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Mutagenic role of Watson–Crick protons in alkylated DNA bases: A theoretical study

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Abstract. Loss of Watson–Crick protons following DNA base alkylation has been proposed as a key event which confers mutation-inducing properties on to alkylated DNA bases. In this theoretical study, the promutagenic O^6-guanine and O^4-thymine sites are clearly distinguished from the nonmutagenic N^7-guanine site on the basis of calculated values of mechanistic indicators for Watson–Crick proton acidity following alkylation at these respective sites. The degree of acidity predicted for these protons for each type of alkylated base accords well with the presence or absence of mutagenicity observed experimentally in each case.

Keywords. Alkylated DNA bases; promutagenicity; Watson–Crick proton acidity.

1. Introduction.

The carcinogenicity and mutagenicity of alkylating agents and *N*-nitroso compounds is attributed to their alkylating action upon DNA (Singer 1975). However, not all of the 16 alkylation sites so far identified are believed to be of carcinogenic or mutagenic significance, this choice being specifically limited only to certain oxygen sites on the DNA base moieties (Pegg 1977).

The promutagenic (mutation-inducing) properties of DNA bases alkylated at the O^6-guanine and O^4-thymine sites have been demonstrated by *in vivo* and *in vitro* studies on the base-pairing properties of O^6-alkyguanines (O^6-RGus) and O^4-alkyl-thymines (O^4-RThs) when incorporated into templates for nucleic acid polymerases (Gerchman and Ludlum 1973; Bhanot and Ray 1986; Abbott and Saffhill 1977; Preston *et al* 1986; Singer *et al* 1986). O^6-RGus possess the ability to induce the GC →AT transitional mutation, while the O^4-Ths can induce the TA →CG mutation. This ability of alkylated DNA to induce point mutations is of significance for chemical carcinogenesis in the light of the somatic mutation theory of cancer, especially when considering the evidence available for the mechanism of point mutation serving as a basis for activation of cancer genes (oncogenes) belonging to the *ras* family (Varmus 1984). The procarcinogenic role of these *O*-alkylated basis is corroborated by evidence indicating a correlation between tumour incidence and the persistence of these lesions in tissues of rats treated with alkylating carcinogens (Goth and Rajewsky 1974; Richardson *et al* 1983).

Such a promutagenic and procarcinogenic role is not indicated for the products of alkylation at the N^7-guanine site. N^7-methylguanine residues lack the capacity to induce base-misincorporation when present in substrates for nucleic acid polymerases (Ludlum 1970). Persistence of N^7-methylguanine in tissues of rats treated

with methylnitrosourea could not be correlated with tumour incidence (Schoental 1969). Such evidence points to the promutagenic role of the O^6-RGus and O^4-RThs in contrast to the nonmutagenicity of the N^7-alkylguanines (N^7-RGus).

The prornutagenicity of chemically modified nucleic acid bases has been investtigated quite extensively by theoretical means. Psoda *et al* (1981) studied the base-pairing properties of O^6-methylguanine and N^4-hydroxycytosine using a perturbational method. An improved technique was employed by Pohorille and Loew (1985) to investigate the potential for base-misincorporation possessed by several *O*-methylated nucleic acid bases. These studies helped define some steric and energetic considerations for successful base-mismatching, but did not serve to provide any clear basis for differentiating between the promutagenic and nonmutagenic types of alkylated bases.

This prompted the need to advance a molecular basis for differentiating between promutagenic and nonmutagenic species. In an earlier paper, Duncan and Davies (1989) had proposed abstraction of the Watson–Crick protons (the N^1-proton for alkylated guanines and the N^3-proton for alkylthymines) as a mechanistic basis for the conferring of mutation-inducing properties on to the alkylated base residue. Theoretically calculated values of certain mechanistic indicators for facility of the deprotonation reaction were found to differentiate quite effectively between promutagenic and nonmutagenic species.

It is, of course, conceivable that the promutagenic property might stem from the direct effect of the steric presence of the alkyl groups at the O^6-guanine and O^4-thymine positions, which might perturb the Watson–Crick hydrogen-bonding schemes. But this would be of mutagenic significance only if such perturbation not only makes normal base-pairing unfavourable, but also favours aberrant base-pairing situations involving incorporation of the wrong base. Now, in the conformation of the *O*-alkylated bases where the alkyl group is *syn* to the hydrogen-bonding side, although normal base-pairing is certainly disrupted, neither is aberrant base-pairing possible in the double-helical configuration due to the steric block introduced by the alkyl group position. The theoretical study of Pohorille and Loew (1985) has indicated that, in the double-helical configuration, no pairing scheme whatsoever is possible for the *syn* conformer with any DNA base, all interactions being strongly repulsive, and so we may conclude that the *syn* conformer of the *O*-alkylated bases is of no relevance for base-pair substitutional mutagenesis in the double-helical configuration.

