so purified d-pT^{h}pA was digested to the mononucleoside level by snake venom phosphodiesterase and calf alkaline phosphatase and analysed by reverse-phase C_{18} HPLC column as described in Experimental Procedures.

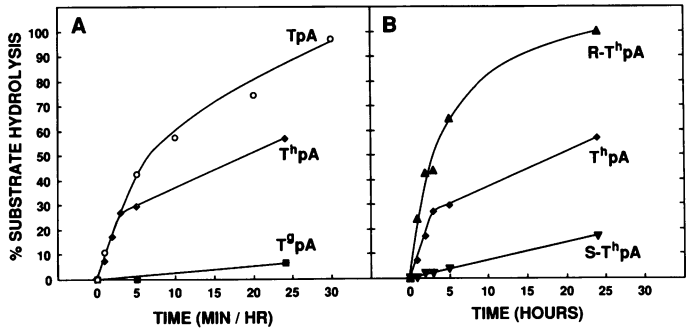


Figure 4. Kinetics of hydrolysis by nuclease P1. (A) Comparison of the rates of hydrolysis of d-TpA with d-T^{h}pA and d-T^{g}pA. Open symbols indicate time units are minutes, closed symbols are hours. (B) Plot showing the stereospecificity of hydrolysis of d-T^{h}pA by nuclease P1.

Hydrolysis by nuclease P1 and S1

The effect of thymidine modification on the rate of hydrolysis by nuclease P1 is shown in Figure 4. The thymine glycol derivative was almost totally refractory to digestion by the enzyme. Hydrolysis of d-T^{g}pA could be raised to 15% after 24 h by increasing the enzyme concentration 12-fold. The rate of digestion by nuclease P1 was also affected by reduction of the thymine, but the influence was dependent on the stereochemistry of the dihydrothymine moiety. The *R*-isomer of d-T^{h}pA is hydrolyzed approximately 20-fold slower than the parent dinucleoside monophosphate, but approximately 50-times faster than the *S*-isomer. As shown in Figure 4B, the stereospecificity of hydrolysis by the enzyme accounts for the biphasic nature of the curve for the rate of reaction with d-T^{h}pA.

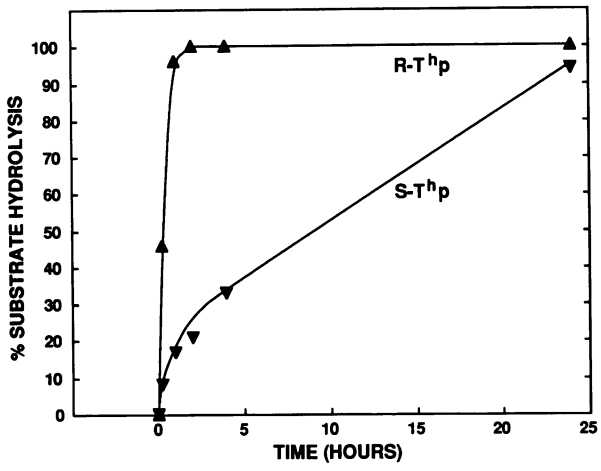


Figure 5. Dephosphorylation of 3′-T^{h}MP by nuclease P1 indicating the stereospecificity of the reaction.

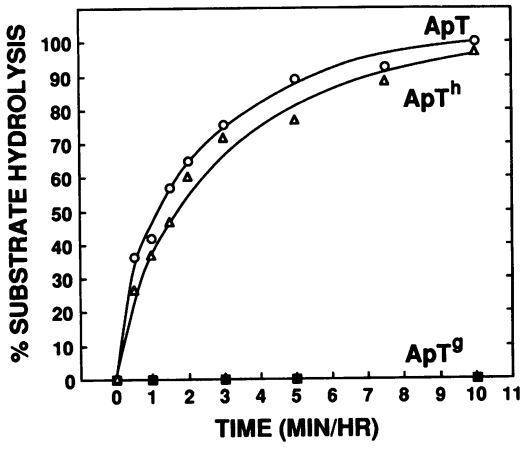


Figure 6. Kinetics of hydrolysis of d-ApT and its ring-saturated pyrimidine derivatives by snake venom phosphodiesterase. Open symbols indicate time units are minutes, closed symbols are hours.

In addition to being an endonuclease, nuclease P1 also has a 3′-phosphatase activity. We, therefore, examined the influence of thymine modification on this activity by measuring the dephosphorylation of thymidine 3′-monophosphate and its reduced derivatives. The outcome was qualitatively similar to the results obtained with the dinucleoside monophosphates, although the enzyme concentration had to be increased 10-fold in order to see measurable levels of hydrolysis of the *S*-isomer. Figure 5 shows the stereospecificty of hydrolysis of 3′-T^{h}MP.

