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On the Relative Acidity and Basicity of the Amino Groups of the Nucleic Acid Bases

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Quantum chemical calculations are reported on the deprotonation and protonation of the amino groups of the nucleic acid bases adenine, guanine and cytosine, in an attempt to compare the relative reactivities of these groups. In the light of renewed interest in the amino groups as reactive sites for certain carcinogenic and carcinostatic agents, we discuss the possible significance of our results for the interpretation of these molecular interactions.

Key words: Amino groups of nucleic acid bases

1. Introduction

Recently, special interest has centered on the reactivity of the amino groups of the nucleic acid bases, adenine, guanine and cytosine. Thus, for instance, experimental evidence [1–4] accumulated during the last years which shows that a class of metabolites of polycyclic aromatic hydrocarbon (PAH) carcinogens termed "bay-region" diol epoxides, considered by a number of investigators as possible "ultimate" forms of these carcinogens, form adducts with nucleic acid base amino groups, both in vitro and in vivo. The largest amount of this experimental work has involved the "bay-region" diol epoxide of benzo[a] pyrene. For this metabolite the absolute configuration of the guanine adduct has been determined [5–7] and for reaction with the synthetic homopolynucleotides poly A, poly G and poly C it has been found that poly G is the most reactive, poly A somewhat less so and that reaction with poly C occurs only slowly [4, 5]. There is also some experimental evidence that the preferential reactivity of the amino group of guanine towards this carcinogen exists in vivo when cellular RNA [4] or DNA [8] are attacked.

Suggestions have been made as to the molecular mechanism involved in forming these base adducts [2, 3, 9, 10]. It was proposed that the oxirane ring of benzo[a]-

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pyrene diol epoxide would open, preceded or followed by the protonation of the epoxide oxygen, to yield a triol carbonium ion which could then attack the amino group of guanine, adenine or cytosine.

The present authors carried out a model quantum chemical study of the reaction mechanism for this triol carbonium ion [11] and proposed the formation of an addition product with the base amino group where this group is quaternary and, formally, positively charged. An hydroxyl ion was then employed to assist with the removal of an amino proton to yield the final substitution product.

Another important reaction involving the NH_2 group of the nucleic acid bases and, again, in particular that of guanine, concerns the interaction of glyoxal with these bases. The reaction has drawn attention in relation to Szent-Gyorgyi's theory [12, 13] on the possible importance of glyoxal and its derivatives in problems of chemical carcinogenesis. It results in the formation of an adduct in which the glyoxal forms a supplementary cyclic ring attached to the amino N_2 and imino N_1 atoms of guanine with the concommitant migration of the N-bonded H atoms to the oxygens of glyoxal. The mechanism of this reaction has been investigated recently theoretically in our laboratory [14, 15] and it was found that the most plausible mechanism involves as the first step the interaction of a carbonyl group of glyoxal with the amino group of guanine.

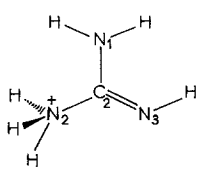
The reactivities of the amino groups of the nucleic acid bases, especially of guanine, seem thus of possibly greater biochemical significance than considered some years ago. In the present paper we propose to explore some of the fundamental electronic properties of these groups, having a bearing on their reactivities, in particular their proton donor and proton acceptor abilities.

2. Method

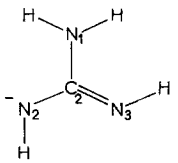
The SCF *ab initio* calculations reported here were carried out using the Gaussian 70 program [16] and the minimal basis set of Gaussian type orbitals due to Clementi *et al.* [17], that is [7s 3p/3s] contracted to [2s 1p/1s].