Now, if the alkyl group is in the *anti* conformation, since a lone pair is present on the oxygen, there is no reason why normal base-pairing should not be possible, *provided* the Watson–Crick protons are still present. However, if these protons are abstracted for some reason, the stage could be set for unusual base-pairing situations, where modified (alkylated) guanine pairs with thymine instead of cytosine, and modified thymine pairs with guanine instead of adenine. The *anti* conformation of the alkyl group here should provide no steric hindrance to such aberrant base-pairing in the double-helical configuration. So it may be inferred that the primary factor for aberrant (mutagenic) base-pairing in the double-helical configuration is not the mere steric presence of the alkyl group, but rather the absence of the relevant Watson–Crick protons. This point may be further illustrated by referring to the ideas advanced by Pullman and Pullman (1962, 1964) concerning the mutation-inducing properties of free (unalkylated) DNA bases in their tautomeric forms,

where no alkyl group is present, but the Watson–Crick protons are not present in their usual positions.

This work focusses on the hypothesis of Duncan and Davies (1989), seeking to furnish additional substantiation of the role of Watson–Crick protons for promutagenicity. A comparison is made between the promutagenic and nonmutagenic species of the calculated affinity between the concerned Watson–Crick proton and a candidate deprotonating agent. Note is also taken of the reaction running *reverse* to the Watson–Crick deprotonation reaction of figure 1, *viz.,* the reaction of N^1-protonation for alkylguanines and of N^3-protonation for alkylthymines (see figure 1). If Watson-Crick proton acidity is to be correlated with promutagenicity, then calculated indices for the feasibility of this reaction should predict a greater acidity for the protons of the promutagenic *O*-alkylated bases as compared with those of the nonmutagenic N^7-RGus.

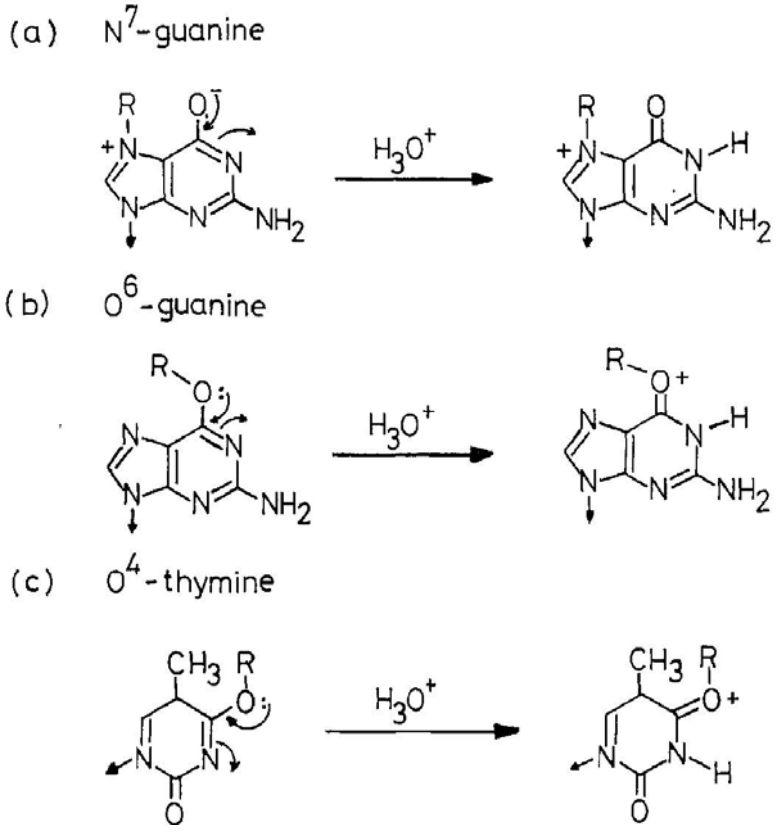

Figure 1. The Watson–Crick protonation for DNA bases alkylated at (a) the N^7-guanine, (b) the O^6-guanine and the (c) the O^4-thymine positions.

