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Comparison of alkyl group labilities in O- and N-alkylated DNA bases: A semiempirical molecular orbital study

R H DUNCAN LYNGDOH

Institute of Self-Organising Systems and Biophysics, North-Eastern Hill University, Mawkynroh-Umshing, Mawlai, Shillong 793 022 India

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Abstract. All O-alkylated DNA bases and nucleosides possess alkyl groups considerably more labile than those in N-alkylated bases and nucleosides, being prone to degradation through loss of the alkyl group at strongly acidic *p*H. The strength of the bond between the alkyl group and the atom on the base to which it is bound is calculated here using the semiempirical INDO–SCF–MO method, comparison being made between O^6-alkylguanines, O^4-alkylthymines and N^7-alkylguanines. The results, calculated for many different alkyl groups, predict that the strength of this bond at acidic *p*H would be appreciably lower for the O-alkylated bases than for the N^7-alkylguanines, but that increase of *p*H would serve to stabilise this bond for the O-alkylated bases. These predictions are in good accord with experimental findings.

Keywords. Alkylated DNA bases; alkyl group lability; molecular orbital calculations.

1. Introduction

The role of DNA alkylation for carcinogenesis and mutagenesis by N-nitroso compounds and alkylating agents is well-established (Lawley 1984; Osborne 1984; Preussmann and Stewart 1984). Out of the 16 different alkylation sites so far characterised (Singer 1975; Pegg 1977), only the O^6-guanine and the O^4-thymine positions are believed to be of relevance for cancer and mutagenesis when alkylated (Margison and Kleihues 1975; Abbott and Saffhill 1977; Richardson *et al* 1983; Bhanot and Ray 1986; Preston *et al* 1986). The usually more abundantly alkylated N^7-guanine site is apparently of no carcinogenic or mutagenic significance (Schoental 1969; Ludlum 1970). Figure 1 portrays the products of alkylation at the O^6-G, O^4-T and N^7-G sites, where species I, II and III represent the cationic forms of the O^6-alkylguanines (O^6-RGus), the O^4-alkylthymines (O^4-RThs) and the N^7-alkylguanines (N^7-RGus) respectively. Species IV, V and VI represent their respective conjugate bases–the alkylated bases in their neutral (deprotonated) form. The protons involved in the acid–base equilibria here are the Watson–Crick protons, viz. the N^1-proton for the alkylguanines and the N^3-protons for the alkylthymines, as inferred from the suggestions of Loveless (1969) and of Kusmierek and Singer (1976).

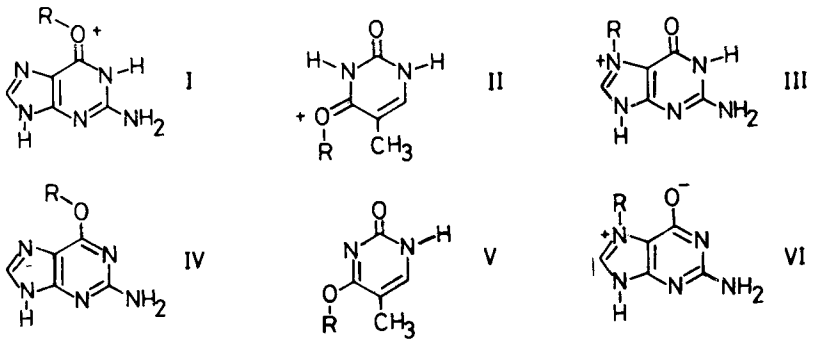

Figure 1. The cationic and deprotonated (neutral) forms of the products of alkylation at the N^7-G, O^6-G and O^4-T sites on DNA bases.

