THEORETICA CHIMICA ACTA
Theoret. Chim. Acta (Berl.) 50, 75–79 (1978)

Short Communications

A Quantum-Mechanical Study of the Interaction of Methylglyoxal with Guanine

Daniel Demoulin, Anne-Marie Armbruster and Bernard Pullman

Institut de Biologie Physico-Chimique, Laboratoire de Biochime Théorique associé au C.N.R.S., 13, rue P. et M. Curie, F-75005 Paris, France

Ab initio self-consistent field molecular orbital computations on the relative stabilities of the different possible intermediate adducts for the reactions between methylglyoxal and guanine, as well as the evaluation of the relative stabilities of the two different possible final cyclic products (IIIb and IIIc) point all to the conclusion that it is the addition product in which the methyl group is close to the amino nitrogen which is the most stable one.

Key words: Methylglyoxal, interaction of ~ with guanine – Guanine, interaction of methylglyoxal with ~

1. Introduction

In a recent study [1], we have investigated the interaction of glyoxal (I) with guanine (II) by *ab initio* SCF molecular orbital calculations. The product of this interaction is a covalent adduct (IIIa) in which the glyoxal attaches itself to the N_1 imino and N_2 amino nitrogen atoms of guanine, to form a stable saturated pentacycle [2]. Our results indicate that the reaction should occur in two steps and that the most favorable route involves a nucleophilic addition of a carbonyl group on the N_2 amino nitrogen atom followed by a similar interaction at the N_1 imino nitrogen atom, rather than the reverse order of interaction. The energy barrier was smaller and the intermediate product was more stable when addition occurred first at N_2.

Experimentally, one can find indirect support for a greater stability of the mono-adduct on the amino nitrogen in the fact that periodate degradation of the guanine-glyoxal adduct leads exclusively to the N_2-formylguanine [2], and that the dominant product of the addition of formaldehyde to guanine, at neutral pH, is also N_2-formylguanine [3].

0040-5744/78/0050/0075/$01.00

When substituted glyoxals R–CO–COH (methylglyoxal or pyruvaldehyde, R=CH_3; ketoxal, R=$CH_3CH(OC_2H_5)$) are made to interact with guanine, the substituent-bearing carbon atom is always bound to the N_2 amino nitrogen atom in the final product (IIIb) [2]. Shapiro *et al.* [2] tried to rationalize this orientation effect by considering that the aldehydic carbonyl group, assumed more reactive, would react first with the imino nitrogen atom of guanine also assumed more reactive; then a slower cyclization step would occur between the ketone group of the substituted glyoxal and the amino group of guanine. The supposition that the imino group would react before the amino group is based on the results of the interaction of *formaldehyde* with guanine [3], which indicate that, while the N_2-amino adduct is more stable at neutral pH, the rate of formation of the N_1-imino adduct is greater. Our calculations [1] show, however, that for the interaction of *glyoxal* with guanine, the formation of the N_2-amino adduct in the first step of the reaction is favored both by kinetic factors (lower energy barrier) and by thermodynamic factors (greater stability). Thus it seems that formaldehyde and glyoxal do not act in exactly the same way. This may be traced back [1] to unfavorable steric repulsions between the non-reacting COH group of glyoxal and the guanine cycle, which are greater when the attack occurs at the endocyclic N_1 imino nitrogen than on the exocyclic N_2 amino group.

In the present paper we wish to extend our studies of Ref. [1] to some aspects of the interaction of methylglyoxal $CH_3–\overset{O}{\overset{\|}{C}}–\overset{O}{\overset{\|}{C}}–H$ with guanine in order to account for the stereospecificity of the reaction leading preferentially to type IIIb adduct.

2. Method

As in Ref. [1] a simplified model compound IV was used to represent guanine. In the present work we have limited our study to the determination of the relative stabilities of the four possible monoadducts of methylglyoxal with guanine, symbolized by Vb, Vc, VIb, VIc, and also of the relative stabilities of the two isomers of the final, cyclic adduct IIIb and IIIc. We thus investigate only the thermodynamic aspects of the reaction.

To compare the relative energies of the four monoadducts, it is essential to determine in each case its optimal conformation. This refers to the conformation of the "side chain" (glyoxal fragment) with respect to the guanine ring (as symbolized by IV). We have chosen to use for this sake the relatively rapid PCILO method [4], whose successes in dealing with problems of molecular conformations have been well substantiated [5–8].