2. Methods of calculation.

The molecular wave function for all species was obtained by the semiempirical INDO SCF-MO method (Pople *et al* 1967), complete optimization of all molecular

geometries being performed by an improved version (Duncan 1989) of an analytical gradient procedure (Kanakavel *et al* 1976). Self-consistent field convergence was accelerated by an extrapolation procedure involving the density matrix elements (Duncan 1991). The INDO SCF-MO method gives a reliable estimate of the molecular charge distribution (atomic charges, bond orders and dipole moments). While this method tends to overestimate enthalpies of bond-making and bond-breaking reactions, it has been shown by Duncan (1989) that the INDO values of enthalpies for such reactions within a homologous series reproduce the same trends of magnitude orderings as those given by more sophisticated and time-consuming quantum chemical theories.

Starting geometries for the alkylated bases (cationic form) were obtained by imposing standard geometries for the alkyl groups on to the geometries of the DNA bases got from crystal structure data (Thewalt *et al* 1971; Gerdil 1961). Starting geometries for the neutral (deprotonated) forms of the modified bases were constructed by simply removing the concerned proton from the structure of the cationic modified base. Since it has been argued earlier that the *anti* conformers of the *O*-alkylated bases are the conformers relevant for promutagenic base-pairing, only the *anti* conformers of the O^6-RGhs and O^4-RThs were taken into consideration for this study. All molecular species were optimized with respect to the cartesian coordinates of all atoms till the square of the gradient norm crossed a threshold of 0·002 N mdyn^2 for each molecule, where N is the number of atoms present in the molecule.

The following theoretically calculated indices were utilized to compare acidities of the Watson–Crick protons of interest (the N^1-protons for alkylguanines and the N^3-protons for alkylthymines).

2.1 *Interaction energy between cationic alkylated base and deprotonating agent.*

This quantity refers to the affinity between the concerned protons of the alkylated DNA base and the candidate deprotonator. Using a hydroxide anion as a candidate deprotonator, the interaction energy E_{dp} between the alkylated base and the deprotonator was calculated for an intermolecular configuration where the anion approaches the concerned proton in the plane of the base from the hydrogen-bonding side.

The approach used here to calculate non-bonded affinities between the electrophilic alkylated bases and the nucleophilic hydroxide anion stems from the approach of Klopman (1968) who introduced the concepts of charge-and frontier-controlled aspects of reactivity. The first aspect pertains to the charge distribution of the two interacting species, which here is approximated by a monopole-monopole truncation of the infinite series for the interaction between two separate charge distributions. The monopoles (atom charges) were calculated from a Mulliken population analysis performed on each of the interacting systems following a semiempirical MO calculation (INDO SCF-MO method) with full geometry optimization, as described above.

The second aspect refers to the beginning of the formation of the covalent bond between the interacting species, and is here approximated by an expression derived from the frontier orbital concept, where only the frontier orbitals of the two species are held to be of prime importance regarding this aspect. Since the two aspects

of charge and frontier-controlled contributions both involve terms that invariably increase as the interatomic distance between the atoms of interest is minimized, no scope is left for optimization of the intermolecular configuration unless a repulsive interaction term is incorporated as well.

The objective here is to incorporate all three aspects of charge-controlled interaction, overlap-controlled interaction and repulsive interaction in the simplest manner as may be sufficient to advance the point of this study through. Here, the energy of interaction $E_{dp,}$ between the alkylated base A and the deprotonating agent B is expressed as a sum of the electrostatic contribution $E_{es,}$ the covalent interaction term $E_{cv,}$ and a repulsive interaction term $E_{rp,}$ so that

$$E_{dp} = E_{es} + E_{cv} + E_{rp}, \tag{1}$$

where

$$E_{es} = \sum_{a}^{A} \sum_{b}^{B} Q_a Q_b / \varepsilon R_{ab}, \tag{2}$$

Q_a and Q_b being the coulombic point charges on the atoms a and b of systems A and B respectively, R_{ab} being the internuclear distance for each interacting pair of atoms, and ε the dielectric constant for the medium, which is assigned a value of 4.0 following the suggestion of N Yathindra (personal communication).

