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THEORETICAL STUDY OF THE MALONDIALDEHYDE-ADDUCTS FORMED BY REACTION WITH DNA-BASES

ESTUDIO TEÓRICO DE LOS ADUCTOS-MALONDIALDEHÍDO FORMADOS POR LA REACCIÓN CON BASES ADN

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ABSTRACT

Malondialdehyde (MDA) is a major genotoxic carbonyl compound generated by lipid peroxidation and is also a by-product of the arachidonic acid metabolism in the synthesis of prostaglandins. MDA has been shown to be mutagenic in bacterial and mammalian systems and carcinogenic in rodents. Besides, it is known that MDA reacts with DNA to form adducts with deoxyguanosine, dG, deoxyadenosine, dA, and deoxycytidine, dC: M_1G, M_1A and M_1C, respectively.

In this paper we present a density functional theoretical study of the several nucleophilic additions followed by eliminations of MDA with dG, dA, and dC. Due to the size of the adducts, the ribofuranoside chain has not been taking into account in the calculations because this part of the molecule is far of the reaction centers and does not participate in the reactions. Therefore, guanine, adenine, and cytosine have been taken as model compounds and their adducts with MDA have been calculated in order to obtain the reaction profiles and the adducts stabilities. All of the studied reactions have been modeled in the gas phase at 298.15 K and 1 atm. Taking into account the free energies of the reactants and the final adducts, it is observed that the three reactions are endergonic. It would be expected that the adduct with cytosine will be the most abundant and the one with guanine the less. However, the first step of the reaction presents a lower barrier in the reaction of MDA with guanine indicating that probably the kinetic factor is more important in the formation of these adducts.

Keywords: *DFT, computational methods, mutagenesis, malondialdehyde, DNA adducts.*

RESUMEN

Malodialdéhido (MDA) es el mayor compuesto carbonílico genotóxico, generado por la peroxidación lipídica y es también un subproducto del metabolismo del ácido araquidónico en la síntesis de prostaglandinas. Se ha mostrado que es mutagénico en bacterias y en células mamarias y cancerigeno en roedores. Es también conocido que el MDA reacciona con DNA para formar aductos deoxiguinosina, dG, deoxiadenosina, dA, and deoxicitosina, dC: M_1G, M_1A and M_1C, respectivamente.

En este trabajo presentamos un estudio utilizando la teoría de funcionales de densidad(DFT) de las diversas reacciones de adición nucleofílica, seguidas de eliminación (AN-E) de MDA con dG, dA y dC. Debido a el gran tamaño, la parte ribofuranósida no se ha tenido en cuenta dado que está alejada del centro de la reacción. De esta forma, la guanina, adenina y citosina han sido tomadas como compuestos modelos y sus aductos con MDA han sido calculados con el fin de obtener los perfiles de reacción y la estabilidad de los aductos.

Todas las reacciones estudiadas se modelaron en fase gaseosa a 298.15 K y 1 atm. Tomando en cuenta las energías libres de los reactantes y los aductos finales, se observa que las reacciones globales son endergónicas. Se esperaría que el aducto mas abundante sea con citosina y el de menor cantidad con la guanina. No obstante lo anterior, el primer paso de las reacciones presenta una barrera energética baja en la adición de MDA con guanina, indicando que probablemente es el factor cinético el predominante en la formación de este tipo de aductos.

Palabras clave: *DFT, método computacional, mutagénesis, malondialdehido, aductos de DNA.*

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INTRODUCTION

Lipid peroxidation is a complex process known to occur in both plants and animals. This process occurs in three distinct stages: initiation, propagation and termination. Polyunsaturated fatty acids present in biological membranes appear to be particularly sensitive to oxidative damage due to the lowered bond dissociation energy of their allylic hydrogens. Once initiated, the process proceeds as a free radical chain reaction (1). The progress of these reactions can be monitored by determination of: lipid hydroperoxides; secondary products as malondialdehyde, (MDA); ethane and pentane formation, mainly (2). Thus, lipid peroxidation and free radicals have been associated with a number of normal (prostaglandin synthesis, phagocytosis and ageing) and abnormal (inflammation, drug toxicity, carcinogenesis, atherosclerosis and several other pathologies) physiological processes (3).