S1 nuclease is an endonuclease with similar properties to nuclease P1. Not surprisingly, we observed that S1 nuclease was unable to cleave d-T^{g}pA and displayed the same discrimination towards the d-T^{h}pA isomers as nuclease P1 (data not shown).

Hydrolysis by snake venom phosphodiesterase

There was a marked contrast in the response of the two modified d-ApT derivatives towards snake venom phosphodiesterase (Figure 6). While d-ApT^h displayed virtually identical kinetics to the parent compound, d-ApT^g remained totally refractory to

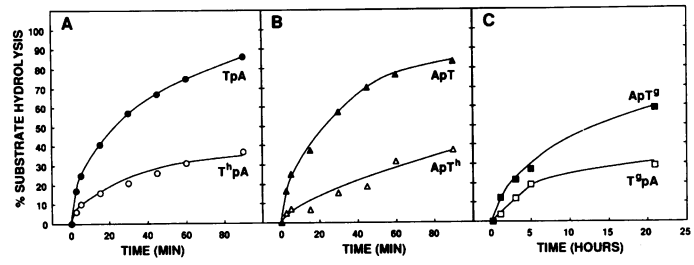


Figure 7. Kinetics of hydrolysis by calf spleen phosphodiesterase of d-TpA and d-ApT series of compounds. Note the time scale for the hydrolysis of the thymine glycol compounds is in hours.

the enzyme at a concentration of 0.002 unit/ml. Only after increasing the concentration of the phosphodiesterase four-fold was a limited degree of reaction with d-ApT^g observed (13% hydrolysis after 5 h). The *R* and *S* isomers of d-ApT^h were digested at the same rate.

Hydrolysis by calf spleen phosphodiesterase

The activity of calf spleen phosphodiesterase is influenced by the 5′ and 3′ bases flanking an internucleotide phosphodiester group (7). Consequently, we looked at the kinetics of hydrolysis of both the d-TpA and d-ApT series of compounds (Figure 7), and in accordance with our previous observations with this enzyme, it was again observed that modification of either one of the bases affected enzyme activity. The dihydrothymidine derivatives showed only moderately reduced (~3-fold) rates of hydrolysis compared to the parent compounds, and no significant difference was observed between the rates of hydrolysis of the *R* and *S* isomers of either d-T^{h}pA or d-ApT^h. A thymine glycol, in either a 3′ or 5′ position, reduced the rate by at least two orders of magnitude. Our results with d-T^{g}pA and calf spleen phosphodiesterase are in good agreement with Maccubbin et al. (25), who, in addition, observed that the 5*R*,6*S*-isomer of d-T^{g}pA was hydrolysed considerably slower than the 5S,6*R*-isomer.

DISCUSSION

NMR analysis of the substrates used in this study has shown that, with the exception of d-T^{g}pA, the distortion caused by reduction and oxidation of the pyrimidine within the dinucleoside monophosphate is confined to the modified base, with no significant change to sugar conformation or glycosidic torsion angle (20). Thus, apart from d-T^{g}pA, the differences in substrate capacity reported here are attributable solely to the base modification. The thymine glycol in d-T^{g}pA appears to have a higher *syn* population than thymine in the parent compound, which may contribute to its poor reactivity. Ogilvie and Hruska (26) showed that replacement of uracil by 6-methyluracil in the dinucleoside ApU changed the predominant conformation of the pyrimidine from *anti* to *syn* and rendered the dimer resistant to snake venom phosphodiesterase.

The primary focus of this study concerns the importance of base aromaticity in enzyme-substrate interactions. It is clear that all five enzymes examined were able to act to an appreciable extent on substrates containing dihydrothymine, implying that base aromaticity is not an absolute requirement for enzyme function. However, polynucleotide kinase, nuclease P1, nuclease

S1, and calf spleen phosphodiesterase reacted more slowly with the dihydrothymine derivatives than with the parent compounds, indicating a potential enhancement to the interaction between enzyme and substrate if the substrate has an aromatic base. In the case of polynucleotide kinase, this was shown to be the result of reduced affinity for the substrate rather than a decrease in the rate of phosphorylation of bound substrate. This would suggest that the substrate binding site in the kinase either has a relatively narrow groove, so that it is advantageous for the substrate to be planar, or that there is a contribution to binding from hydrophobic bonding and London dispersion forces associated with the stacking of aromatic systems. Although the DNA sequence of the *pse*T gene, which codes for T4 polynucleotide kinase, has been determined (27), a substrate binding site has not been unambiguously identified, so it remains to be seen whether it contains aromatic amino acids.