E_{cv} is calculated taking only the first two concerned frontier orbitals of each of the systems A and B, being explicitly calculated only for the immediately interacting atoms. E_{cv} may then be derived from the complete perturbation al treatment of Klopman (1968) to be expressed as

$$E_{cv} = \sum_{h}^{m} \sum_{o}^{n} \sum_{m}^{2\ emp} \sum_{n}^{2\ occ} 2(C_h C_o B_{ho})^2 / (E_n - E_m), \tag{3}$$

C_o and C_h being respectively the atomic orbital coefficients of the Watson–Crick proton of system A and of the oxygen atom of the deprotonating system B in the first two appropriate frontier orbitals of the interacting species, B_{ho} being the corresponding resonance integral, and E_m and E_n being the energies of the concerned frontier orbitals. The integral B_{ho} is approximated as the off-diagonal term in the CNDO/2 core Hamiltonian, given by

$$B_{rs} = (B_h + B_o)S_{rs}/2, \tag{4}$$

where B_h and B_o are CNDO/2 parameters dependent upon the elements hydrogen and oxygen respectively, S_{rs} being the corresponding overlap integral.

E_{rp} is calculated using the repulsive terms of the 6–12 potential of Lennard–Jones, where, for all the atoms a and b of systems A and B respectively,

$$E_{rp} = \sum_{a}^{A} \sum_{b}^{B} D_{ab} / (R_{ab})^{12}, \tag{5}$$

where D_{ab} represents the constant parameter for repulsive interaction between a

pair of atoms a and b of systems A and B respectively, R_{ab} being the internuclear distance.

The total interaction energy $E_{dp'}$ as given by eqn. (1), was minimized by varying the internuclear distance R_{ho} between the proton of system A and the oxygen of system B, the deprotonating agent being oriented in such a manner as to ensure maximum frontier overlap interaction between the interacting atoms at all stages of the optimization, R_{ho} being varied at intervals of 0·10 A. The search in configurational space was simplified by the following considerations (apart from the condition of maximum frontier overlap as just mentioned). The hydroxide anion was placed in the plane of the base with the oxygen atom being collinear with the N–H bond of the alkylated base. This collinearity allows for the maximum distance apart between the negatively charged oxygen and nitrogen atoms, thus contributing to the lowering of the interaction energy. It was also presumed that this in-plane interaction would be the favourable one, since previous studies (Mohammed and Hopfinger 1980) on the non-bonded interactions between nucleophiles and electrophiles involving DNA base systems predicted the favourability of this in-plane interaction over out-of-plane interactions.

2.2 *Indices concerning the reverse reaction of protonation.*

These are derived from the neutral alkylated bases, and include the following calculated indices:

(i) Q_n (the negative charge on the N^1-atom for alkylguanines and on the N^3-atom for alkylthymines), lower values of which would point to greater facility of Watson–Crick protonation.

(ii) E_h (the energy of the highest occupied MO involving these nitrogens), for which higher values would indicate greater ease of protonation, assuming that the lowest empty MO of the protonating species is of higher energy level than these occupied MOs.

(iii) P_z (the pi electron density present in the C^6–N^1 bond for alkylguanines and in the C^4–N^3 bond for alkylthymines), which represents the ease of electron flow during the protonation reaction (see arrows in figure 1), lower values of which being associated with greater ease of flow and of the protonation reaction.

(iv) ΔH_p, the enthalpy of Watson–Crick protonation as given by eqn. (6) below:

$$\Delta H_n = \{\Delta H_f(\mathrm{RBH^+}) + \Delta H_f(\mathrm{H_2O})\} - \{\Delta H_f(\mathrm{RB}) + \Delta H_f(\mathrm{H_3O^+})\}, \tag{6}$$

corresponding to the enthalpy of the reaction of eqn. (7) below:.

$$\mathrm{RB} + \mathrm{H_3O^+} \rightarrow (\mathrm{RBH})^+ \mathrm{H_2O}, \tag{7}.$$

where RB and $(RBH)^+$ stand for the neutral and protonated forms of the alkylated base. The enthalpy term is used here rather than the free energy change, since the entropy changes would have been very tedious to calculate for the large number of fair-sized systems studied here. Furthermore, it has been shown (Venkateswarlu and Lyngdoh, unpublished results) that the AM1 SCF-MO calculated free energy changes for reactions involving DNA bases do not differ much from the corresponding enthalpy changes, since the entropy terms cancel out in approximately equal manner.

Values of the above indices are calculated for the N^7-RGus, the O^6-RGus and the O^4-RThs in order to examine if any good trend of demarcation arises in these values between the promutagenic O-alkylated bases and the nonmutagenic N^7-RGus. Such a predicted difference in Watson–Crick proton acidity between pro- and non-mutagenic species would corroborate the hypothesis proposed, *viz.,* that abstraction of these protons is a key event in the mutagenic pathway following alkylation of DNA.

