CONF-780477--1

METHYLATION EFFECTS ON NUCLEIC ACID CONFORMATIONS

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MASTER

Prepared for

11th Jerusalem Symposium
on Quantum Chemistry and Biochemistry
April 3-7, 1978
Jerusalem, Israel

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INTRODUCTION

Chemical modification of nucleic acids by alkylating agents continues to be extensively explored from the perspective of chemical mutagenesis and carcinogenesis. Largely through the pioneering *in vitro* and *in vivo* studies of Lawley (1961), Lawley and Brookes (1963), Singer (1972), and Reese and coworkers (1965), coupled with theoretical predictions of Pullman (1974), a detailed profile has emerged of the principal alkylation sites for purine and pyrimidine nucleotides. Generally, alkylation of nucleic acids produces a multiplicity of reaction products dependent upon factors such as nature of base ring, electrophilicity of the alkylating agent (Lawley, 1976), and presence of activating enzymes (Magee and Barnes, 1967). Thus, for example, alkylation of adenine nucleotides can occur at six sites, Figure 1. Strong electrophiles such as N-methyl-N-nitrosourea (MNUA) and N-methyl-N¹-nitro-N-nitrosoguanidine (MNNG) couple preferentially with available oxygen donors, e.g., phosphate group, 2' oxygen in ribonucleotides, while weakly electrophilic agents dimethyl sulfate and methyl methane sulfonate tend to show a higher proportion of ring nitrogen methylation at N1, N3, and N7 of purines.

Figure 1. Potential methylation sites on an adenine nucleotide.

*Work supported by the U.S. Department of Energy.

A facet of this problem only marginally investigated to the present is concerned with the impact of carcinogen modification upon structural/conformational features of nucleic acids. Yet, methylation effects on structure may be quite substantial, notably in instances where charge redistribution and/or electrostatic charges are altered. In the present communication, we explore this aspect in some detail using dinucleoside monophosphates as simple models. Of specific interest was the effect of N1 methylation in purines on base stacking and overall conformation. Since purine-rich regions of RNA and DNA are presumptive nucleating sites for ordered stacked structures, introduction of positive charges into the base ring by methylating agents can have a profound influence on total biopolymer structure.

MATERIALS AND METHODS

Methylation at the adenine N1 position of a series of adenylate dinucleoside monophosphates, ApA, ApU, and UpA, was carried out following procedures described by Reese et al. (1965). Figure 2 shows a schematic illustration of the overall process. In a typical reaction, dimethyl sulfate was added slowly to an aqueous solution of the dimer under conditions of strict pH control (pH = 7.0). Following ether extraction of the mixture, the products were separated by column chromatography and characterized further by electrophoresis, UV, and NMR spectra. Yields of the N1 methylated dimers ranged from 20-35%.

Figure 2. Schematic illustration of adenylyl-3'-5'-adenosine, ApA, methylation reaction. Similar reactions can be written for methylation of adenylate fragments in ApU and UpA.

Samples for NMR measurements were lyophilized from 99.8% D₂O, then dissolved in 100% D₂O and the solutions adjusted to the desired concentration, 0.004-0.007 M and pD. A trace amount of TSP added to the sample served as an internal reference. Proton spectra were recorded in the FT mode on a Varian 220 MHz spectrometer equipped with a Nicolet FT accessory and 20 K data system. All of the spectra were measured at 20 ± 2°C and no sample degradation occurred in the time span of the measurements. Spectral simulations were made on a Nicolet 1080 computer using appropriate simulation programs.

