Molecular Orbital Elucidations on Mechanisms for Repair of Alkylated Nucleosides

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Experimental findings have indicated that purine nucleosides alkylated at the N^7-guanine site are repaired by removal of the entire base moiety through spontaneous depurination or glycosylase-mediated base-excision. Repair of nucleosides alkylated at the O^6-guanine and O^4-thymine positions, however, is mediated by an alkyltransferase which removes only the alkyl group. The chemical reasons behind these specific choices of repair mechanisms are brought to light through semiempirical molecular orbital calculations, which provide a correct assignment of mechanism to each class of alkylated nucleosides in good accord with experiment.

Chemical carcinogenesis by *N*-nitroso compounds and alkylating agents being attributed to mutations induced by alkylation of DNA, it may be expected that an error-free repair of DNA alkylated by these compounds would serve an important role as a deactivating path which detracts from the carcinogenic effect. The organ-specificity of tumour incidence in rodents, which is a noteworthy feature of *N*-nitroso carcinogenesis, has been related to organ-specific differences in the capacity to repair the critical lesions induced. The persistence of certain *O*-alkylated DNA lesions (in the tissues of animals treated with alkylating carcinogens) has been correlated with the incidence of tumours. The persistence of O^6-alkylguanine residues in tissues of rats following administration of *N*-nitroso carcinogens has been found by several groups of workers to correlate well with tumour incidence in the target organs.[1–4] The link between persistence of O^4-methylthymine and tumour incidence has been demonstrated in rats.[5] In contrast, persistence of N^7-alkylguanine (the chief alkylation product for methylating and ethylating agents) cannot be correlated with tumour incidence for any case examined.[6] This observation, coupled with that concerning the non-miscoding property of N^7-methylguanine,[7] suggests that this alkylated DNA base has possibly little or no role to play in the processes of mutagenesis and cancer induction.

The above evidence points to the very likely importance of DNA repair for determining the carcinogenic effect. This paper attempts to conduct a theoretical investigation on the molecular mechanisms for repair of alkylated DNA nucleosides. Two basic mechanisms are studied, of which one is particularly relevant to carcinogenesis. These two mechanisms (base-excision repair and alkyltransferase repair) are found by experiment to operate in general for two different classes of alkylated nucleosides, *viz.* for the *N*-alkylated purine nucleosides, and for the *O*-alkylated nucleosides (notably, O^6-alkyldeoxyguanosines and O^4-alkyldeoxythymidines), respectively. Focus is cast here upon three types of alkylated nucleosides, *viz.* the N^7-alkyldeoxyguanosines (important because of their greater abundance of formation when DNA is alkylated), and two kinds of *O*-alkylated nucleosides, *viz.* the O^6-alkyldeoxyguanosines and O^4-alkyldeoxythymidines (important owing to their promutagenic and procarcinogenic significance).[1–5] This study aims to see if theoretical calculations would predict the facility of base-excision repair for the N^7-alkylguanosines (over the *O*-alkylated nucleosides), and the facility of alkyltransferase repair for the *O*-alkylated nucleosides (over the N^7-alkylguanosines). To the author's knowledge, this study is the first attempt at using theoretical chemistry to study the mechanisms of DNA repair. Being an enzymatic process, DNA repair might not seem amenable to quantum chemical treatment, but it will be shown that simple physicochemical criteria for reactivity, as calculated by molecular orbital theory, could be of great importance in deciding the choice of repair mechanism for each case.

Repair Mechanisms for N-*Alkylated Nucleosides.*—The products of alkylation at the N^3-G, N^3-A, N^7-G and N^7-A sites in DNA may be repaired either enzymatically (through action of glycosylases or endonucleases) or through spontaneous depurination by hydrolytic cleavage of the glycoside linkage. While repair by endonuclease excision of the whole nucleotide may also operate as one mode of repair mechanism, this study focuses on repair *via* loss of the base moiety only, whether through glycosylase action or spontaneous depurination.

Evidence exists for mammalian DNA glycosylases which are specific for *N*-alkylated nucleosides. N^3-Methyladenosine glycosylase activity has been found to be present in human lymphoblastoid cell extracts,[8] which have been purified 30-fold.[9] Glycosylase activity from human lymphoblasts has been detected for a number of N^3- and N^7-alkylpurines, including N^7-methylguanine.[10] A rat liver glycosylase excising N^7-methylguanine from liver nuclei has been identified.[11] None of these glycosylase activities has been shown to be effective for repairing O^6-methylguanine.[10,11] A glycosylase has also been demonstrated to operate in bacteria for excising ring-opened and unopened N^7-methylguanine.[12,13]

Alkylation at the purine ring-nitrogen sites apparently destabilises the glycosyl bond, inducing a tendency towards spontaneous depurination. Alkylation at the N^3-guanine, N^7-guanine, N^3-adenine and N^7-adenine sites is believed to initiate spontaneous depurination at neutral pH for alkylated DNA.[14] However, all O^4-alkylated deoxyribopyrimidine and ribopyrimidine nucleosides resemble the unalkylated nucleosides in possessing a stable glycosyl bond.[15] Likewise, alkylation at the O^6-guanine site does not destabilise the glycosyl bond.[16] It is suggested that the decreased strength of the glycosyl bond upon alkylation at these *N*-sites is responsible for this tendency towards base-excision repair, which does not occur for *O*-alkylated nucleosides. The free (unalkylated) nucleosides are, of course, associated with a minimal tendency towards base-loss or base-excision.