Bond distances and bond angles are taken following Ref. [1] and are indicated in VIIa and VIIb for the adducts on N_2 and N_1 respectively.

The torsion angles considered in the conformational studies are indicated in Table 1. Following the classical definition [5–8], the torsion angle τ about the

Table 1. Optimized conformations and energies (in kcal/mole)

A) Adduct on N_2 amino nitrogen (V)

Species	Energy	Torsion angles		
		$\tau(N_1-C_2-N_2-C_{10})$	$\tau(C_2-N_2-C_{10}-O_{10})$	$\tau(N_2-C_{10}-C_{11}-O_{11})$
Va	−77850.4	210°	330°	120°
Vb	−83321.9	210°	330°	120°
Vc	−83303.4	210°	330°	120°

B) Adduct on N_1 imino nitrogen (VI)

Species	Energy	Torsion angles	
		$\tau(C_2-N_1-C_{10}-C_{11})$	$\tau(O_{11}-C_{11}-C_{10}-N_1)$
VIa	−77834.1	315°	150°
VIb	−83293.8	315°	150°
VIc	−83307.6	315°	330°

C) Final cyclic product (III)

Species	Energy	
IIIb	−83478.5	All ring atoms coplanar except C_{10}
IIIc	−83476.1	$\tau(N_1-C_2-N_2-C_{10})=20°$

bond B–C in the sequence of atoms A–B–C–D is the angle through which the far bond C–D is rotated relative to the near bond A–B. The *cis*-planar position of bonds A–B and C–D, represents $\tau=0°$. The torsion angles are considered positive for a right-handed rotation: when looking along the bond B–C, the far bond C–D rotates clockwise relative to the near bond A–B. Alternatively, the positive angles are defined as 0 to 180°, measured for a clockwise rotation and negative angles as 0 to −180°, measured for a counter-clockwise rotation.

3. Results

The optimized torsion angles and the corresponding total energies of the adducts are given in Table 1. For adducts on the amino nitrogen N_2, V, the torsion angles around C_2–N_2 and N_2–C_{10}, were first simultaneously varied, then the torsion angle around C_{10}–C_{11} was optimized. For adducts on the imino nitrogen N_1, VI, only two torsion angles need be varied, around N_1–C_{10} and around C_{10}–C_{11} and they have been varied simultaneously. In all cases, the elementary variation step was 30°.

In the case of the reaction with glyoxal itself, in the conformations taken from Ref. [1], we find by the PCILO method that the adduct on the amino nitrogen (Va) is more stable than the adduct on the imino nitrogen (VIa) by 10.6 kcal/mole. In Ref. [1] that same energy difference was 12.8 kcal/mole using the *ab initio* SCF method with the STO-3G basis set. This shows that the PCILO method reproduces quite adequately the *ab initio* energy differences and is thus well suited for our purpose. Optimization of the dihedral angles leads to a further stabilization of Va compared to VIa and the final energy difference is 16.3 kcal/mole.

The most stable monoadduct of methylglyoxal results from the addition of the ketone carbonyl group to the amino group (Vb). In order of increasing energy, we then find the adduct VIc (interaction of the aldehyde carbonyl group with the imino group) at +14.3 kcal/mole, Vc (interaction of the aldehyde carbonyl group with the amino group) at +18.5 kcal/mole, and VIb (interaction of the ketone carbonyl group with the imino group) at +28.1 kcal/mole. Thus, as with glyoxal, the most stable monoadduct (Vb) results from the addition of methylglyoxal on the amino group of guanine. Moreover, this most stable adduct corresponds to the interaction of the ketone carbonyl group with the amino group (Vb), prefiguring thus the formation of IIIb as the final cyclic product. If we consider the initial addition at the imino N_1H group, then the methyl substituent will be preferentially on the unreacted carbonyl group (VIc), and in both events, the final cyclic reaction product (IIIb) will thus bear the methyl substituent on the carbon adjacent to the N_2 amino nitrogen, in agreement with experimental data.