RESULTS AND DISCUSSION

Spectral Assignments, Analysis, and Comparisons

Figure 3A illustrates a typical spectrum for a methylated dimer, ^mApU ,[†] recorded at $\text{pD} = 7.0$ and 20°C . Except for differences in chemical shifts, the overall pattern of signal multiplets conforms to that observed for the corresponding parent dimer ApU , (Ezra et al., 1977) and related N6 methylated ApU derivatives (Altona, 1975). Similar correlations between parent and methylated derivatives are seen for the other compounds. Assignment of signals to specific protons followed procedures developed in earlier dimer studies (Kondo and Danyluk, 1976; Lee et al., 1976; Ezra et al., 1977). Because of the near equivalence in methyl shifts, no assignment is possible for these signals in $^m\text{Ap}^m\text{A}$. Final best-fit values of chemical shifts and coupling constants are summarized in Tables I and II, respectively.

Table I
NMR Parameters for Methylated Dinucleoside Monophosphates*

Chemical Shifts [†] ppm	$^m\text{Ap}^m\text{A}$		^mApU		Up^mA	
	$^m\text{Ap}-$	$-p^m\text{A}$	$^m\text{Ap}-$	$-p\text{U}$	$\text{Up}-$	$-^m\text{pA}$
2	8.540	8.540	8.543	-	-	8.551
8	8.540	8.609	8.561	-	-	8.623
^mAp	3.903	-	3.966	-	-	-
$p^m\text{A}$	-	3.936	-	-	-	3.927
5	-	-	-	5.772	5.818	-
6	-	-	-	7.861	7.859	-
1'	6.141	6.177	6.168	5.868	5.795	6.200
2'	4.864	4.818	4.882	4.309	4.386	4.745
3'	4.773	4.573	4.759	4.327	4.514	4.577
4'	4.391	4.391	4.468	4.277	4.227	4.395
5'	3.836	4.282	3.895	4.291	3.773	4.191
5''	3.814	4.220	3.968	4.150	3.818	4.123

* $\text{pD} = 7.0$; $T = 20^\circ\text{C}$; Concentration = 0.005 M.

[†] Shifts are accurate to ± 0.005 ppm.

[†] Abbreviations used: ApA , adenylyl-3'-5'-adenosine; ApU , adenylyl-3'-5'-uridine; UpA , uridylyl-3'-5'-adenosine; $^m\text{Ap}^m\text{A}$, N-1 methyl adenylyl-3'-5'-N-1 methyl adenosine, etc.; $m = \text{CH}_3$ group.

Figure 3. The 220 MHz proton spectrum for $mApU$, 0.003 M, measured at $pD = 7.0$ and $20^\circ C$ (upper trace). A simulated spectrum is shown in trace B.

Table II
NMR Parameters for Methylated Dinucleoside Monophosphates*

Coupling Constants Hz [†]	^m Ap ^m A		^m ApU		Up ^m A	
	^m Ap-	-p ^m A	^m Ap-	-pU	Up-	- ^m pA
1'2'	5.7	5.2	4.4	3.6	4.5	4.5
2'3'	5.2	5.1	5.2	5.1	5.2	5.1
3'4'	3.8	4.0	4.8	5.6	5.1	4.9
4'5'	<u>2.5</u>	2.8	3.8	2.2	3.5	(3.5)
4'5''	<u>2.5</u>	3.3	2.6	2.8	2.7	3.5
5'5''	-13.1	-12.1	-13.1	-11.4	-13.0	-11.0
5'P	-	3.7	-	3.5	-	(4.5)
5''P	-	3.7	-	4.6	-	4.5
3'P	8.2	-	8.2	-	8.0	-
4'P	-	(2.0)	-	(2.0)	-	(2.0)
56	-	-	-	8.0	8.1	-

* pD = 7.0; T = 20°C; Concentration = 0.005 M

[†] Couplings are accurate to ± 0.2 Hz except for values in parentheses.

Comparison of the shift data with values for parent dimers reveals extensive deshielding of base and ribose protons in all three methylated compounds, Table III, the changes being most pronounced for H2 and H8 protons of ^mAp A, Figure 4. This behavior is not surprising since methylation at N1 introduces a net positive charge into the base ring. Qualitatively the deshielding arises from a combination of base-ring charge deficiency, and base destacking due to repulsive interactions between adjacent positively charged adenines in ^mAp^mA. Both methylation and destacking affect H2 to a greater extent than H8;[†] also, the effects are, as expected, less marked in hetero-dimers.