The above evidence points to the mechanisms of spontaneous depurination or glycosylase-mediated base-excision repair for purine nucleosides alkylated at the N-7 or N-3 positions. The general mechanism for repair of N^7-alkylguanine *via* base-loss (whether enzymatic or spontaneous) is illustrated in Fig. 1(*a*), which involves hydrolytic cleavage of the glycosyl C–N bond of

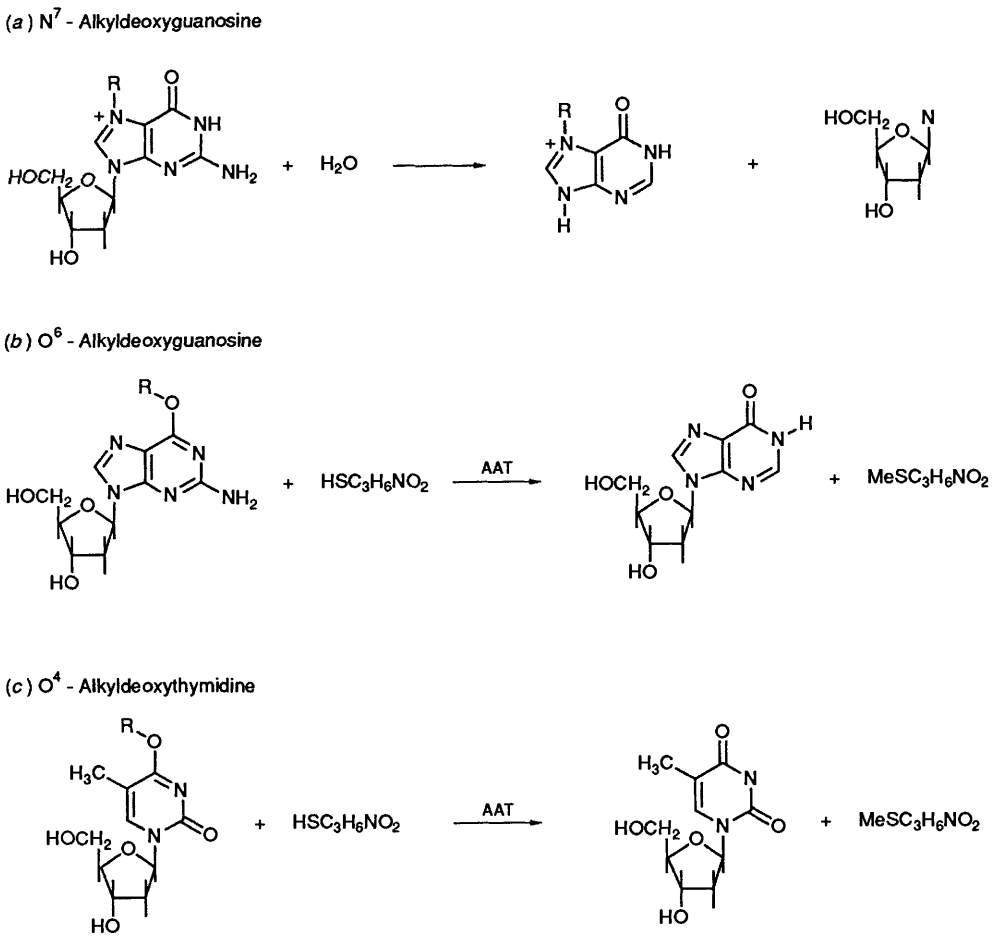

Fig. 1 Repair mechanism pathways for nucleosides alkylated at the (*a*) N^7-guanine, (*b*) O^6-guanine and (*c*) O^4-thymine positions, where $HS\text{-}C_3H_6NO_2$ stands for cysteine

the modified nucleoside. Removal of the alkyl group alone may be excluded as a likely mechanism for repair of these *N*-alkylated nucleosides.

Repair Mechanisms for O-Alkylated Nucleosides.—Unlike the *N*-alkylated nucleosides, base-excision or spontaneous depurination seems to be ruled out as a chief mode of repair for such lesions.[17] Since 1980, the evidence gathered concerning repair of O^6-alkyldeoxyguanosines has converged towards the finding that this repair is mediated in bacterial and mammalian cells by action of an alkyltransferase enzyme which accepts the alkyl group on to a cysteine residue in the enzyme.[18] The enzymes responsible have been purified for bacteria[19] and for mammalian cells.[20] This enzyme repairs alkylated lesions at the O^6-guanine position in DNA and is called O^6-alkylguanine-DNA-alkyltransferase (AAT). A scheme for the mechanism of O^6-alkyldeoxyguanosine repair by the AAT enzyme is portrayed in Fig. 1(*b*). The scope of this mechanism extends beyond methylated residues to include the ethyl, *n*-propyl, *n*-butyl, isopropyl, 2-hydroxyethyl and chloroethyl groups as well.[21–23] Transfer of the methyl group produces an *S*-methylcysteine residue for both the bacterial and mammalian enzymes.[24–26] The stoichiometry of the reaction indicates that such a mechanism constitutes the sole method for repair of O^6-methyldeoxyguanosine.