1.1 *Experimental background*

The products of DNA alkylation by carcinogens *in vitro* and *in vivo* are separated for characterisation and analysis by a variety of methods for hydrolysing the modified DNA. For a number of years, certain important O-alkylated base residues escaped proper identification and quantification due to their degradation under the strongly acidic conditions used for DNA hydrolysis (Lawley and Thatcher 1970; Frei and Lawley 1975), while the N-alkylated base residues were retained. This loss was due to the lability of the O-alkyl groups under highly acidic *p*H (Frei *et al* 1978; Singer *et al* 1978) which did not, however, render the N-alkyl groups labile to a similar extent. This failure to characterise and quantify the O^6-alkylguanines and O^4-alkylthymines was a significant setback to a fuller understanding of the molecular mechanisms of carcinogenesis, especially considered in the light of the procarcinogenic and promutagenic role assigned to these O-alkylated bases, particularly the O^4-RThs (Singer 1986). The use of milder conditions of acidity and temperature allowed for O^6-methylguanine to be detected in rat tissues treated with methylating N-nitroso carcinogens (Kleihues and Magee 1971; O'Connor *et al* 1972, 1973). Even these conditions failed to detect the more minute levels of O^6-methylguanine produced by action of methane methanesulphonate. A comprehensive and accurate analysis of the various O-alkylation products would call for the use of enzymic hydrolysis at almost neutral (biological) *p*H, when no significant degradation occurs (Singer 1976; Beranek *et al* 1980).

On the other hand, the N-alkylated bases and nucleosides may be characterised by a stronger binding of the alkyl group to the site of attachment. This is apparent from their ability to retain the alkyl group intact when subjected to strongly acidic conditions for DNA hydrolysis. N^7-methylguanine, N^3-methylcytosine, N^7- and N^3-methyladenine have all been separated out and identified upon hydrolysis of treated DNA by 1·0 M HCl at 100°C for 1 hour (Magee and Farber 1962; Lawley and Thatcher 1970; Frei and Lawley 1975; Swann and Magee 1971).

The above observations indicate that alkyl group lability of the alkylated bases (in their conjugate acid form corresponding to the highly acidic *p*H used) is significantly greater for the O-alkylated bases than for the N-alkylated bases. Moreover, the raising of *p*H by employing mildly acid or neutral conditions helps to stabilise the alkyl group binding for the O-alkylated bases.

It may be added that, in the broader perspective of general heterocyclic chemistry, the study of alkylation of heterocyclic compounds has focussed largely on C-alkylation by hard alkylating agents. The N- and O-alkylation dealt with here arise from the action of the softer alkanediazonium ion or of alkylating esters. In any case, the varying labilities of N- and O-alkyl groups in heterocyclic compounds has received serious attention only in the context of the alkylation of DNA constituents, and that too because of the significance of this reaction for mutagenesis and carcinogenesis.

1.2 *Previous theoretical studies*

Molecular orbital studies on alkylated DNA constituents, in particular the modified DNA bases and nucleosides, have been performed by various groups of workers (Mohammed and Hopfinger 1980; Psoda *et al* 1981; Pohorille and Loew 1985; Pederson *et al* 1988; Ford and Scribner 1990). These studies were all chiefly concerned with elucidation of the mechanisms of carcinogenesis and mutagenesis. MNDO–MO studies (Ford and Scribner 1990) calculated the activation energies of O- and N-alkylation of DNA bases by various alkanediazonium ions, relating their values to the O-selectivity of the reaction (a feature of importance for carcinogenesis). Studies using perturbational methods (Psoda *et al* 1981; Pohorille and Loew 1985) treated the base-pairing properties of various O-alkylated DNA bases in relation to their mutagenic properties. The alkyl group affinities in gas phase for various oxygen and nitrogen sites on heterocyclic compounds have been calculated by the MNDO method and also estimated from related experimental data by a number of empirical assumptions (Ford and Scribner 1983), but no attempt was made to relate their values to alkyl group labilities observed experimentally in liquid medium.

The problem of varying alkyl group labilities of modified DNA constituents, so well-documented in experiment, has so far received no definitive treatment by theoreticians. The possible effects of *p*H change upon the properties of DNA constituents has also not been dealt with using theoretical methods. There is also the need to adopt a generalising approach by the incorporation of a large number of different alkyl groups, since previous studies, both experimental and theoretical, have employed only one, or at the most, three different alkyl groups in their studies.