The size of the nucleic acid bases studied makes *ab initio* computations expensive, even when a minimal basis set is employed. Consequently, we have introduced in this study certain simplifications justified by the preliminary nature of this work in which we are primarily interested in the *relative values* of the acidity and the basicity of the three nucleic acid bases possessing an NH_2 group: guanine, adenine and cytosine. Thus we assumed that 1) the bond length joining the amino nitrogen atom to the carbon atom of the base ring (r_{C-NH_2}) does not alter on protonation or deprotonation of the amino group and have maintained it at 1.34 Å which is its length in the three bases in the geometries due to Spencer [18]; 2) the amino groups of the three neutral bases are trigonal and coplanar with the base rings; 3) the protonated amino groups are accurately tetrahedral. In order to establish the minimum energy rotamers of these amino-protonated bases we have carried out rather extensive studies on the model compound I. This compound mimicks

closely the corresponding part of guanine [11]. The study of the energy variation as a function of the torsion of the H_3N^+-group around the C_2–N_2^+ bond indicates then that the minimum energy conformer occurs when one of the N^+–H bonds is *cis*-planar with the C_2–N_3 bond (with a barrier of about 2.2 kcal/mole for the



I **Fig. 1**



II **Fig. 2**

intermediate position at a torsion of 60°). By extension it was assumed that the minimum conformers in the amino protonated adenine and cytosine will have one of their N^+–H bonds *cis*-planar to bonds C_6–N_1 and C_4–N_3 of these molecules, respectively.

For the geometry of the bases with a deprotonated amino group exploratory computations were performed on the model compound II. The minimum energy rotamer occurs when the remaining N_2^-–H bond is *cis*-planar to C_2–N_3 with the angle C_2–N_2–H equal to 104°. (The rotamer with the N_2^- amino proton trans-planar with respect to N_3 is about 7 kcal/mole less stable.) Again, by extension, it was assumed that the minimum energy conformers in the amino deprotonated adenine and cytosine will have the remaining N_{amino}^-–H bond *cis*-planar to their C_6–N_1 and C_4–N_3 bonds, respectively.

For the remaining parts of the bases the geometries due to Spencer [18] were employed.

3. Results and Discussion

We first discuss amino group deprotonation, the results for which are contained in Table 1.

The penultimate column of this table contains the energies for the endothermic process of deprotonating the amino groups while in the final column are the

Table 1. Deprotonation of the base amino groups

Base	Total energy of base (a.u.)[a]	Total energy of deprotonated base (a.u.)	Deprotonation energy (kcal/mole)	Hydroxyl ion assisted deprotonation energy (kcal/mole)
Adenine	−462.55284	−461.97038	365.5	−81.5
Cytosine	−390.93564	−390.35577	363.9	−83.1
Guanine	−537.13942	−536.59251	343.2	−103.8

[a] From Ref. [17].

energies for the exothermic reaction of deprotonation with the assistance of an hydroxyl ion, that is, the energy of the neutral base plus an hydroxyl ion at infinity compared to the energy of the base with a deprotonated, anionic amino group plus a water molecule at infinity. The O–H bond lengths in the hydroxyl ion and in water were taken to be 0.96 Å and the H–O–H bond angle in water was set at 105°. These latter energies may then be taken to model a base-catalysed amino group deprotonation.

Whichever set of deprotonation energies is considered it can be seen that the values for adenine and cytosine are very similar, while the deprotonation of guanine is more favorable by approximately 20 kcal/mole. These results are supported by the recent experimental work of Stewart and Harris [19] who assign identical pKa's of 16.7 to the amino groups of adenine and cytosine, while that of guanine is found to be more acidic with a pKa of 14.6. This support may be taken as evidence that the geometrical assumptions we have made do not obscure the fundamental relative reactivities of the base amino groups.

Table 2. Protonation of the base amino groups

Base	Total energy of base (a.u.)[a]	Total energy of protonated base (a.u.)	Protonation energy (kcal/mole)	Hydroxyl ion assisted deprotonation energy of the quaternary product (kcal/mole)
Adenine	−462.55284	−462.79158	−149.8	−297.2
Cytosine	−390.93564	−391.15256	−136.1	−310.9
Guanine	−537.13942	−537.34154	−126.8	−320.2

[a] From Ref. [17].

In Table 2 the results for amino group protonation are presented. The penultimate column contains the amino group exothermic protonation energies, while the final column contains the energies, also exothermic, for the process when an hydroxyl ion is assumed to assist in the deprotonation of the quaternary product, that is, the energy of the quaternary amino protonated base plus that of an hydroxyl ion at infinity is compared to the energy of the neutral base plus that of a water molecule at infinity. Both these processes are of interest as models of our proposed reaction mechanism of benzo[a]pyrene triol carbonium ion with the base amino groups and this aspect of the study will be discussed shortly.