As with the shifts, methylation causes alterations in magnitudes of coupling constants; however, the effects are more selective with J_{1'2'}, J_{3'4'} of the ^mAp^mA ribose rings showing the largest changes. The increase in J_{1'2'} and decrease of J_{3'4'} closely parallel the behavior seen on base protonation of corresponding adenylate mononucleotides (Danyluk, Nelson, and Ezra, unpublished results).

Conformational Properties

The utilization of chemical shifts and coupling constants for conformational analyses of short-chain nucleic acid segments has been extensively detailed previously (Altona and Sundaralingam, 1973; Kondo

[†] Protonation of adenine is formally analogous to methylation. The former occurs preferentially at N1 and produces deshieldings in order $\delta 2 > \delta 8$ (Danyluk and Hruska, 1968).

Table III
Methylation Effects on Chemical Shifts
 $\Delta\delta^\dagger$ (ppm)

Proton	m_{ApU}		$m_{Ap} m_A$		$U_p m_A$	
	m_{Ap-}	-pU	m_{Ap-}	-p m_A	Up-	- m_{pA}
2	0.362	-	0.659	0.487	-	0.326
8	0.250	-	0.364	0.364	-	0.212
5	-	0.186	-	-	0.056	-
6	-	0.107	-	-	0.103	-
1'	0.114	0.123	0.306	0.220	0.070	0.100
2'	0.044	0.070	0.190	0.233	0.131	0.035
3'	0.111	0.035	0.105	0.061	0.042	0.037
4'	0	0.028	0.032	0.013	0.038	0.020
5'	0	-0.048	-0.064	-0.098	0.025	-0.073
5''	-0.046	0.014	-0.027	0.035	0.020	-0.032

*Data at 20°C and pD = 7.0.

$\dagger\Delta\delta = \delta_{CH_3} - \delta_0$; (values for δ_0 obtained from Ezra et al., 1976 and Lee et al., 1977).

Figure 4. Comparison of methylation effects upon the proton spectrum of ApA at pD = 7.0 and 20°C.

and Danyluk, 1976; Altona, 1975; Ts'o and coworkers, 1969; Lee et al., 1976; Ezra et al., 1977). Briefly the ribofuranose ring can be represented by two puckered modes 2E and 3E undergoing rapid interconversion via pseudorotation, i.e., ${}^2E \rightleftharpoons {}^3E$. Since $J_{2,3'}$ (5.2 Hz) and $J_{1,2'} + J_{3,4'}$ ($9.4 \pm .2$ Hz) are identical to values for dinucleoside monophosphates, no significant change in pseudorotational angle or degree of pucker takes place on methylation. Equilibrium populations were accordingly evaluated as for regular dimers and yielded 3E populations listed in Table IV. A marked shift is noted from a favored 3E form in both nucleotidyl residues of ApA (% E values are given in parentheses, (Table IV) to an 2E conformation in ${}^m\text{Ap}^m\text{A}$. Values in the latter are in fact close to those for corresponding monomers (33 and 38% 3E for 3'-AMP and 5'-AMP, respectively (Davies and Danyluk, 1974, 1975), and ApA at elevated temperatures (Lee et al., 1976). This trend is qualitatively indicative of decreased base stacking in ${}^m\text{Ap}^m\text{A}$ versus ApA. In contrast, introduction of a positive charge into the adenine ring of ${}^m\text{ApU}$ and Up^mA has minimal direct or indirect effect on 3E populations in either dimer.