1.3 *Scope of this study*

This study employs semiempirical molecular orbital theory to examine the susceptibility to dealkylative degradation of alkylated DNA bases, comparison being made between O-alkylated bases (O^6-RGus and O^4-RThs) and one class of N-alkylated bases (N^7-RGus). Conditions under high and low *p*H are treated here by considering both the neutral (deprotonated) forms of the alkylated bases as well as their conjugate acids. The aims of this study may be delineated as follows.

(a) To compare alkyl group lability between the O-alkylated bases and the N-alkylated bases, both in their cationic form, to corroborate the finding that, in strongly acidic *p*H, the O-alkyl groups are more labile than the N-alkyl groups.
(b) To compare alkyl group lability between the neutral and cationic forms of the O-alkylated bases to corroborate the finding that raising of *p*H serves to increase the strength of binding between the alkyl group and the base.

2. Theoretical

2.1 *Theoretical method*

The wavefunctions for all molecular species studied were calculated using the semi-empirical INDO–SCF–MO method (Pople *et al* 1967). Owing to the prolonging of the self-consistent procedure by oscillatory behaviour of the wavefunction, SCF convergence was effectively accelerated by two-point extrapolation of the density matrix elements (Duncan 1991). The INDO method, being parametrised to reproduce *ab initio* values of dipole moments, may be regarded as quite reliable in evaluating physical quantities immediately derived from the density matrix, such as charge densities and bond orders.

Complete optimisation of all geometries was performed by an analytical gradient method (Kanakavel *et al* 1976), starting geometries being obtained from crystal structure data (Gerdil 1961; Thewalt *et al* 1971) and from standard data (Pople and Beveridge 1967). The conformers used for the O-alkylated bases had the exocyclic O-alkyl *anti* to the Watson–Crick hydrogen-bonding side, since this conformer is the one which would allow for effective base-mismatching (Pohorille and Loew 1985; Pederson *et al* 1988).

2.2 *Theoretical indices of alkyl group lability*

The following theoretical indices were employed to gauge the susceptibility to dealkylation for the alkylated bases.

(1) The strength of the bond C–X between the α-carbon C of the alkyl group and the nucleophilic atom X on the base moiety to which it is attached, measured by the Wiberg bond index W_{cx} (Wiberg 1968) and the Mulliken bond order P_{cx} as given below. The INDO–SCF–MO method furnishes fairly reliable values of bond indices based on the density matrix elements.

$$W_{cx} = \sum_{m}^{c} \sum_{n}^{x} P_{mn}^2, \tag{1}$$

$$P_{cx} = \sum^{c} \sum^{x} P_{mn} S_{mn}. \tag{2}$$

(2) The positive Mulliken charge Q_c on the α-carbon of the alkyl group, higher values of which would render it more prone to nucleophilic attack of water during hydrolytic dealkylation.

(3) The tendency of the alkylated base to lose its alkyl group, which may be assessed through the enthalpy ΔH_{dr} of the formal dealkylation reactions given below for the cationic alkylated base and the neutral alkylated base, respectively.

$$(\mathrm{RB})^+ \rightarrow \mathrm{B}^0 + \mathrm{R}^+ + \Delta H_{dr}^+, \tag{3}$$

$$(\mathrm{RB})^0 \rightarrow \mathrm{B}^- + \mathrm{R}^+ + \Delta H_{dr}^0. \tag{4}$$

Here, $(\mathrm{RB})^+$ and $(\mathrm{RB})^0$ stand for the cationic and neutral alkylated base, respectively, while B^0 and B^- represent, respectively, the free DNA base and its conjugate base without the Watson–Crick proton.

