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On the Sites and Mechanisms of Alkylation in the Nucleic Acids

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The intrinsic capacity (computed by *ab initio* molecular orbital techniques) of ethyl and methyl cations to bind to the anionic oxygens of the phosphate groups of the nucleic acids is found to be larger than their affinity for the carbonyl oxygens or for the nitrogens of the bases cytosine and guanine. Analysis of the components of the binding energies indicates that the preference of the ethyl cation for the oxygens of the phosphate or for those of the bases is due to different reasons. The results are discussed in connection with the available experimental observations.

Key words: Nucleic acids, alkylation of ~

In a previous study [1] we have investigated the possible reasons for the preference of some ethylating agents to alkylate the carbonyl oxygens of cytosine [2] and of guanine [3] rather than the most "basic" nitrogens of these rings. It was shown that the attack by an ethyl cation was directed to the position corresponding to the "least exchange repulsion" (the carbonyl oxygens) in contrast to protonation which occurs at the position of strongest attraction (nitrogen N_3 in cytosine or N_7 in guanine). The attack by a methyl cation was shown to have no marked intrinsic preference between the two sites. The consequences of these findings for the possible mechanisms of the reaction of the various reagents were discussed.

We present here complementary informations concerning the affinity of these cations for the anionic phosphate group which appears to be the major site of ethylation by ethylnitrosourea in TMV RNA [3] and in DNA [4]. The approach of the ethyl and methyl cations has been studied towards one of the anionic oxygens of dimethyl phosphate taken as a model of the phosphodiester group in the nucleic

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acids. As in Ref. [1] the attacked molecule has been maintained undeformed. The cations have been taken in the geometry optimized in the study of their attack on cytosine. The computations have been done as in Ref. [1] by the SCF *ab initio* method using the same basis set.

Table 1. The binding energy ΔE, (and its components[a]), kcal/mole, for the alkylation of the anionic oxygen of the phosphate compared to the alkylation of O_2 and N_3 of cytosine.

Ethylation (d=1.48 Å for O, 1.50 Å for N)					
Site	ΔE	E_C	E_{DEL}	E_{AT}	E_{EX}
O^- Phosphate	−332.6	−208.0	−249.1	−451.6	124.5
O_2 Cytosine	−156.4	−104.7	−187.8	−292.5	136
N_3 Cytosine	−151.0	−136.6	−193.2	−329.8	178.8
Methylation (d=1.46 Å for O, 1.49 Å for N)					
Site	ΔE	E_C	E_{DEL}	E_{AT}	E_{EX}
O^- Phosphate	−383.3	−224.3	−257.9	−482.2	98.8
O_2 Cytosine	−202.0	−105.5	−201.9	−307.4	105.4
N_3 Cytosine	−201.7	−136.6	−199.3	−336.0	134.3

[a] E_C=electrostatic, E_{DEL}=delocalization=polarization+charge transfer, E_{AT}=total attractive component, E_{EX}=exchange repulsion (see [1]), d=equilibrium distance.

The results, given in Table 1, lead to the following main conclusions:

1) The addition of $C_2H_5^+$ to the phosphate is by far more favorable than the addition to either the O or N atom of cytosine. This explains why the major product of ethylation by ethylnitrosourea occurs on the phosphate, and is consistent with an S_N1 mechanism of attack, involving the dissociated cation (see [1]).

2) The examination of the components of the binding energy indicates that the reason for the observed preference of $C_2H_5^+$ for the phosphate oxygen is different from the reason of its preference for the oxygen of the base rather than for its nitrogen: while the last preference was dictated by the "least repulsion" favoring the oxygen, playing against the global attraction which would favor the nitrogen, the preferential ethylation of the phosphate with respect to the two ring atoms is favored by *both* a stronger global attraction (electrostatic and delocalization terms) *and* by a smaller repulsion. Thus the observed preference [3] of ethylnitrosourea for alkylating the oxygens of the phosphate and of the guanine moiety is due to different reasons. In other words, the affinity of the two kinds of oxygens towards ethylnitrosourea is not due to a common characteristic but is the result of a complex interplay of differently weighted factors.

3) The results for CH_3^+ indicate that the attachment of the cation to the phosphate is intrinsically more favorable than its attachment to the atoms of the base. This result, associated with the experimental observation [3] that methylation of the

secondary phosphates is less efficient than their ethylation concurs with our previous conclusion [1] that the mechanism of methylation has less of S_N1 character, even when the methylating agent is methylnitrosourea.

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