Malondialdehyde (MDA) is a major genotoxic carbonyl compound generated by lipid peroxidation (4,5) and is also a by-product of the arachidonic acid metabolism in the synthesis of prostaglandins (6). It has been shown to be mutagenic in bacterial and mammalian systems (7) and carcinogenic in rodents (8).

MDA reacts with DNA to form stable adducts to deoxyguanosine, dG, deoxyadenosine, dA, and deoxycytidine, dC: M_1G, M_1A and M_1C, respectively (9-13), that are possible promutagenic lesions (see Figure 1). There are several methods for the determination of M_1G. Chaudhary et al. (14) demonstrated the existence of this adduct in human liver, white blood cells and pancreas. M_1G residues were detected in all of these tissues at levels ranging from below the limits of detection to as high as 1.2 adducts per 10^6 nucleosides (approximately 6500 adducts per cell). M_1G also has been detected in human breast tissue by ^{32}P-post-labeling and in rodent tissues (15,16).

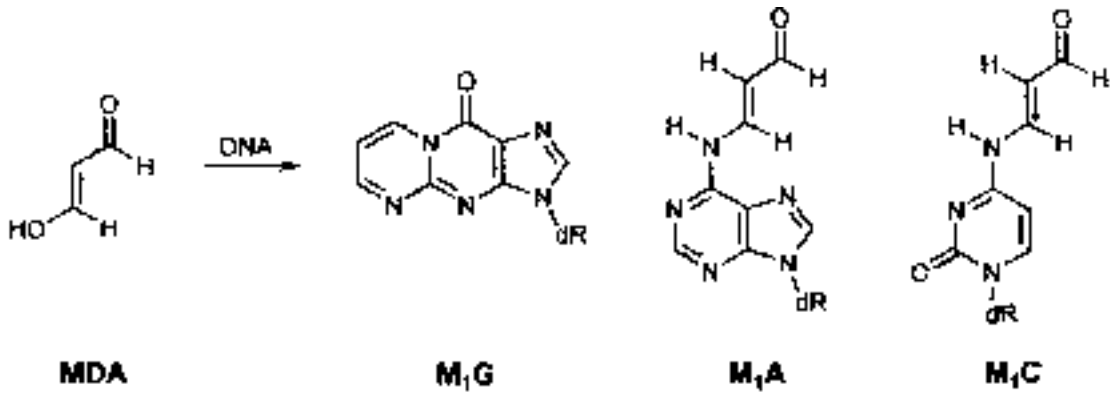

Figure 1. Structures of MDA-DNA adducts.

Modification of a single-stranded bacteriophage genome with MDA followed by transformation of SOS-induced E. coli strains causes frameshift and base pair substitution mutations in the lacZα gene carried on the vector (17). Base pair substitutions are observed at G, A and C residues which are assumed to arise from the corresponding MDA-DNA adduct at that position (G→ T, A→ G and C→ T). Site-specific experiments confirm that M_1G is mutagenic in E. coli, inducing transversions to T and transitions to A (18). Transformation of adducted genomes into repair-deficient strains suggest that M_1G is repaired by both bacterial and mammalian nucleotide excision repair pathways and is also repaired in E. coli by mismatch repair (19,20).

Very recently (21), a study has been devoted to answer the question, is MDA mutagenic in human cells? Sequence analysis revealed that the majority of MDA-induced mutations occurred at GC base pairs. The most frequent mutations were large insertions and deletions, but base pairs substitutions were also detected.

The condensation product of MDA with dG, with loss of two water molecules includes N2 and exocyclic amine group (N2) and forms the

exocyclic pyrimido[1,2-a]purin-10(3H)-one, abbreviated M_1G (22, 23). M_1G is a mutagenic DNA lesion and a terminal product of lipid peroxidation in vivo that may be implicated in cancer related to lifestyle and diet (23).