3. Results and discussion

Tables 1–3 present optimized values of the interaction energy E_{dp} (together with its constituent terms) for the three sets of alkylated DNA bases, alkylated at the N^7-G, O^6-G and O^4-T sites respectively. A total of nine different alkyl groups were considered in each set for the sake of generality, being abbreviated as follows: methyl (Me), ethyl (Et), *n*-propyl (Pr), isopropyl (Pr^i), *n*-butyl (Bu), *n*-pentyl (Pe), 2-hydroxyethyl (HE), 2-acetoxyethyl (AE) and cyanomethyl (CM). For the sake of

Table 1. Optimized values of E_{dp} (with constituent terms and optimum R_{ho} distance) for interaction between hydroxide anion and N^7-alkylguanines.*

Alkyl group	E_{es}	E_{cv}	E_{rp}	E_{dp}	R_{ho}
Me	−15·25	−0·27	0·80	−14·72	2·40
Et	−15·07	−0·25	0·80	−14·52	2·40
Pr	−15·04	−0·26	0·80	−14·51	2·40
Pr^i	−15·06	−0·27	0·80	−14·53	2·40
Bu	−14·95	−0·26	0·80	−14·41	2·40
Pe	−14·91	−0·26	0·80	−14·37	2·40
CM	−15·47	−0·17	0·80	−14·83	2·40
HE	−15·05	−0·18	0·80	−14·43	2·40
AE	−15·59	−0·20	0·80	−14·99	2·40

*Values of energy quantities in kcal/mol and of R_{ho} in Å.

Table 2. Optimized values of E_{dp}, (with constituent terms and optimum R_{ho} distance) for interaction between hydroxide anion and O^6-alkylguanines.*

Alkyl group	E_{es}	E_{cv}	E_{rp}	E_{dp}	R_{ho}
Me	−18·83	−0·72	1·28	−18·26	2·30
Et	−18·73	−0·66	1·29	−18·11	2·30
Pr	−18·70	−0·66	1·29	−18·07	2·30
Pr^i	−18·32	−0·74	1·26	−17·80	2·30
Bu	−18·43	−0·65	1·29	−17·79	2·30
Pe	−18·64	−0·65	1·29	−18·00	2·30
CM	−19·54	−1·13	2·16	−18·51	2·20
HE	−18·88	−0·68	1·30	−18·27	2·30
AE	−18·57	−0·67	1·29	−17·95	2·30

*Values of energy quantities in kcal/mol and of R_{ho} in Å.

Table 3. Optimized values of E_{dp} (with constituent terras and optimum R_{ho} distance) for interaction between hydroxide anion and O^4-alkylthymines.*

Alkyl group	E_{es}	E_{ev}	E_{rp}	E_{dp}	R_{ho}
Me	– 18·13	– 1·83	2·10	– 17·86	2·20
Et	– 18·04	– 1·39	2·10	– 17·33	2·20
Pr	– 17·95	– 1·29	2·10	– 17·15	2·20
Pr^i	– 17·38	– 0·77	1·25	– 16·90	2·30
Bu	– 18·01	– 1·24	2·10	– 17·14	2·20
Pe	– 17·87	– 1·24	2·10	– 17·01	2·20
CM	– 18·80	– 3·70	3·70	– 18·88	2·10
HE	– 18·15	– 1·41	2·10	– 17·46	2·20
AE	– 18·82	– 1·18	2·06	– 17·94	2·20

* Values of energy quantities in kcal/mol and of R_{ho} in Å.

making equable comparisons, the values of E_{dp} at a fixed internuclear distance R_{ho} of 2·50 A are also entered into table 4 for the three sets of alkylated bases, along with the optimized values. Table 5 presents values for the various indices for Watson–Crick protonation facility, *viz.*, Q_n, E_h, P_z and ΔH_p, data being given again for the three sets of alkylated bases, six different alkylating groups being taken into account.

Comparison of the values of all these indices between the nonmutagenic N^7-alkylguanines and the promutagenic *O*-alkylated bases reveals that a clear demarcation is quite evident between the two types in the values of these indices for Watson–Crick proton acidity. This trend of differentiation may be summarized by noting the value ranges for each index corresponding to the three sets of alkylated bases, as given in table 6.