The INDO values for the bond indices P_{cx} and W_{cx} and for the charge index Q_c may be regarded as fairly reliable, as noted above. The INDO method, however, tends to overestimate the magnitudes of the enthalpies of bond-making and bond-breaking reactions due to an overestimation of electron correlation inherent as an artifact of this method. For the INDO values of ΔH_{dr}^{+} for the protonated alkyl bases, resort was made to a scaling procedure for making the values more acceptable, through the linear transformation

$$\Delta H_{dr}^{+}(\text{scaled}) = m.\Delta H_{dr}^{+}(\text{INDO}) + c. \tag{5}$$

Here, the values are transformed to fit the MNDO calculated values of ΔH_{dr}^{+} for N^7-methylguanine and O^6-propylguanine, which were derived from a joint consideration of various MNDO calculations (Ford 1986; Ford and Scribner 1990). The values of m and c work out to be 0·209053 and 8·264, respectively. The scaled values of ΔH_{dr}^{+} all fall within an acceptable range, representing a "contraction" of the original INDO values. Since the scaled values bear among themselves the same relationships that the original values did, they serve to make the same qualitative predictions as the unscaled INDO values, having the advantage of being more realistic in range.

For the neutral alkylated bases, values of ΔH_{dr}^{0} given by (4) were calculated from a consideration of (6) and (7) below, together with (3) above,

$$B^0 \rightarrow B^- + H^+ + \Delta H_a, \tag{6}$$

$$(RB)^+ \rightarrow (RB)^0 + H^+ + \Delta H_b. \tag{7}$$

By considering together (3), (4), (6) and (7), it may be seen that for the neutral alkylated base

$$\Delta H_{dr}^{0} = \Delta H_{dr}^{+} + \Delta H_a - \Delta H_b. \tag{8}$$

Values of ΔH_{dr}^{+} are obtained by (5) above. Values of ΔH_a for the DNA bases were obtained by equating them to the ΔG value of 17·72 kcal/mol associated with the proton loss in aqueous medium, which value is derived from their pK_a value of about 13 (Dunn and Hall 1975). The values of ΔH_b were obtained from the INDO calculated values of the gas-phase enthalpy of the Watson–Crick deprotonation (Duncan and Davies 1989) by a scaling procedure similar to that of (5) above.

3. Results and discussion

Table 1 presents a comparison of the INDO calculated values of the bond strength indices W_{cx} and P_{cx} between the O-alkylated bases and the N^7-alkylguanines, all in their cationic (Watson–Crick protonated) forms. Table 2 makes a similar comparison using the Q_c and ΔH_{dr}^{+} indices of susceptibility to dealkylation. Table 3 compares values of the W_{cx}, P_{cx} and ΔH_{dr} indices between neutral and conjugate acid forms of the O^6-alkylguanines, while table 4 makes a similar comparison for the O^4-alkylthymines. Nine different alkyl groups were incorporated for this study, this number

Table 1. INDO values of the W_{cx} and the P_{cx} indices of C–X bond strength for the cationic N^7-RGus, O^6-RGus and O^4-RThs*.

	W_{cx}			P_{cx}		
R	N^7-RGu	O^6-RGu	O^4-RTh	N^7-RGu	O^6-RGu	O^4-RTh
Me	1·000	0·972	0·967	0·969	0·589	0·592
Et	0·957	0·923	0·915	0·667	0·562	0·566
Pr	0·955	0·922	0·911	0·664	0·561	0·562
Pr^i	0·916	0·873	0·872	0·644	0·537	0·544
Bu	0·956	0·923	0·910	0·666	0·562	0·562
Pe	0·955	0·922	0·910	0·665	0·562	0·562
CM	0·959	0·930	0·925	0·674	0·567	0·571
HE	0·967	0·935	0·927	0·672	0·566	0·569
AE	0·967	0·937	0·926	0·671	0·567	0·568

* All values in atomic units

Table 2. Values of the Q_c and ΔH_{dr}^+ indices for proneness towards dealkylation of the cationic O^6-RGus, O^4-RThs and N^7-RGus*.

	Q_c			ΔH_{dr}^+		
R	N^7-RGu	O^6-RGu	O^4-RTh	N^7-RGu	O^6-RGu	O^4-RTh
Me	0·134	0·256	0·243	91·3	90·4	83·0
Et	0·156	0·274	0·268	74·6	73·8	66·1
Pr	0·148	0·267	0·261	72·1	71·3	63·5
Pr^i	0·158	0·190	0·250	62·4	58·6	53·3
Bu	0·146	0·264	0·257	71·5	70·6	62·7
Pe	0·146	0·264	0·258	71·4	70·5	62·4
CM	0·157	0·270	0·262	78·1	76·7	69·0
HE	0·115	0·233	0·226	78·8	77·9	70·3
AE	0·110	0·228	0·223	72·4	71·2	63·3

* Values of Q_c in atomic units, values of ΔH_{dr}^+ in kcal/mol

Table 3. Comparison of calculated values of the W_{cx}, P_{cx} and ΔH_{dr} indices for alkyl group lability between neutral and conjugate acid forms of O^6-alkylguanines*.