The condensation products with dA and dC arise by addition-elimination of one of the carbonyl equivalents of MDA with the exocyclic amine groups to form an oxopropenyl derivate. No evidence for cyclization of these products has been observed (5).

Very recently (24), it has been developed a novel strategy for the synthesis of the MDA nucleoside adducts, which significantly improves their availability.

In this paper we present a density functional theory study of the several nucleophilic additions followed by eliminations of MDA with dG, dA, and dC. Owing to the size of the adducts, the ribofuranoside chain has not been taking into account in the calculations because this part of the molecule is far of the reaction centers and does not participate in the reactions. So, guanine, adenine, and cytosine have been taken as model compounds and their adducts with MDA (Figures 2, 3, and 4, respectively) have been calculated in order to obtain the reaction profiles and the stabilities of the adducts.

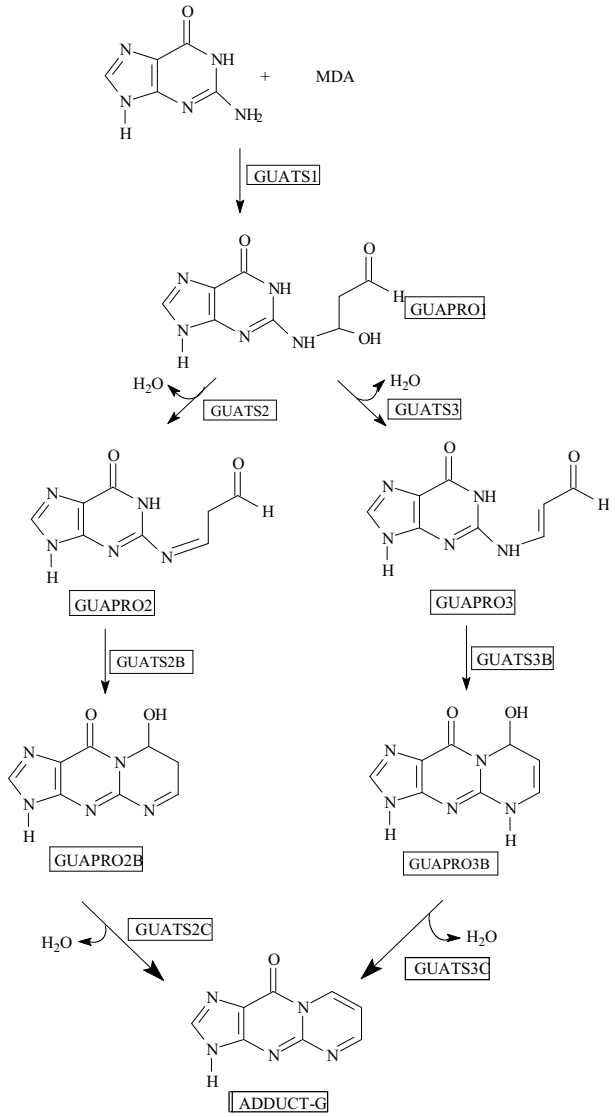

Figure 2. Structures of all the intermediates in the formation of the adduct of MDA to guanine.