The reduced reactivity of d-T^{h}pA with nuclease P1 would be anticipated from the structure of the enzyme recently determined by X-ray crystallography (4). Two binding sites have been proposed on the basis of soaking enzyme crystals with d-Ap(S)A (an uncleavable thiophosphorylated substrate analog), and both appear to involve potential stacking interactions with aromatic amino acids. In one case, the substrate is sandwiched between two tyrosine side chains, and the other has the substrate stacked between a phenylalanine and a valine.

A curious feature of the enzyme-substrate model put forward by Volbeda et al., (4) is that the enzyme appears to bind to the 3′-nucleoside in d-Ap(S)A. For the following reasons, we consider it far more likely that nuclease P1 binds to the 5′-nucleoside of a normal (i.e. non-thiophosphorylated) dinucleoside monophosphate: (i) the enzyme has a 3′-phosphatase activity capable of removing the phosphate group from nucleoside 3′-monophosphates, (ii) the enzyme, as demonstrated here, shows an identical pattern of reactivity towards the sets of substrates derived from d-TpA and 3′-TMP, suggesting that the phosphatase and phosphodiesterase activity of nuclease P1 involve the same active site(s), and (iii) there is no discernable effect on the rate of hydrolysis when an abasic site lies 3′ to the phosphodiester bond, but the isomeric compound with a 5′ abasic site is refractory to the enzyme (7).

Nuclease P1 and S1 reacted considerably slower with the *S*-isomer of d-T^{h}pA than with the *R* isomer. The NMR analysis of both isomers shows that the saturated pyrimidine ring takes up a distorted half-chair conformation, with C5 and C6 on opposite sides of a plane defined by N1, C2, N3, and C4, and that the methyl substituent at C5 lies in a pseudo-equatorial position. Thus, a possible cause for the difference in reactivity of the two isomers is the steric disposition of the pseudo-axial protons at C5 and C6.

Clearly, loss of base aromaticity contributes to only a minor extent, if at all in the case of snake venom phosphodiesterase, to the grossly diminished rates of reaction shown by all the enzymes towards the thymine glycol containing substrates. The poorer substrate capacity must be primarily due to the addition of the two hydroxyl substituents which could disrupt interaction with the enzymes by steric hindrance, altered hydrophobicity, and potentially aberrant hydrogen bonding.

A practical outcome of this study is the bearing it has on the use of these enzymes in postlabeling assays for dihydrothymine, thymine glycol and other saturated pyrimidine species produced in DNA by ionizing radiation and various oxidative processes. Indeed, one of our objectives was to see if the loss of pyrimidine ring aromaticity would generally favour one technique over another. This we have plainly shown not to be the case. The three main postlabeling procedures require digestion of damaged DNA by different sets of nucleases: (a) calf spleen phosphodiesterase and micrococcal nuclease, which release the lesion as a nucleoside 3′-monophosphate (28); (b) nuclease P1 and phosphatase, which release some lesions in the form of a dinucleoside monophosphate with the damaged base at the 5′-end (29); and (c) DNase I, snake venom phosphodiesterase and phosphatase, which release some lesions as a dinucleoside monophosphate with the damaged base at the 3′ end (30). In all cases the damage-containing species are end-labeled by polynucleotide kinase and [γ-^{32}P]ATP. Several groups have used the calf spleen phosphodiesterase approach to detect thymine glycols (12,13) but, as with d-T^{g}pA, thymidine glycol 3′-monophosphate is a poor substrate for polynucleotide kinase and the efficiency of labeling is low. We have previously demonstrated that thymine glycol can be readily detected by the snake venom phosphodiesterase-based procedure (30). (An advantage of this protocol is that the kinase acts on the normal 5′-nucleoside in the lesion-containing dinucleoside monophosphate.) It is apparent from the present study, however, that dihydrothymine will not be detectable by this approach since the lesion does not prevent venom phosphodiesterase from cleaving the adjacent 5′-phosphodiester bond. On the other hand, the lesion should be detectable as the labelled 5′,3′ nucleoside diphosphate following digestion of damaged DNA by calf spleen phosphodiesterase and micrococcal nuclease since 3′-T^{h}MP, like d-T^{h}pA, is a good substrate for polynucleotide kinase. It may also be possible, with careful use of nuclease P1 or S1, to assay specifically for the *S*-isomer of dihydrothymine as a dinucleoside monophosphate. Although it appears that addition of relatively bulky substituents to C5 and C6 of thymine (e.g. thymine glycols and cyclobutane pyrimidine dimers) would favour the snake venom phosphodiesterase procedure, the best method for quantifying other saturated pyrimidine lesions will probably need to be determined empirically.