This trend of demarcation between the N^7alkylguanines and the *O*-alkylated bases is portrayed in figures 2 and 3 for the E_{dp}, E_h, and Q_n indices respectively. All of these indices clearly predict lower favourability of the protonation reaction

Table 4 Values of E_{dp} at fixed R_{ah} distance of 2·50 Å along with optimized values for N^7-alkylguanines (N^7-RGus), O^6-alkylguanines (O^6-RGus) and O^4-alkylthymines (O^4-RThs).*

Alkyl group	N^7-RGus		O^6-RGus		O^4-RThs	
	2·5 Å	optd	2·5 Å	optd	2·5 Å	optd
Me	– 14·59	– 14·72	– 17·76	– 18·26	– 16·83	– 17·86
Et	– 14·40	– 14·52	– 17·65	– 18·11	– 16·65	– 17·33
Pr	– 14·38	– 14·51	– 17·62	– 18·07	– 16·54	– 17·15
Pr^i	– 14·40	– 14·53	– 17·30	– 17·80	– 16·41	– 16·90
Bu	– 14·29	– 14·41	– 17·37	– 17·79	– 16·58	– 17·14
Pe	– 14·25	– 14·37	– 17·55	– 18·00	– 16·45	– 17·01
CM	– 14·73	– 14·83	– 17·97	– 18·51	– 17·16	– 18·88
HE	– 14·34	– 14·43	– 17·79	– 18·27	– 16·76	– 17·46
AE	– 14·88	– 14·99	– 17·47	– 17·95	– 17·21	– 17·94

*Values of E_{dp} in kcal/mol and of R_{ho} in Å

Table 5. INDO calculated indices for facility of Watson–Crick protonation of neutral alkylated DNA bases.*

Species	Q_n	E_h	P_z	ΔH_p
N^7-alkylguanines				
N^7-MeG	−0·432	−0·326	−0·485	−149·9
N^7-EtG	−0·432	−0·323	−0·487	−152·0
aN^7-Pr^{i}G	−0·429	−0·328	−0·498	−149·6
N^7-CMG	−0·429	−0·331	−0·488	−149·3
N^7-HEG	−0·432	−0·325	−0·486	−151·3
N^7-AEG	−0·430	−0·326	−0·487	−151·6
O^6-alkylguanines				
O^6-MeG	−0·365	−0·395	−0·630	−110·1
O^6-EtG	−0·367	−0·387	−0·633	−113·9
O^6-Pr^{i}G	−0·366	−0·365	−0·634	−107·5
O^6-CMG	−0·361	−0·397	−0·635	−107·7
O^6-HEG	−0·364	−0·389	−0·632	−111·5
O^6-AEG	−0·365	−0·394	−0·634	−116·9
O^4-alkylthymines				
O^4-MeT	−0·341	−0·419	−0·745	−99·1
O^4-EtT	−0·348	−0·414	−0·744	−100·8
O^4-Pr^{i}T	−0·349	−0·411	−0·738	−105·1
O^4-PeT	−0·348	−0·412	−0·745	−101·5
O^4-CMT	−0·340	−0·423	−0·749	−95·1
O^4-HET	−0·345	−0·416	−0·747	−99·1
O^4-AET	−0·344	−0·421	−0·747	−102·1

* Values of Q_n, E_h and P_z in atomic units and of ΔH_p in kcal/mol.

Table 6. Range of values* for calculated indicators of Watson–Crick proton acidity, comparison being made for the N^7-RGus, the O^6-RGus and the O^4-RThs.

Index	N^7-RGus	O^6-RGus	O^4-RThs
E_{hp}	−14·4 to −15·0	−17·8 to −18·5	−16·9 to −18·9
Q_n	around −0·43	around −0·36	around −0·35
E_h	around −0·33	around −0·39	around −0·42
P_z	around 0·49	around 0·63	around 0·74
ΔH_p	−149 to −152	−107 to −117	−95 to −105

* Values of Q_n, E_h and P_z in atomic units and values of E_{hp} and ΔH_p in kcal/mol.

and greater Watson–Crick proton acidity for the promutagenic *O*-alkylated bases as compared with the nonmutagenic N^7-alkylguanines. The general trend followed for proton acidities as shown by the ΔH_p index turns out to be O^4-RThs > O^6-RGus > N^7-RGus. Each of the different alkyl groups demonstrates this trend, which may be held to be independent of the nature of the alkylating group.