From Table 2 it can be seen that the amino group of adenine is most readily protonated while protonating the same group of cytosine is less favorable by almost 14 kcal/mole and the reaction for guanine is less favorable still by a further 9 kcal/mole. The order of favorability is naturally reversed for the hydroxyl ion assisted deprotonation of the quaternary adduct to return to the neutral bases.

The results for amino group protonation are in line with an earlier theoretical study of the relative proton affinity of the amino groups of nucleic acid bases [20]

where electrostatic molecular potentials derived from the same minimal basis *ab initio* calculations predicted the order of this affinity to be:

adenine > cytosine > guanine

There does not seem to exist direct experimental evidence pertaining to this ease of protonation. Indirect evidence is inconclusive. Thus the preceding order seems to differ from that proposed by McGhee and von Hippel [21] who predict pKa's for the amino groups of −3.0 for dAMP, −2.6 for dGMP and −1.7 for dCMP, that is, protonation energies in the order dCMP > dGMP > dAMP. These results were based on an assumed linear relationship between amino group protonation pKa and the forward reaction rate constants of a series of aromatic amines with formaldehyde, the pKa's of the nucleic acid amino groups being consequently obtained from a linear fit to the rate data for the series of amines. However, it must be underlined that these deductions concern the nucleotides while our computations refer to the bases and that, moreover, as the authors themselves state, the accuracy of their predicted values cannot be ascertained.

The above results suggest a few comments in conjugation with our previously mentioned attempt to interpret the electronic aspects of the relative reactivity of the metabolic triol carbonium ions of carcinogenic PAH with the amino groups of the nucleic acid bases, a reaction which occurs preferentially with guanine. This preference correlates with the present evaluation of the relative acidity of the amino groups in free or quaternized bases and would thus correlate with the second phase of our proposed mechanism, namely the deprotonation of the cationic intermediate adduct between the base and the PAH metabolite. On the contrary, for the first phase of the reaction, namely the formation of this adduct, guanine would be expected to be the least reactive inasmuch, of course, as results relative to the protonation of the free bases may be of significance for the much more complex reaction of the PAH metabolite with base moieties in polynucleotides or nucleic acids. There are many reasons why this significance could be very limited indeed. In the first place, steric effects may play a very important role in the biological reaction between the large systems, as already considered in Ref. [11]. The amino group of guanine, situated in the minor groove of DNA seems sterically more accessible (although even so only within a number of fairly restricted areas of conformational space) than those of adenine or cytosine, to the approach of the huge metabolite. In the second place, recent computations from this laboratory [22] indicate that the order of the relative basicity of the amino groups of the bases may undergo a modification when the bases are included in hydrogen bonded or polymeric systems. For instance these results show that when the complementary hydrogen-bonded A–T and G–C base pairs are considered it is the guanine amino group which becomes the most basic one among those of these purines and pyrimidines. The problem is therefore obviously open for a deeper investigation.

Another question which may be raised in conjunction with the mechanism of the reaction between the "bay region" diol epoxides of PAH with the amino groups of the nucleic acid bases concerns the possible involvement in such a reaction of the

anionic deprotonated amino group, which could then react with the PAH diol epoxide. That such a possibility is improbable stems from some of our studies on the reactivity of the diol epoxides, the details of which will be published shortly. Here we would only like to report that we find that the opening and protonating of the epoxide ring of a PAH diol epoxide model is exothermic by some 250 kcal/mole. (For oxirane, where the carbonium ion of the opened ring lacks any stabilization due to the presence of a conjugated system, a value of 191 kcal/mole has recently been calculated by Hopkinson *et al.* [23] using a double zeta basis set and geometry optimization.) The comparison of this energy to the ease of deprotonating the base amino groups, exothermic by roughly 100 kcal/mole when base catalyzed, shows that opening the epoxide ring is considerably more favorable, in support of the originally proposed reaction mechanism. The possibility that the reaction could occur between two ions, the cationic opened and protonated epoxide and the anionic deprotonated amino group may be thought much less likely on kinetic grounds.

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