ACKNOWLEDGMENTS

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REFERENCES

1. Dimicoli, J.L. and Hélène, C. (1971) *Biochemistry* **13**, 714–723.
2. van Amerongen, H., Kuil, M.E., Scheerhagen, M.A., and van Grondelle, R. (1990) *Biochemistry* **29**, 5619–5625.
3. Lenz, A., Cordes, F., Heinemann, U. and Saenger, W. (1991) *J. Biol. Chem.* **266**, 7661–7667.
4. Volbeda, A., Lahm, A., Sakiyama, F. and Suck, D. (1991) *EMBO J.* **10**, 1607–1618.
5. Weinfeld, M. and Livingston, D.C. (1986) *Biochemistry* **25**, 5083–5091.
6. Liuzzi, M., Weinfeld, M. and Paterson, M.C. (1989) *J. Biol. Chem.* **264**, 6355–6363.
7. Weinfeld, M., Liuzzi, M. and Paterson, M.C. (1989) *Nucleic Acids Res.* **17**, 3735–3745.
8. Weinfeld, M., Liuzzi, M. and Paterson, M.C. (1989) *J. Biol. Chem.* **264**, 6364–6370.

9. Weinfeld, M., Liuzzi, M. and Paterson, M.C. (1990) *Biochemistry* **29**, 1737–1743.
10. Beach, A.C. and Gupta, R.C. (1992) *Carcinogenesis* **13**, 1053–1074.
11. Weinfeld, M. and Buchko, G.W. In Phillips, D. H., Castegnaro, M., and Bartsch, H. (eds.), Postlabelling Methods for the Detection of DNA Damage, IARC Scientific Publications No. 124, Lyon (in press).
12. Hegi, M.E., Sagelsdorff, P. and Lutz, W.K. (1989) *Carcinogenesis* **10**, 43–47.
13. Reddy, M.V., Bleicher, W.T. and Blackburn, G.R. (1991) *Cancer Communications* **3**, 109–117.
14. Ide, H. and Wallace, S. S. (1988) *Nucleic Acids Res.* **16**, 11339–11354.
15. Cadet, J., Voituriez, L., Hruska, F.E. and Grand, A. (1985) *Biopolymers* **24**, 897–903.
16. Kan, L-S., Voituriez, L. and Cadet, J. (1988) *Biochemistry* **27**, 5796–5803.
17. Hruska, F.E., Sebastian, R., Grand, A., Voituriez, L. and Cadet, J. (1987) *Can. J. Chem.* **65**, 2618–2623.
18. Vaishnav, Y., Holwitt, E., Swenberg, C., Lee, H-C. and Kan, L-S. (1991) *J. Biomol. Struct. Dyn.* **8**, 935–951.
19. Hilmoe, R.J. (1961) *Biochem. Prep.* **8**, 105–109.
20. Baleja, J.D., Buchko, G.W., Weinfeld, M. and Sykes, B.D. (1993) *J. Biomol. Struct. Dyn.* (In press).
21. Iida, S. and Hayatsu, H. (1971) *Biochim. Biophys. Acta* **240**, 370–375.
22. Cohn, W.E. and Doherty, D.G. (1956) *J. Am. Chem. Soc.* **78**, 2863–2866.
23. Lineweaver, H. and Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666.
24. Lillehaug, J.R. (1977) *Eur. J. Biochem.* **73**, 499–506.
25. Maccubbin, A., Evans, M., Paul, C.R., Budzinski, E.E., Pryzbyszewski, J. and Box, H.C. (1991) *Radiat. Res.* **126**, 21–26.
26. Ogilvie, K.K. and Hruska, F.E. (1976) *Biochem. Biophys. Res. Commun.* **68**, 375–378.
27. Midgley, C.A. and Murray, N.E. (1985) *EMBO J.* **4**, 2695–2703.
28. Randerath, K., Reddy, M.V. and Gupta, R.C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6126–6129.
29. Randerath, K., Randerath, E., Danna, T.F., van Golen, K.L. and Putman, K.L. (1989) *Carcinogenesis* **10**, 1231–1239.
30. Weinfeld, M. and Soderlind, K-J.M. (1991) *Biochemistry* **30**, 1091–1097.