In a fashion analogous to that for O^6-alkyldeoxyguanosines and alkylphosphotriesters, repair of O^4-alkyldeoxythymidines is also believed to occur in bacteria through action of an alkyltransferase enzyme.[18] In *E. coli*, the rate of repair is slower than that for O^6-alkyldeoxyguanosines,[27] while in mammalian cells, the repair rate is markedly slow,[28] indicating an inefficient repair process. A human DNA repair capacity specific for O^4-ethyldeoxythymidine has been identified and partially characterised.[29] The slowness of O^4-alkyldeoxythymidine repair in mammals (as compared to O^6-alkyldeoxyguanosines) has led to the inference that the former might be more significant for carcinogenesis than the latter.[30] Fig. 1(*c*) depicts the reaction corresponding to this form of repair, which is analogous to AAT repair of O^6-alkyldeoxyguanosines.

Theoretical

Methods of Calculation.—All calculations were performed using the semiempirical CNDO/2 and INDO SCF MO methods[31,32] subjecting all molecular species to full geometry optimisation by an analytical gradient method.[33] The cationic form of the N^7-alkyldeoxyguanosines is considered here, and not the neutral form, in accordance with the inferences of previous theoretical work[34] that this form might be their preferred mode of existence *in vivo*. Conversely, the neutral (and not the cationic) forms of the *O*-alkylated nucleosides are the ones considered here, since these are the likely structures *in vivo* as the same work indicates.

Due to limitations in the ability to handle large molecular systems, models were used to represent the free nucleosides, the alkylated bases and the alkylated nucleosides, especially in connection with the study of base-excision repair. Fig. 2 portrays the structures for the systems used to provide molecular models for the actual species. Xanthine (**1**) was used in place of guanine, while thymine (**2**) was small enough to be treated as such. The deoxyribose molecule was replaced by the molecule 1-methoxyethanol (**3**), the conformation chosen for

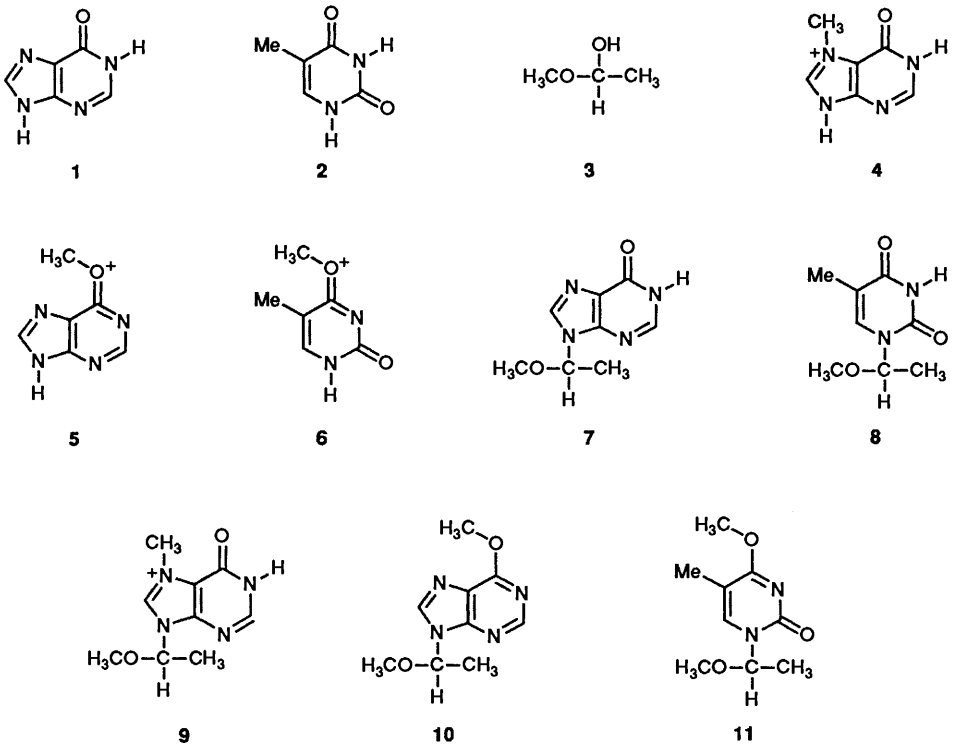

Fig. 2 Structural formulae for model nucleosides and other molecules studied

which was made to simulate that for the actual deoxyribose molecule. The models used to represent the alkylated bases for the N^7-G, O^6-G and O^4-T sites are depicted by the structures **4**, **5** and **6**, respectively, the O^4-methylthymine molecule **6** being small enough to be treated as such. The model nucleosides representing deoxyguanosine and deoxythymidine are given as **7** and **8**, respectively. N^7-Methyldeoxyguanosine is represented by the model given as **9**, O^6-methyldeoxyguanosine as **10**, and O^4-methyldeoxythymidine as **11**. Standard data on molecular geometries[35] was used to build up the starting geometries for these structures, taking the crystal structures of the DNA bases[36,37] as a basis.