	W_{cx}		P_{cx}		ΔH_{dr}	
R	Neutral	Acid	Neutral	Acid	Neutral	Acid
Me	1·019	0·972	0·608	0·589	104·9	90·4
Et	0·980	0·923	0·586	0·564	87·3	73·8
Pr	—	0·922	—	0·561	85·0	71·3
Pr^i	0·931	0·873	0·559	0·537	73·4	58·6
Bu	—	0·923	—	0·562	84·2	70·6
Pe	—	0·922	—	0·562	84·1	70·5
CM	0·981	0·930	0·587	0·567	91·6	76·7
HE	0·987	0·935	0·588	0·566	92·1	77·9
AE	0·988	0·937	0·588	0·567	84·5	71·2

* Values of W_{cx} and P_{cx} in atomic units, ΔH_{dr} in kcal/mol

Table 4. Comparison of calculated values of the W_{cx}, P_{cx} and ΔH_{dr} indices for alkyl group lability between neutral and conjugate acid forms of O^4-alkylthymines*.

	W_{cx}		P_{cx}		ΔH_{dr}	
R	Neutral	Acid	Neutral	Acid	Neutral	Acid
Me	1·020	0·967	0·615	0·592	99·2	83·0
Et	0·978	0·915	0·592	0·566	82·1	66·1
Pr	—	0·911	—	0·562	79·3	63·5
Pr^i	0·940	0·872	0·571	0·544	68·6	53·3
Bu	—	0·910	—	0·562	78·5	62·7
Pe	0·973	0·910	0·589	0·562	78·3	62·4
CM	0·987	0·925	0·595	0·571	85·9	69·0
HE	0·985	0·927	0·594	0·569	86·5	70·3
AE	0·988	0·926	0·594	0·568	79·0	63·3

* Values of W_{cx} and P_{cx} in atomic units, ΔH_{dr} in kcal/mol

3.1 *Structural aspects*

Discussion of structural aspects focusses here chiefly on the differences in the geometries between the cationic and the neutral (deprotonated) bases. The optimised INDO and CNDO/2 values of bond lengths are known to be shorter than those obtained by *ab initio* methods in general (along with force constant values about twice the *ab initio* values) owing to the inherent overestimation of electron correlation.

The calculated length of the C–X bond (between the α-carbon C of the alkyl group and the electronegative atom X of the base to which it is bound) is longer for the N-alkylated bases than for the O-alkylated bases. For the cationic bases, the C–X bond length range is 1·416 to 1·431 Å for the N-alkylated bases, and 1·394 to 1·423 Å for the O-alkylated bases. The value ranges for the neutral bases are 1·418 to 1·440 Å (N-alkylated) and 1·373 to 1·394 Å (O-alkylated), respectively. Thus it is predicted that the C–X bond length invariably decreases with deprotonation, indicating a strengthening of the bond for the neutral species, more appreciably so for the O-alkylated bases. Furthermore, the C–O bond between the C^6 and O^6 atoms (for O^6-alkylguanines) and between the C^4 and O^4 atoms (for the O^4-alkylthymines) increases with deprotonation, as seen from the value range of 1·30 to 1·35 Å for the cationic species compared with the range of values 1·37 to 1·38 Å for the neutral species, indicating the adoption of greater single bond character with deprotonation. For comparison's sake, the C–O bond values for the unmodified guanine and thymine are 1·284 and 1·281 Å, respectively, which is concomitant with the strong double bond character for these bonds.