The limited experimental data on acid dissociation constants for alkylated nucleosides seem to confirm these trends. The pK_a values calculated for N^7-alkylguanosines are generally somewhat higher than the neutral pH of 7, being estimated as 6·7 to 7·3 for N^7-methylguanosine (Hendler *et al* 1970; Singer 1972) and as

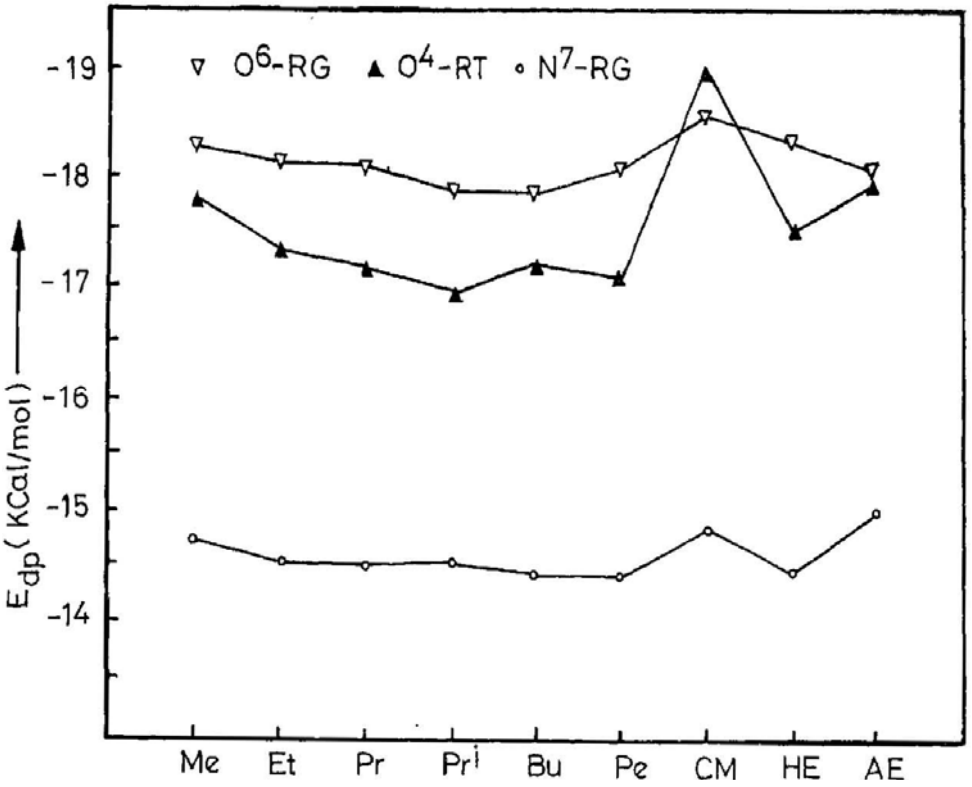

Figure 2. Differentiation in Watson–Crick proton acidity between the O-alkylated bases and the N^7-alkylguanines, as demonstrated by the E_{dp} index.

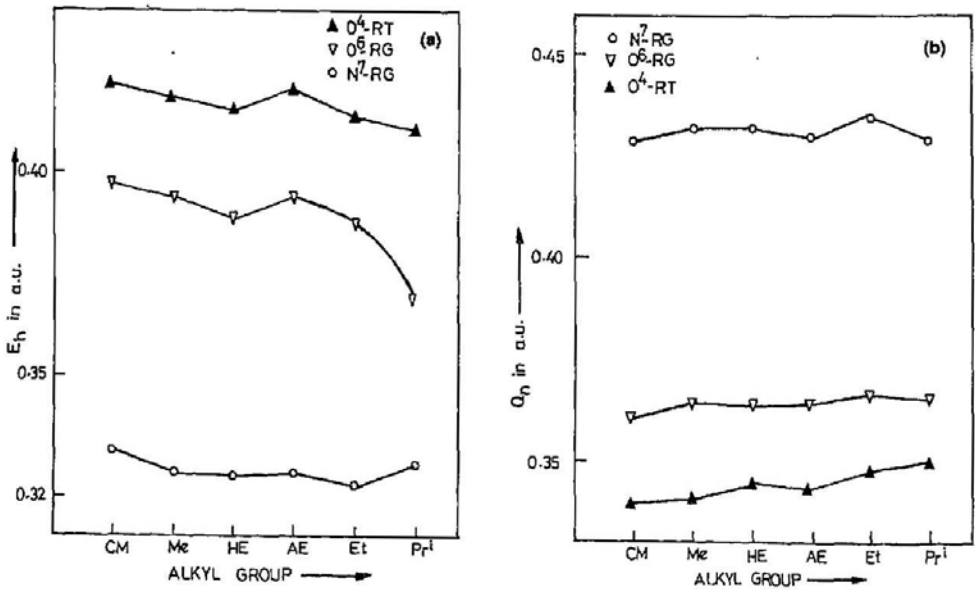

Figure 3. Differentiation in Watson–Crick proton acidity between the O-alkylated bases and the N^7-alkylguanines, as demonstrated by (a) the *Eh* index, and (b) the *Qn* index.