Table IV

Conformational Properties for Methylated Dinucleoside Monophosphates

Property	${}^m\text{Ap}^m\text{A}$		${}^m\text{ApU}$		Up^mA	
	${}^m\text{Ap-}$	${}^m\text{-pA}$	${}^m\text{Ap-}$	-pU	Up-	$\text{-p}^m\text{A}$
<u>Ribose Ring</u>						
Stacked ^a	8(38)		27(37)		10(15)	
${}^3E^b$	40(58)	42(61)	50(57)	59(59)	54(53)	51(51)
<u>Backbone</u>						
$\text{gg}(\psi_+)^c$	85(79)	78(74)	75(89)	87(95)	77(74)	70(79)
$\text{g}'\text{g}'(\phi_+)^c$	-	85(90)	-	81(85)	-	77(80)
θ PH	$\pm 35^\circ$		$\pm 35^\circ$		$\pm 33^\circ$	

^aComputed using $J_{1,2'} + J_{3,4'} = 9.5$ Hz (dimers) and 9.3 Hz (monomers);

% stacked = $J_{3,4'}(\text{dimer}) - J_{3,4'}(\text{monomer}) / 9.5 - J_{3,4'}(\text{monomer})$.

^b% ${}^3E = J_{3,4'} / J_{1,2'} + J_{3,4'}$

^cRotamer equations: $\psi_+ = (13.7 - \Sigma) / 9.7$; $\phi_+ = (25 - \Sigma^1) / 20.8$.

Rotamer populations along the phosphodiester backbone, C4'-C5', ψ_+ ; C5'-O5'; ϕ_+ ; and C3'-O3', can be evaluated using expressions, Table IV, footnote, derived by the usual staggered rotamer formalism (Wood et al., 1973; Sarma et al., 1974; Evans and Sarma, 1974; Lee and Sarma, 1976). The values summarized in Table V are not significantly different from those for unmodified dimers (parentheses) further exemplifying the extraordinary conformational integrity of these three conformational bonds across a wide spectrum of charged and uncharged dimers.

As with all short chain nucleic acid fragments, the overall three-dimensional solution structure is predominantly determined by two factors, the extent of base-base interactions and the swivel nature of

P-03' and P-05' bonds. Although there is no single method for precise evaluation of base stacking, a reasonable semi-quantitative estimate can be made from magnitudes of $J_{3,4'}$ (or $J_{1,2'}$). The underlying rationale assumes that ribofuranose rings adopt an 3E form (100%) in fully-stacked dimers and a monomer ${}^3E/{}^2E$ distribution in unstacked states (Hruska and Danyluk, 1968; Altona, 1975; Kondo and Danyluk, 1976). With this approach, stacked populations are estimated to range from 8% in ${}^mAp{}^mA$ to 27% in mApU , Table V. It is apparent that methylation produces striking changes in the homo-adenylate and somewhat smaller but not insignificant alterations in hetero-dimers. Whereas stack populations increase in order, $UpA < ApU < ApA$, methylation shifts the trend to ${}^mAp{}^mA < Up{}^mA < {}^mApU$. This behavior is clearly linked to more pronounced repulsive forces between two adjacent positively charged adenine rings in ${}^mAp{}^mA$. Charge neutralization at high pH removes this interaction and shifts the equilibrium towards base stacking.

Two additional stacking indices of a somewhat more qualitative nature are dimerization-induced shift changes for pyrimidine H5 signals and relative differences in shift non-equivalence for 5',5'' protons of 5'-nucleotidyl fragments (-pX). In each instance, increased stacking correlates with increased dimerization shifts and 5'5'' nonequivalence (Ezra et al., 1977). Table V summarizes relevant data for a series of hetero-dimers including mApU and $Up{}^mA$. Both methylated hetero-dimers follow the general patterns observed for normal dimers and are consistent with a diminished stacking in methylated derivatives.