3.2 *Alkyl group labilities for protonated bases*

The calculated indices for alkyl group lability of the alkylated DNA bases in their cationic form (tables 1 and 2) all point to the same trend that the O-alkylated bases

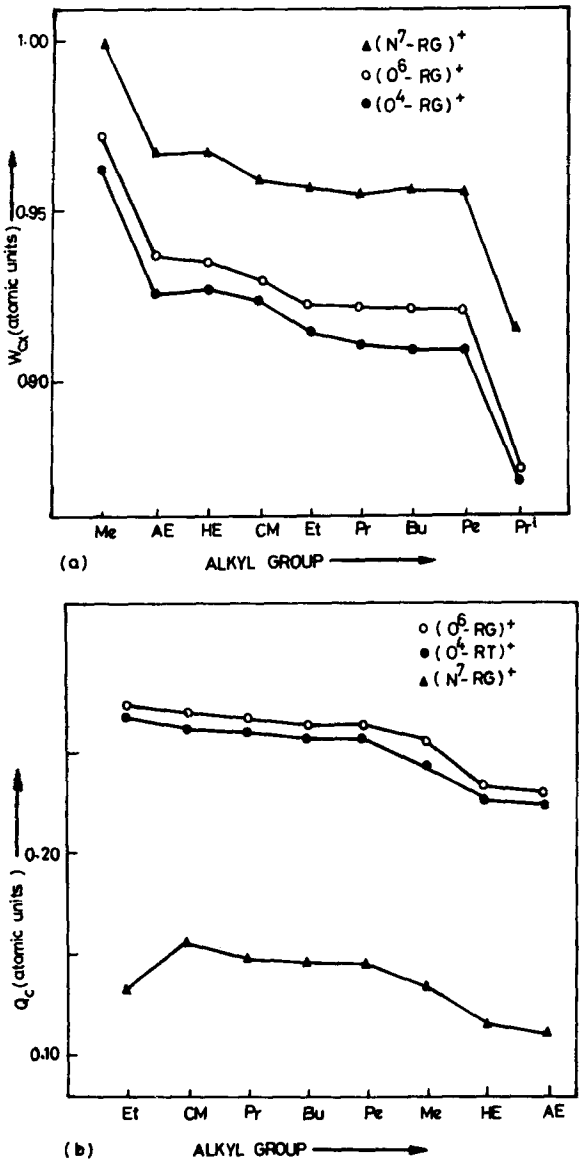

Figure 2. Maintenance of the trend of lower alkyl group lability for the N^7-alkylguanines over the O-alkylated bases as demonstrated by (**a**) the W_{cx} index, and (**b**) the Q_c index.

the O- and N-alkylated bases, predicting higher likelihood of nucleophilic attack of the hydrolysing agent at the α-carbon of the alkyl group. The ΔH_{dr} index likewise indicates a greater affinity between alkyl group and base for the N^7-RGus than for the O^6-RGus and O^4-RThs. This trend of greater alkyl group lability for the O-alkyla-

of the alkyl groups of the O-alkylated bases as compared with the N^7-alkylguanines and other N-alkylated bases.

3.3 *Comparison of neutral and protonated O-alkyl bases*

The data of tables 3 and 4 serve to predict that alkyl group lability of the O-alkylated bases would decrease as the transition is made from the cationic species to the neutral species. Experimentally determined values of the pK_a for the alkylated bases help us to see at which *p*H value ranges each type predominates. At neutral or biological *p*H, it may be expected that the neutral form of the O^6-RGus and the O^4-RThs predominates, as seen from the experimentally determined pK_a values of 2·4 and of −0·32 for O^6-methylguanosine and O^4-methylthymidine respectively (Singer 1975; Allore *et al* 1983). At highly acidic *p*H (below 2·4 for O^6-RGus and below −0·3 for O^4-RThs), the cationic form would predominate. Milder acidic conditions (between 2·4 and 7·0 for O^6-RGus and between −0·3 and 7·0 for O^4-RThs) would see the neutral