CNDO/2 optimised structures for water polymers[38] were used in an attempt to simulate an aqueous environment for the base-excision repair reactions. Use was made of standard molecular data[35] to construct the starting geometries for cysteine, cationic and neutral *S*-methylcysteine. For the study of alkyltransferase repair, the alkylated DNA bases in deglycosylated form were also considered, being studied in their complete (not model) molecular structures (which were built up from the crystal structure data on DNA bases).

The CNDO/2 method[31] was used to obtain the SCF wave function and all molecular properties for all the model species considered in the study of base-excision repair. The INDO MO method[32] was used to calculate the wave function and molecular properties for the alkylated DNA bases when investigating the susceptibility towards alkyltransferase action, a number of different alkylating groups being considered. All molecular species considered were fully optimised with respect to the coordinates of all atoms until the prescribed threshold was reached, *viz.* the square of the gradient norm cross a threshold of $0.002N$ mdyn^2, N being the number of atoms in the molecule.

Abbreviations Used.—Names for the various molecular species studied are abbreviated by symbols given in brackets as follows: Guanine (G), adenine (A), thymine (T), cytosine (C), xanthine or model guanine (X), deoxyribose model (D), deoxyguanosine model (XD), deoxythymidine model (TD), cationic N^7-alkylguanine (N^7-RG$^+$), model cationic N^7-alkylguanine (N^7-RX$^+$), model O^6-alkylguanine (O^6-RX), O^6-alkylguanine (O^6-RG), O^4-alkylthymine (O^4-RT), model N^7-alkyldeoxyguanosine (N^7-RXD$^+$), model O^6-alkyldeoxyguanosine (O^6-RXD), model O^4-alkyldeoxythymidine (O^4-RTD). The alkyl groups (R) are abbreviated as methyl (Me), ethyl (Et), *n*-propyl (Pr), *n*-butyl (Bu), *n*-pentyl (Pe), isopropyl (Pr^i), 2-hydroxyethyl (HE), 2-acetoxyethyl (AE) and cyanomethyl (CM).

Indices for Facility of Base-loss Repair.—The chemical reaction for this form of DNA repair may basically be expressed as the nucleophilic attack of a water molecule upon the C-1′ carbon of the sugar moiety so as to result in hydrolytic cleavage of the glycosyl bond. The properties relevant to this mechanism would be concerned with the electrophilicity of the C-1′ carbon, with the strength of the glycosyl bond, and also with the thermodynamics of the base-loss reaction. Frontier orbital and electrostatic indicators for tendency of the C-1′-carbon to nucleophilic attack may be provided by Q_c (the positive charge on this atom), by E_f [the energy of the lowest unoccupied molecular orbital (LUMO) significantly involving this carbon], and by Q_f (the corresponding frontier electron density at this atom). The strength of the glycosyl bond C–N may be measured by the Mulliken bond index P_{cn} and the Wiberg bond index[39] W_{cn}, where, for any bond between atoms A and B, eqns. (1) and (2) hold.

$$P_{ab} = \sum_{m}^{A}\sum_{n}^{B} P_{mn}S_{mn} \quad (1)$$

$$W_{ab} = \sum_{m}^{A}\sum_{n}^{B} P_{mn}^2 \quad (2)$$

The thermodynamics of the base-excision reaction may be represented by the enthalpy ΔH_{db} of the reaction for each case as given by reactions (3)–(5).

Here, $(H_2O)_n$ refers to the water polymer with n mole-

Table 1 CNDO/2 calculated frontier orbital and electron distribution indices for facility of depurination or depyrimidination of various nucleoside species[a]

Species	Q_c	E_f	Q_f	W_{cn}	P_{cn}
N^7-MeXD$^+$	0.283	0.084	0.316	0.915	0.646
O^6-MeXD	0.290	0.225	0.130	0.976	0.681
O^4-MeTD	0.291	0.229	0.160	0.960	0.663
XD	0.288	0.234	0.234	0.971	0.678
TD	0.294	0.228	0.171	0.957	0.664

[a] All values in atomic units.

$$N^7\text{-MeXD}^+ + (H_2O)_n \longrightarrow N^7\text{-MeX}^+ + DOH + (H_2O)_{n-1} + \Delta H_{db} \quad (3)$$

$$O^6\text{-MeXD} + (H_2O)_n \longrightarrow O^6\text{-MeX} + DOH + (H_2O)_{n-1} + \Delta H_{db} \quad (4)$$

$$O^4\text{-MeTD} + (H_2O)_n \longrightarrow O^4\text{-MeT} + DOH + (H_2O)_{n-1} + \Delta H_{db} \quad (5)$$

cules. Use is made here of systems with n from 1–5. Values of the above reactivity indices may be compared between the model N^7-alkyldeoxyguanosines on one hand, and the model *O*-alkylated nucleosides and free nucleosides on the other.