Table V
Variation in Stacking Parameters of Dimers

Dinucleotide	$\Delta\delta_{H5}^a$	$\Delta J_{1,2'}^b$		%	Δ^c
		Pyrimidine	Purine		
GpC	0.476	1.9	3.2	45	0.190
ApC	0.449	1.6	2.6	38	0.143
ApU	0.350	1.4	2.4	34	0.133
GpU	0.234	1.4	1.4	27	0.111
CpA	0.280	1.1	1.5	24	0.152
CpG	0.215	1.0	1.8	25	0.129
UpA	0.153	0.6	1.1	15	0.109
UpG	0.122	0.1	1.1	10	0.068
mApU	0.200	1.3	1.7	27	0.127
$Up{}^mA$	0.097	0.5	1.1	10	0.035

^a $\delta_{H(5)}(\text{monomer}) - \delta_{H(5)}(\text{dimer})$, ppm.

^b $J_{1,2'}(\text{monomer}) - J_{1,2'}(\text{dimer})$, Hz.

^c $[(\delta_{5'} - \delta_{5''})_{\text{dimer}} - (\delta_{5'} - \delta_{5''})_{\text{monomer}}]$ for the 5'-nucleotidyl unit.

Base proton and H1' dimerization shifts have been used in the past to confirm existence of base-stacking (Ts'o et al., 1969; Chan and

Nelson, 1969; Danyluk and Hruska, 1968) and, in combination with theoretical isoshielding profiles (Giessner-Prettre and Pullman, 1970; Giessner-Prettre and Pullman, 1977), to fix relative orientations of bases in stacks. As noted earlier, a more complex situation prevails in methylated dimers where base protons are influenced by at least two major factors, the presence of a delocalized positive charge (deshielding), and base stacking (shielding increase). Nevertheless, a qualitative measure of base overlap is possible from a consideration of dimerization shifts[†] ${}^m\delta(\text{dimer}) - {}^m\delta(\text{monomer})$. Table VI summarizes $\Delta\delta_d$ values for protons of all three methylated dimers. Several features are apparent when one compares $\Delta\delta_d$ values in Table VI with corresponding $\Delta\delta_d$ for parent dimers (Ezra et al., 1977; Lee et al., 1976). In each instance, the absolute magnitudes of dimerization shifts are substantially less for methylated compounds, particularly for base and H1' protons. However, the general trend of $\Delta\delta_{H2(5)}^m > \Delta\delta_{H8(6)}^m$ follows the previous pattern. From this it can reasonably be concluded that base-base orientations are not radically different in normal and methylated dimers, even though stacked populations are reduced sharply, as in ${}^m\text{Ap}^m\text{A}$. A further quantitation of base orientations does not seem warranted given the relatively small magnitudes of $\Delta\delta_d^m$. Similarly, no assessment can be made of glycosyl torsion angles, χ_{CN} , in methylated derivatives, though any major change, viz. anti $\rightarrow$ syn domains is quite unlikely on methylation or dimerization.

Table VI
Dimerization Shifts* for Methylated Dimers

Proton	${}^m\text{Ap}^m\text{A}$		${}^m\text{ApU}$		Up^mA	
	${}^m\text{Ap-}$	$-p^m\text{A}$	${}^m\text{Ap-}$	$-p\text{U}$	Up-	$-p^m\text{A}$
2(5)	0.026	0.126	0.023	0.166	0.097	0.115
8(6)	- .008	.135	- .013	.130	.046	.121
1'	.059	.134	.032	.122	.165	.111
2'	.015	.024	- .003	.054	.073	.097
3'	- .061	.021	- .047	.065	.058	.017
4'	.058	.061	- .019	- .003	.073	.057
5'	.070	- .091	.011	- .131	.075	0
5''	.142	- .048	- .012	- .060	.110	.049
CH_3	0	0	0			- .009

* $\Delta\delta_d^m = \delta(\text{monomer}) - \delta(\text{dimer})$ in ppm.

From the preceding discussion a generalized conformational model for methylated dimers emerges that is similar to that of normal dimers except for a higher population of extended forms. Both the shift and coupling data are consistent with a rapidly interconverting mixture of stacked right helical, loop stack, and extended forms (Kondo and

[†]It is assumed that ring current distributions are unaltered in positively charged monomers and dimers.