7·2 to 7·4 for N^7-ethylguanosine (Brookes *et al* 1968; Singer 1972). But the O^6-alkylguanosines and O^4-alkylthymidines are invariably associated with pK_u values lower than 7·0. This is seen in the greater proton acidity of the O^6-alkylguanosines, whose pK_a is estimated as 2·4 for O^6-methylguanosine and as 2·5 for O^6-ethylguanosine (Singer 1972). The O^4-alkylated pyrimidines are associated with even greater Watson–Crick proton acidity, as is evinced by the pK_a values of 0·8,0·7 and 0·3 estimated by Allore *et al* (1983) for O^4-methylated uridine, deoxyuridine and deoxythymidine respectively, and also by the pK_a values of 0·66 and –0·32 obtained by Singer *et al* (1978) for O^4-ethylated deoxyuridine and deoxythymidine respectively. The free (unalkylated) bases and nucleosides have pK_a values which point to the high resistance of the Watson–Crick protons to abstraction, being around 13 or more (data compiled by Dunn and Hall 1975).

Experimental data thus points to the following order of magnitude for Watson–Crick proton acidity with respect to variation in species type: O^4-RTh > O^6-RGu > N^7-RGu > free base or nucleoside. The pK_a value ranges for each type suggest that the O^4-RThs and O^6-RGus would readily deprotonate in neutral or biological pH, while the N^7-RGus and the free bases/nucleosides would retain their Watson–Crick protons at biological pH. This is precisely what lies behind the differences in the mutation-inducing properties of the O^4-RThs and O^6-RGus on one hand and the N^7-RGus and free nucleosides on the other.

It may be pointed out that the physical origin of these differences in Watson–Crick proton acidity could lie in the effect of distance of the electron-withdrawing alkyl group from the concerned protons, this distance being appreciably larger for the case of the N^7-alkylguanines than for the cases of the *O*-alkylated bases. While the effect of distance upon proton acidity is an interesting phenomenon in itself, the point to be stressed here is that it is precisely this distance effect which finally manifests itself as a difference in promutagenic potential, being mediated through the role that the Watson–Crick protons play for base-pairing.

The suggestion may now be made that the *O*-alkylated bases would exist in biological pH in their deprotonated form as "frozen tautomers" which resemble the transient tautomeric forms G* and T* (Pullman and Pullman 1962, 1964) in possessing promutagenic properties. The N^7-alkylguanines, however, would exist in the cationic (protonated) form *in vivo,* resembling the free base guanine in its normal form (which does not possess promutagenic properties).

Upon considering the results of this study in conjunction with the previous theoretical study of Pohorille and Loew (1985), two conditions for successful base-mismatching emerge, *viz.,* that (i) the Watson–Crick protons of the alkylated guanine and thymine be abstracted, and (ii) the *O*-alkyl group should not sterically hinder the base-mismatching scheme. Since the study of Pohorille and Loew (1985) treated only promutagenic *O*-alkylated bases (with the Watson–Crick protons absent), a more complete picture can arise only after considering both promutagenic and nonmutagenic alkylated bases (*N*- and *O*-alkylated), subjecting them to the various alternatives open for base-pairing, and incorporating the various possibilities considered, *viz.,* the presence or absence of the Watson–Crick protons as well as the conformational role of the alkyl group. This work is now currently being carried out here.

4. Conclusions

Theoretical treatment of Watson–Crick acidity from the viewpoint of the protonation reaction (concerning the neutral alkylated base) consistently predicts a clear differentiation in this acidity between the promutagenic *O*-alkylated DNA bases and the nonmutagenic N^7-alkylguanines, the former being associated with greater Watson–Crick proton acidity than the latter. This good correlation between Watson–Crick proton acidity and promutagenicity of these alkylated bases supports the proposal that abstraction of these protons is a key event which confers promutagenic properties on to the alkylated DNA base.

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