Indices for Facility of Alkyltransferase Repair.—This form of repair involves the nucleophilic attack of the sulfur atom of a cysteine residue upon the α-carbon of the alkyl group of the alkylated DNA base, resulting in the formation of an *S*-methylcysteine residue. Criteria determining feasibility of this reaction would be concerned with the electrophilic reactivity of the α-carbon of the alkyl group, and also with the strength of the bond between this atom and the DNA base site (which bond is cleaved during transfer of the alkyl group). The thermodynamics of the process may also be invoked as a possible factor.

Accordingly, the indices used for this process are the frontier orbital and charge distribution indicators for electrophilicity of the α-carbon, *viz.* Q_a (the positive charge on this atom), E_l (energy of the LUMO significantly involving this carbon) and Q_l (the corresponding frontier electron density for this carbon atom). The strength of the bond C–X between the α-carbon and the DNA base site to which is attached may be estimated from the bond index P_{cx} calculated from eqn. (1).

The thermodynamics of the alkyl group transfer are considered with the following possibilities in view: (*a*) the product of alkyl group transfer to cysteine may be neutral *S*-methylcysteine or cationic (*S*-protonated) *S*-methylcysteine; and (*b*) alkyl group transfer may take place in the gas-phase or mediated by a water molecule. Accordingly, the enthalpies of the alkyl transfer reaction for the three cases of alkylation at the N^7-G, O^6-G and O^4-T sites correspond to the reactions (6)–(14).

$$N^7\text{-MeXD}^+ + Cys \longrightarrow MeCys + XD + H^+ \quad (6)$$

$$N^7\text{-MeXD}^+ + Cys + H_2O \longrightarrow MeCys + XD + H_3O^+ \quad (7)$$

$$N^7\text{-MeXD}^+ + Cys \longrightarrow (MeCysH)^+ + XD \quad (8)$$

$$O^6\text{-MeXD} + Cys \longrightarrow MeCys + XD \quad (9)$$

$$O^6\text{-MeXD} + Cys + H^+ \longrightarrow (MeCysH)^+ + XD \quad (10)$$

$$O^6\text{-MeXD} + Cys + H_3O^+ \longrightarrow (MeCysH)^+ + XD + H_2O \quad (11)$$

$$O^4\text{-MeTD} + Cys \longrightarrow MeCys + TD \quad (12)$$

$$O^4\text{-MeTD} + Cys + H^+ \longrightarrow (MeCysH)^+ + TD \quad (13)$$

$$O^4\text{-MeTD} + Cys + H_3O \longrightarrow (MeCysH)^+ + TD + H_2O \quad (14)$$

The three alkylated nucleosides studied here in model form, *viz.* the N^7-methyldeoxyguanosine, O^6-methyldeoxyguanosine and O^4-methyldeoxythymidine are examined using the above theoretical indices for alkyltransferase repair facility to see whether the *O*-alkylated nucleosides are predicted to be more prone to this mechanism of repair than the N^7-alkyldeoxyguanosines.

Results and Discussion

Treatment of Base-loss Repair.—Table 1 presents CNDO/2 values of the frontier orbital and electron distribution indices for facility of base-excision or spontaneous base-loss, values having been calculated for the model systems N^7-MeXD$^+$, O^6-MeXD, O^4-MeTD and also for the model nucleosides XD and TD. Table 2 presents CNDO/2 values of the enthalpy of the base-loss reaction (using water polymers where n = 1–5) for each of these five model nucleoside systems.

The Q_c index does not differentiate well between the model N^7-methylguanosine and the other four model nucleosides. A good demarcation is afforded by the frontier orbital indices E_f and Q_f. The lower values of E_f and higher value of Q_f for N^7-MeXD as compared with those for the other systems speak for a greater susceptibility of the former towards nucleophilic (hydrolytic) attack at the C-1′-carbon of the model nucleoside. It is especially worth noting that the strength of the glycosyl bond, as given by the W_{cn} and P_{cn} bond indices, is calculated to be appreciably lower for the N^7-MeXD$^+$ species than for the others. This data accords well with the observations that N^7-G alkylation destabilises the glycosyl bond while alkylation at the O^6-G and O^4-T positions are not thought to destabilise the glycosyl bond. The values of these calculated indices for the O^6-MeXD and O^4-MeTD species are, in fact, quite close to those for the unalkylated model nucleosides XD and TD. This may be interpreted to indicate that these *O*-alkylated nucleosides would closely approach the unalkylated nucleosides with regard to a tendency towards base-loss or base-excision. This is significant since the free nucleosides are obviously associated with a minimal tendency towards spontaneous base-loss or enzyme-mediated base-excision.

The calculated thermodynamic data of Table 2 predict that base-loss is best favoured for N^7-MeXD$^+$, the heat of base-loss being 4.3 kcal mol^{-1} lower than that for O^6-MeXD and 8.7 kcal mol^{-1} lower than that for O^4-MeTD. The calculated heats of base-loss for the unalkylated model nucleosides XD and TD correspond quite closely to those for O^6-MeXD and O^4-MeTD, respectively, again predicting that the *O*-alkylated nucleosides would have about the same tendency towards base-excision repair as the free nucleosides. The base-loss reaction becomes thermodynamically more facile as the number of water molecules in the polymer increases.