Danyluk, 1976; Lee et al., 1976; Ezra et al., 1977). Whether the blend of these forms differs from normal dimers cannot be ascertained from the present data.

Effects of pD Change

Previous optical studies by Macon and Wolfenden (1968) found an apparent pK_a value of the conjugate acid of 1-methyladenosine, $N1^mAdo^+ \rightleftharpoons N1^mAdo$, to be 8.25 at 25°C. Since the conjugate base is uncharged, it is of interest to test whether neutralization of positive charges in both adenylate rings of $^mAp^mA$ leads to enhanced base

Figure 5. Variation of selected base proton shifts of $^mAp^mA$ with pD at 20°C.

stacking. Titration of $^m\text{Ap}^m\text{A}$ with base produces striking upfield shifts of base and H1' signals, Figure 5, and a decrease in $J_{1,2'}$ magnitudes, Figure 6 (the latter are accompanied by mirror-image increases of $J_{3,4'}$). Both sets of parameters plateau above $\text{pD} \sim 11.0$ at values very close to those for ApA (Figure 4), e.g., $\delta\text{H8 Ap}^- = 7.88 \text{ ppm}$ versus 7.90 ppm for $^m\text{Ap}^-$; $J_{1,2'} \text{ Ap}^- = J_{1,2'} ^m\text{Ap}^- = 3.6 \text{ Hz}$. Interestingly, pK_a is slightly lower, 9.0 ± 0.05 for $^m\text{Ap}^-$ than for $-\text{p}^m\text{A}$, 9.25 ± 0.05 ; both are stronger bases than corresponding mononucleotides.

Figure 6. Variation of $J_{1,2'}$ couplings for $^m\text{Ap}^m\text{A}$ with pD at 20°C .

Clearly, the transition from a zwitterionic form for adenylate fragments to a mono-anion (bases uncharged) greatly enhances base stacking by all NMR criteria. Somewhat surprisingly, the total stacked population (right helical + loop forms) is effectively the same as in ApA, whereas purine aggregation studies suggest an increase in the former.

Relevance to Polynucleotide Structure

Base modified nucleosides are fairly common in t-RNA and m-RNA (Hall, 1967; Nishimura, 1972; Rich and RajBhandary, 1976) and are generally believed to play important and specific structural roles. Amongst the most prevalent are methylated bases such as N3 methylcytidine, N7 methylguanosine, and N1 methyladenine (Dunn, 1961; Hall, 1967; Rich and RajBhandary, 1976). N1 Methyladenylate residues are found in nearly all t-RNA molecules for which accurate sequence information is available. Significantly, the recent crystallographic structure determinations for tRNA^{Phe} (Jack, Ladner, and Klug, 1976; Quigley et al., 1975; Kim et al., 1975; Brennan et al., 1975) show N1^m Ado at position 58 as the linking nucleotidyl residue between T ψ C arm (stem) and T ψ C loop regions. At this point, the ordered stacked structure of the stem region alters into a more open loop conformation. Generalizations from solution conformational data for dimers to expectations in polynucleotide structures are risky but it is nevertheless of interest that the highly disordered nature of N1 methylated adenylylate dimers is not inconsistent with the crystallographic results. It is tempting to assign a role for methylated adenylylate residues (and by inference N7^mGuo) as conformational mediators between ordered and open domains of t-RNA and m-RNA molecules. From this perspective, methylation of available base sites by carcinogens produces irreversible conformational changes and concomitant alterations of biological function.