A possible explanation of this orientation effect can be obtained from the inspection of the STO-3G atomic charge distribution in the species involved in the interaction. This shows that the aldehydic carbon of methylglyoxal carries an electronic charge nearly identical to that found in glyoxal, while the ketonic carbon is less electron rich (glyoxal: 5.877e; methylglyoxal aldehydic: 5.879e; ketonic: 5.814e). STO-3G atomic charges in the guanine model IV are 7.379 at N_1 and 7.436, at N_2. Using the atomic charges on the carbons as an approximate first order indication of their electrophilicity, and those on the nitrogens as a similar measure of their nucleophilicity, one would predict that the most favorable interaction should occur between the ketone group of methylglyoxal and the amino group of guanine. One would also predict that the interaction with the imino nitrogen is less favorable than with the amino nitrogen. However, to rationalize the fact that, when the attack occurs on the imino nitrogen, interaction with the aldehyde group of methylglyoxal is favored, one would have to invoke steric effects. It is in fact reasonable that steric crowding should play a greater role for additions to the endocyclic imino nitrogen than for additions to the exocyclic amino nitrogen; it is also reasonable that in this case, the adduct VIb, with the methyl much closer to the guanine ring, should experience more favorable steric interactions than the adduct VIc.

Inspection of Table 1 shows that the preferred conformation of the monoadduct is dictated essentially by the site of attachment of methylglyoxal to the guanine cycle, and not by the presence or the position of the methyl substituent. We must observe, however, that the potential surfaces present many valleys and that, for the methylglyoxal adducts, there are secondary minima with different sets of torsion angles, some of which are less than 1 kcal/mole above the glyoxal minimum. The existence of these secondary minima is of no consequence for our general argument. The existence of the valleys means that no large energy barrier is to be expected when the torsion angles will be modified to start closing the ring in the second half reaction.

We have also calculated the energy of the final cyclic product for the two possible positions of the methyl substituents: close to the amino nitrogen (IIIb) or close to

the imino nitrogen (IIIc). The geometry once again was taken from Ref. [1]. The guanine fragment is as in IV, with $R_{C_2-N_2} = 1.46$ Å; $R_{N-C_{11}} = 1.48$ Å; $R_{C_{11}-C_{10}} = 1.51$ Å and $R_{C_{10}-N_2} = 1.50$ Å; $R_{C_{10}-O_{10}} = R_{C_{11}-O_{11}} = 1.428$ Å and other parameters are as in VIIa and b. The final energies are indicated at the bottom of Table 1 and show that the product IIIb, which is observed experimentally, is more stable than IIIc. The difference is 2.4 kcal/moll and thus smaller than those found for the monoadducts.

4. Conclusions

The calculations of the relative stabilities of the different possible intermediate monoadducts for the reactions between methylglyoxal and guanine, as well as the evaluation of the relative stabilities of the two different possible final cyclic products point all to the same conclusion namely that it is the addition product in which the methyl substituent is close to the amino nitrogen which is the most stable one. As the most probable sequence of reactions we propose the formation of Vb (the addition of the ketone carbon of methylglyoxal to the amino group of guanine), followed by cyclization to IIIb. A possible attack of methyl glyoxal on the imino nitrogen of guanine, which following our studies should lead preferentially to VIc would also result through final cyclization in the formation of IIIb. All evaluations lead thus to the preference for IIIb over IIIc.

Acknowledgement. This work has been carried out as a part of a program sponsored by the National Foundation for Cancer Research to which the authors wish to express their gratitude for the support which made this work possible. They also wish to express their thanks to Professor Albert Szent-Gyorgyi for his stimulative activity in the field of research involving the biological role of glyoxal and its derivatives and for many fruitful and exciting discussions on this subject.

References

1. Demoulin, D., Armbruster, A.-M., Pullman, B.: Theoret Chim. Acta (Berl.) **48**, 143 (1978)
2. Shapiro, R., Cohen, B. I., Shiucy, S.-J., Maurer, H.: Biochem. **8**, 238 (1969)
3. McGhee, J. D., Von Hippel, P. H.: Biochem. **14**, 1281 (1975)
4. Diner, S., Malrieu, J. P., Claverie, P.: Theoret. Chim. Acta (Berl.) **13**, 1 (1969)
5. Pullman, B., Pullman, A.: Advan. Protein Chem. **28**, 347 (1974)
6. Pullman, B., Saran, A.: Progr. Nucleic Acid Res. Mol. Biol. **18**, 215 (1976)
7. Pullman, B.: Advan. Quantum Chem. **10**, 251 (1977)
8. Pullman, B.: Quantum mechanics of molecular conformations. New York: John Wiley and Sons 1976

Received May 31, 1978