Frontier orbital, electron distribution and thermodynamic criteria for stability and strength of the glycosyl bond for all the cases examined in model form thus predict greater facility of base-loss for the N^7-alkyldeoxyguanosines as compared with the *O*-alkylated nucleosides (which closely resemble the free unmodified nucleosides). The free nucleosides being associated with a minimal tendency towards base-loss or base-excision,

Table 2 CNDO/2 values of ΔH_{dh}, the enthalpy of depurination or of depyrimidination for various nucleoside species, different hydrolysing agents being considered.[a] The last column (indicated by 'rel') gives values relative to that for N^7-MeXD$^+$, where ΔH_{db} is set at 0.0.

Species	H_2O	$(H_2O)_2$	$(H_2O)_3$	$(H_2O)_4$	$(H_2O)_5$	Rel.
N^7-MeXD$^+$	−20.0	−21.7	−22.0	−22.9	−23.1	0.0
O^6-MeXD	−15.7	−17.4	−17.7	−18.6	−18.8	4.3
O^4-MeTD	−11.3	−13.0	−13.3	−14.2	−14.4	8.7
XD	−15.8	−17.5	−17.8	−18.7	−18.9	4.2
TD	−8.2	−9.9	−10.2	−11.1	−11.3	11.8

[a] All values in kcal mol^{-1}.

Table 3 Frontier orbital and electron distribution indices for facility of alkyl group transfer from alkylated DNA bases and nucleosides (CNDO/2 values for model nucleosides, and INDO values for deglycosylated bases)[a]

Species	Q_a	E_l	Q_l	P_{cx}
N^7-MeXD$^+$	0.064	0.064	0.215	0.698
O^6-MeXD	0.163	0.275	0.396	0.608
O^4-MeTD	0.154	0.254	0.438	0.614
N^7-MeG$^+$	0.134	0.090	0.279	0.696
O^6-MeG	0.280	0.256	0.171	0.608
O^4-MeT	0.272	0.221	0.294	0.615
N^7-EtG$^+$	0.156	0.085	0.311	0.667
O^6-EtG	0.278	0.242	0.152	0.586
O^4-EtT	0.273	0.235	0.286	0.592
N^7-Pr^{i}G$^+$	—	0.092	0.333	0.641
O^6-Pr^{i}G	0.192	0.260	0.195	0.559
O^4-Pr^{i}T	0.177	0.212	0.253	0.571
N^7-HEG$^+$	0.115	0.083	0.277	0.672
O^6-HEG	0.237	0.252	0.181	0.588
O^4-HET	0.234	0.229	0.250	0.594
N^7-AEG$^+$	0.110	0.086	0.284	0.671
O^6-AEG	0.234	0.219	0.096	0.588
O^4-AET	0.228	0.190	0.117	0.594
N^7-CMG$^+$	0.157	0.061	0.315	0.674
O^6-CMG	0.269	0.237	0.368	0.587
O^4-CMT	0.265	0.213	0.168	0.595

[a] All values in atomic units.

these *O*-alkylated nucleosides may not be expected to undergo this form of repair under normal conditions of temperature and pH.

Treatment of Alkyltransferase Repair.—Table 3 presents the calculated values of various frontier orbital and electron distribution indices related to facility of alkyltransferase repair for the N^7-MeXD$^+$, O^6-MeXD and O^4-MeTD species, as well as for several alkylated bases without the sugar moiety. Since the alkyl group transfer occurs fairly distant from the glycosyl bond, it might seem a fair approximation to also consider the deglycosylated DNA base as an acceptable replacement for the complete nucleoside, since only trends are being sought. The CNDO/2 method was used for the model nucleosides while the INDO method was used for the deglycosylated bases. Each set of results treats the cases for alkyl group transfer from the N^7-G, O^6-G and O^4-T sites in turn. Besides the model methylated nucleosides (studied using the CNDO/2 method), the other cases examined (using the INDO method) consider the methyl (Me), ethyl (Et), isopropyl (Pr^i), *n*-pentyl (Pe), 2-hydroxyethyl (HE), 2-acetoxyethyl (AE) and cyanomethyl (CM) groups,

Table 4 CNDO/2 enthalpy indices for facility of alkyl group transfer from alkylated DNA base sites to cysteine.[j] Data under headings MeCys and (MeCysH)$^+$ correspond to formation of neutral and of *S*-protonated and methylcysteine, respectively. Data under headings 'gas' and 'H_2O' correspond to consideration of the gas-phase or of water molecule participation, respectively.

	MeCys		(MeCySH)$^+$	
Species	Gas	H_2O	Gas	H_2O
Model nucleosides				
N^7-MeXD$^+$	363.6[a]	94.7[b]	125.6[c]	—
O^6-MeXD	26.6[d]	—	−211.4[e]	57.5[f]
O^4-MeTD	0.1[g]	—	−237.9[h]	31.0[i]
Deglycosylated bases				
N^7-MeX$^+$	359.4[a]	90.5[b]	121.4[c]	—
O^6-MeX	26.7[d]	—	−211.3[e]	57.6[f]
O^4-MeT	−3.1[g]	—	−241.1[h]	27.8[i]

[a–i] Values suffixed by the latters *a* to *i* correspond to the enthalpies of reactions given by reactions (6)–(14), respectively, making appropriate substitutions for the cases of the deglycosylated bases. [j] All values in kcal mol^{-1}.

this wide choice of alkyl groups being made for the sake of generality.