REFERENCES

- Altona, C., and Sundaralingam, M., J. Amer. Chem. Soc. 95, 2333 (1973).
- Altona, C., Proceedings of the Fourth Annual Harry Steenbock Symposium, Madison, WI, M. Sundaralingam and S. T. Rao, ed., University Park Press, 1975, p. 613.
- Brennan, T., McMullan, R. K., Rubin, J., Ichikawa, T., Rao, S. T., and Sundaralingam, M., Fourth Annual Steenbock Symposium, Madison, WI, M. Sundaralingam and S. T. Rao, ed., University Park Press, 1975, p. 39.
- Brinacombe, R. L. C., Griffin, B. E., Haines, J. A., Haslam, W. J., and Reese, C. B., Biochemistry 11, 2452 (1965).
- Chan, S. I., and Nelson, J. H., J. Amer. Chem. Soc. 91, 168 (1969).
- Danyluk, S. S., and Hruska, F. E., Biochemistry 7, 1038 (1968).
- Davies, D. B., and Danyluk, S. S., Biochemistry 13, 4417 (1974).
- Davies, D. B., and Danyluk, S. S., Biochemistry 14, 543 (1975).
- Dunn, D. B., Biochim. Biophys. Acta 46, 198 (1961).

- Evans, F. E., and Sarma, R. H., FEBS Letters 41, 253 (1974).
- Ezra, F. S., Lee, C-H, Kondo, N. S., Danyluk, S. S., and Sarma, R. H., Biochemistry 16, 1977 (1977).
- Giessner-Prettre, C., and Pullman, B., J. Theor. Biol. 27, 87 (1970).
- Giessner-Prettre, C., and Pullman, B., J. Theor. Biol. 65, 189 (1977).
- Hall, R. H., Methods Enzymol. 12, 305 (1967).
- Hruska, F. E., and Danyluk, S. S., J. Amer. Chem. Soc. 90, 3266 (1968).
- Jack, A., Ladner, J. E., and Klug, A., J. Mol. Biol. 108, 619 (1976).
- Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A. H. J., Seeman, N. C., and Rich, A. Fourth Annual Steenbock Symposium, Madison, WI, M. Sundaralingam and S. T. Rao, ed., University Park Press, 1975, p. 7.
- Kondo, N. S., and Danyluk, S. S., Biochemistry 15, 757 (1976).
- Lawley, P. D., J. Chem. Phys. 1011 (1961).
- Lawley, P. D., and Brookes, P., Biochem. J. 89, 127 (1963).
- Lawley, P. D. Screening Tests in Chemical Carcinogenesis, International Agency for Research on Cancer, 1976, p. 181.
- Lee, C-H., Ezra, F. S., Kondo, N. S., Sarma, R. H., and Danyluk, S. S., Biochemistry 15, 3627 (1976).
- Lee, C-H., and Sarma, R. H., Biochemistry 15, 2460 (1976).
- Macon, J. B., and Wolfenden, R., Biochemistry 10, 3453 (1968).
- Magee, P. N., and Barnes, J. M., Advance in Cancer Res. 10, 163 (1967).
- Nishimura, S., Progr. Nucl. Acids Res. Mol. Biol. 12, 50 (1972).
- Pullman, A., Chemical Carcinogenesis, P. O. P. Ts'o and J. A. DiPaolo, ed., Part A, Marcel Dekker, Inc., 1974, Chapt. 16.
- Quigley, G. J., Wang, A. H. J., Seeman, N., Suddath, F. L., Rich, A., Sussman, J. L., and Kim, S. H., Proc. Nat. Acad. Sci. U.S.A. 72, 4866 (1975).
- Rich, A., and RajBhandary, U. L., Ann. Rev. Biochem. 45, 805 (1976).
- Sarma, R. H., Lee, C-H., Evans, F. E., Yathindra, N., and Sundaralingam, M., J. Amer. Chem. Soc. 96, 7337 (1974).

Singer, B., and Kusmierek, J. T., Biochemistry 15, 5052 (1976).

Ts'o, P. O. P., Kondo, N. S., Schweizer, M. P., and Hollis, D. P.,
Biochemistry 8, 997 (1969).

Wood, D. J., Hruska, F. E., Mynott, R. J., and Sarma, R. H., Can. J.
Chem. 51, 2571 (1973).