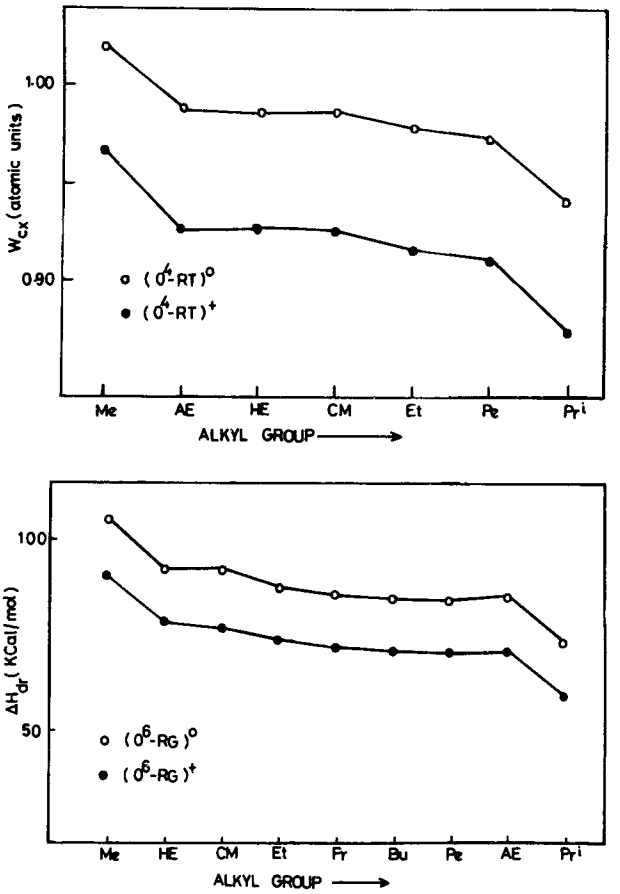

Figure 3. Maintenance of the trend of lower O-alkyl group lability for the cationic form over the neutral form of O-alkylated bases as demonstrated by (**a**) the W_{cx} index for the O^4-alkylthymines and by (**b**) the ΔH_{dr} index for the O^6-alkylguanines.

form of the O-alkylated bases predominating to a greater or lesser extent, depending upon the *p*H.

Now, the indices of tables 3 and 4 for O^6-RGus and O^4-RThs respectively predict a clearcut decrease in alkyl group lability for the neutral alkylated bases as compared with the cationic alkylated bases. For both O^6-RGus and O^4-RThs, the W_{cx}, P_{cw} and ΔH_{dr} indices uniformly predict stronger alkyl group binding for the neutral species than for the protonated species. In fact, the values of these indices compared with the corresponding indices for the cationic N^7-alkylguanines indicate a degree of binding as strong or stronger for the neutral O-alkylated bases than for the N^7-alkylguanines (compare data of tables 3 and 4 with data of tables 1 and 2). Figures 3a and b depict the trend of stronger binding for the neutral over the protonated species as demonstrated, respectively, by the W_{cx} index for the O^4-alkylthymines and by the ΔH_{dr} index for the O^6-alkylguanines. These observations hold for every alkyl group studied, implying a general trend independent of alkyl group identity and structure. Coupling these predictions on alkyl group binding strength with the pK_a values noted above in the last paragraph, it can be inferred that the O-alkylated bases would be characterised by a stronger binding of the alkyl group (i.e. lower alkyl group lability) at neutral or mildly acidic *p*H value ranges than at strongly acidic *p*H. This inference fits in very well with the observations regarding the sensitivity of alkyl group lability to *p*H changes. It also points to the need for employing mildly acidic *p*H (for hydrolytic analysis) or neutral *p*H (for enzymatic analysis) in order to preserve the fragile O-alkylated bases intact without any appreciable degradation.

4. Conclusions

These semiempirical MO calculations serve to furnish indices of alkyl group lability and binding strength for various O- and N-alkylated DNA bases which can be utilised to corroborate, rationalise and generalise the following experimental findings:

(1) At strongly acidic *p*H, the O-alkylated bases are much more prone to dealkylation than the corresponding N^7-alkylguanines (which remain intact under these conditions).
(2) By raising the *p*H to mildly acidic or neutral levels, the O-alkylated bases assume the neutral deprotonated form characterised by strengthening of alkyl group binding so that they can be separated out intact from the DNA on hydrolysis.

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