The Q_a index functions well to distinguish between the N^7-G alkylated bases and the *O*-alkylated bases, the latter having a higher positive charge at the α-carbon atom. This trend followed by the calculated values is portrayed in Fig. 3(*a*). The E_l frontier orbital index also points towards the expected trend of greater electrophilicity of the α-carbon for the *O*-alkylated bases than for the N^7-alkylguanines, this trend being depicted in Fig. 3(*b*). But the frontier orbital electron density index Q_l does not display these trends. Apparently, the frontier orbital approximation breaks down here due to several frontier orbitals lying close to each other. Collective consideration of all the empty MOs together, as in the original expression of Klopman,[40] would perhaps correct the impressions gleaned here from the simple frontier orbital picture.

The calculated strengths of the C–X bond between the alkyl group carbon and the DNA base site, as given by the P_{cx} index, quite clearly predicts that the *O*-alkylated bases may be expected to possess a marked tendency to undergo the bond cleavage and alkyl group transfer, in comparison with the N^7-alkylguanines. Calculated values of P_{cx} for the N^7-alkylguanines range from 0.67–0.70, while values for the *O*-alkylated bases range *ca.* 0.56–0.62, thus providing a clear trend of demarcation as portrayed in Fig. 4. These trends are shown by all the alkyl groups studies, suggesting that the identity of the alkyl group has nothing to do with this trend. Many different types of alkyl groups have, in fact, been experimentally shown to be susceptible to transferase repair by the same enzyme system.[21–24]

Table 4 presents CNDO/2 calculated values of the enthalpies of the alkyl transfer reaction for the three cases, with various alternatives in view, as indicated in reactions (6)–(14). Two sets of data are presented, corresponding, respectively, to the model nucleosides (N^7-MeXD$^+$, O^6-MeXD and O^4-MeTD) and to the deglycosylated bases (N^7-MeG$^+$, O^6-MeG and O^4-MeT). The data for the deglycosylated bases correspond to the enthalpies calculated from reactions (6)–(14), the appropriate substitutions being made. The various alternatives considered correspond to (*a*) consideration of the gas-phase or of the participation of a water molecule, and (*b*) consideration of the formation of neutral or of protonated *S*-methylcysteine.

A clear demarcation in the calculated CNDO/2 values of the enthalpy of alkyl group transfer may be noted between the *N*-alkylated species and the two *O*-alkylated species, regardless of

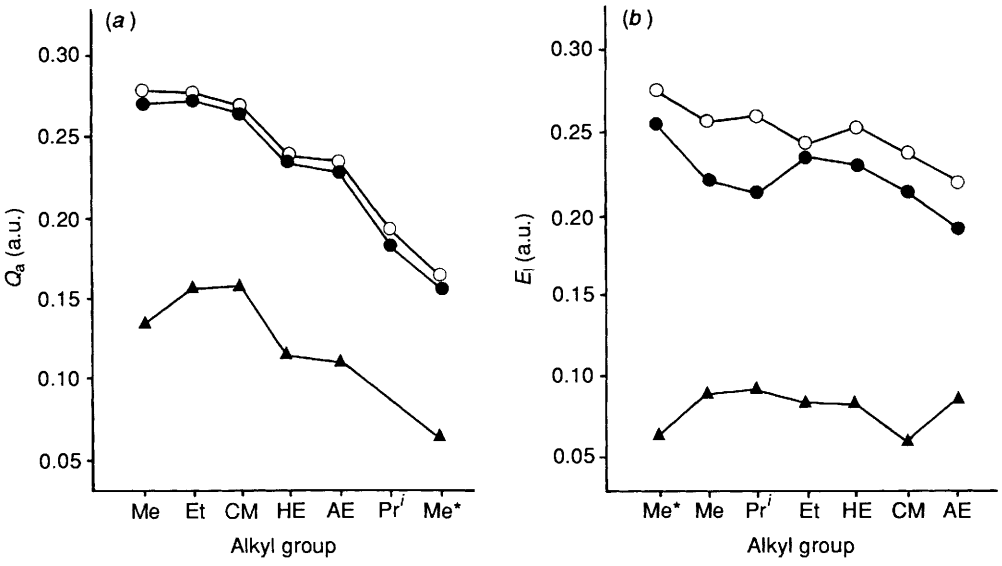

Fig. 3 Demarcation between *O*-alkylated bases and N^7-alkylguanines as shown by the Q_a and E_l indices for alkyltransferase repair, where Me stands for the methylated model nucleosides. ○, O^6-RG; ●, O^4-RT; ▲, N^7-RG$^+$.

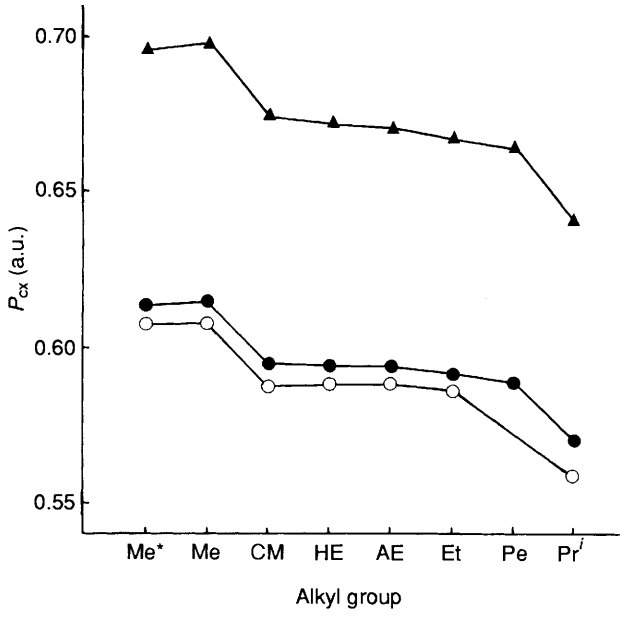

Fig. 4 Demarcation between N^7-alkylguanines and *O*-alkylated bases as shown by the P_{cx} index for alkyltransferase repair, where Me* stands for the methylated model nucleosides. ▲, N^7-RG; ●, O^+-RT; ○, O^6-RG.

whatever situation is considered. The gas-phase heat of alkyl group transfer demonstrates this demarcation very clearly, values being 363.6, 26.6 and 0.1 kcal mol^{-1}, respectively, for N^7-MeXD$^+$, O^6-MeXD and O^4-MeTD, and 359.4, 26.7 and −3.1 kcal mol^{-1} for N^7-MeG$^+$, O^6-MeG and O^4-MeT, respectively. If allowance is made for participation of a water molecule during the reaction (to take account of the proton exchange occurring), the values for the heat of alkyl group transfer come out to be 94.7, 26.6 and 0.1 for N^7-MeXD$^+$, O^6-MeXD and O^4-MeTD, respectively, and 90.5, 26.7 and −3.1 kcal mol^{-1} for N^7-MeG$^+$, O^6-MeG and O^4-MeT, respectively. If consideration is made for the possibility of formation of the *S*-protonated *S*-methylcysteine species (MeCysH)$^+$, the gas-phase heats of alkyl group transfer come out to be 125.6, −211.4 and −237.9 kcal mol^{-1} for N^7-MeXD$^+$, O^6-MeXD and O^4-MeTD, respectively, and 121.4, −211.3 and −241.1 kcal mol^{-1} for N^7-MeG$^+$, O^6-MeG and O^4-MeT, respectively. Allowance for the process of proton transfer from H_3O^+ to methylcysteine in the case of the *O*-alkylated bases gives the values as 125.6, 57.5 and 31.0 kcal mol^{-1} for N^7-MeXD$^+$, O^6-MeXD and O^4-MeTD, respectively, and 121.4, 57.6 and 27.8 kcal mol^{-1} for N^7-MeG$^+$, O^6-MeG and O^4-MeT, respectively. Thus, we see that all the sets of data pertaining to each situation coincide in maintaining the trend that the mechanism of alkyltransferase repair is favoured for the *O*-alkylated nucleosides as compared to the case of N^7-methyldeoxyguanosine, regardless of whether gas-phase or simulated aqueous phase is used, or whether neutral or protonated *S*-methylcysteine is considered.

Frontier orbital, electron distribution and thermodynamic considerations thus all point towards the greater feasibility of the *O*-alkylated nucleosides to undergo alkyltransferase repair as compared to the N^7-alkyldeoxyguanosines. This trend is maintained regardless of alkyl group identity.

Chemical Reactivity and Choice of Repair Mechanism.— From the way the repair mechanism is successfully predicted on the basis of simple criteria for chemical reactivity like atomic charges, frontier electron densities, bond orders and thermodynamic data, it would appear that, to a large extent, the choice of repair mechanism for each case is determined by such criteria, besides more complex stereochemical considerations which are not accessible to simple quantum chemical treatment. While the complexity of interaction between substrate and enzyme cannot be ruled out for these cases, the simple manner in which this quantum chemical study of repair mechanism furnishes successful predictions and rationalisations suggests that reactivity criteria based on single atoms or bonds do play a significant role in deciding the choice of repair mechanism for the cases studied here.

Conclusions

On the basis of reactivity criteria for single atoms and bonds obtained from molecular orbital calculations, alkylation at the N^7-G site is predicted to appreciably destabilise the glycoside linkage, rendering N^7-alkyldeoxyguanosines reactive towards the choice of spontaneous depurination or glycosylase-mediated base-excision as a mode of repair for these lesions. Alkylation at the O^6-G and O^4-T sites is not predicted to destabilise the glycosyl bond. The covalent bond between the alkyl group carbon and the alkylation site is predicted to be weaker for the *O*-alkylated nucleosides than for the N^7-

alkyldeoxyguanosines, which makes the former more susceptible to alkyltransferase repair than the latter.

The above predictions arrived at by these calculations are in good accord with experimental observations. It is noteworthy that simple criteria for chemical reactivity can be quite successful in assigning the correct mechanism for repair of alkylated nucleosides, which, being an enzymatic process, would have been expected to possess complex macromolecular and stereochemical aspects not amenable to simple quantum chemical treatment.

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