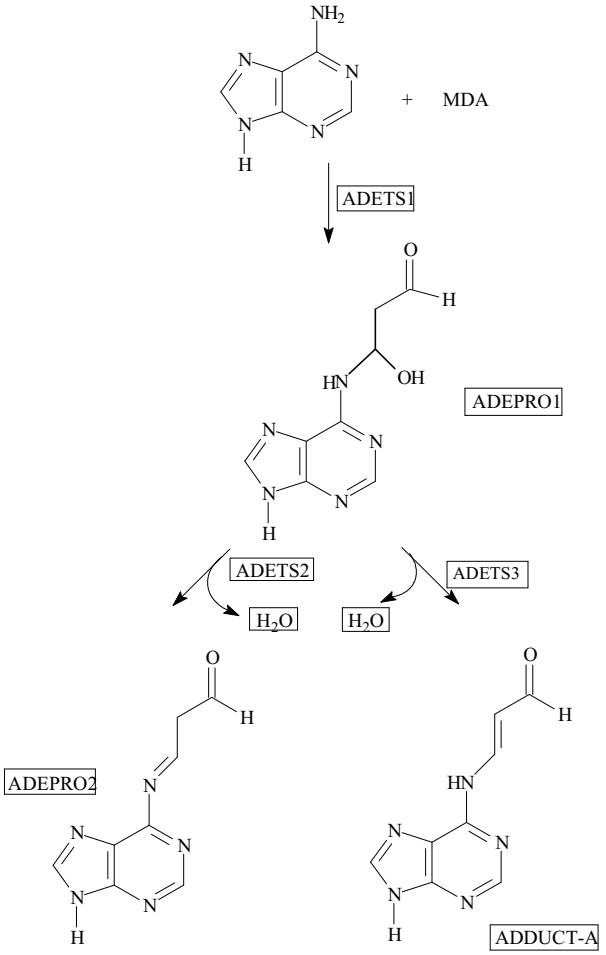

Figure 3. Structures of all the intermediates in the formation of the adduct of MDA to adenine.

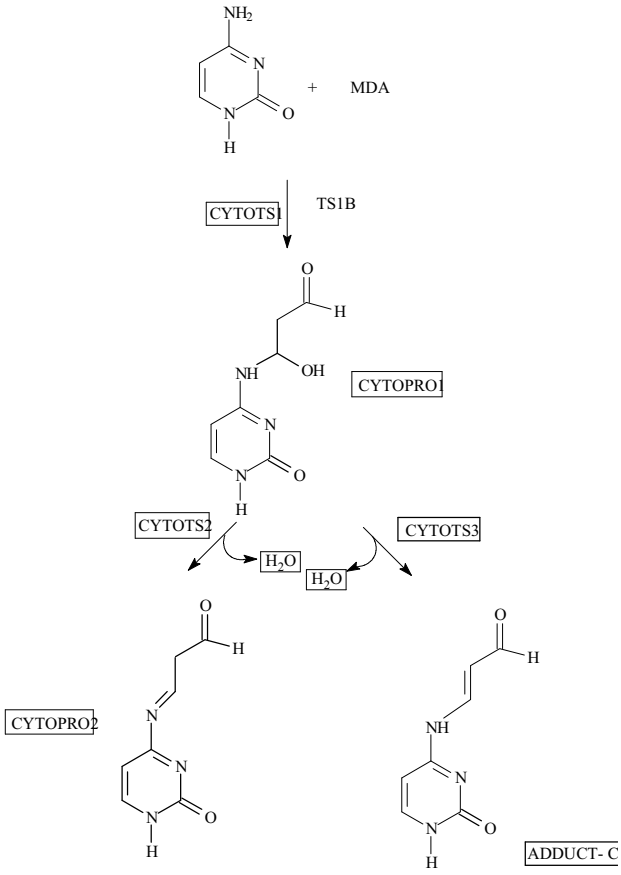

Figure 4. Structures of all the intermediates in the formation of the adduct of MDA to cytosine.

Computational Details

Density functional calculations were performed with the Gaussian98 series of programs (25). Among the various proposed functionals, we have used the combination of Becke's three-parameter hybrid exchange functional (26) with the Lee, Yang and Parr correlation functional (27), denoted B3LYP (28).

The geometric parameters for all the reactants, the transition states (TS), and the products of the reactions studied were fully optimized at the B3LYP/6-31G(d) level (29). Each stationary structure was characterized as a minimum or a saddle point of first order by analytical frequency calculations. A scaling factor (30) of 0.9806 for the zero-point vibrational energies has been used. Thermal corrections to enthalpy and entropy values have been evaluated at the temperature of 298 K, according to standard thermodynamics (31).

Intrinsic reaction coordinate (IRC) calculations (32) have been performed in all cases to verify that the localized transition state structures connect with the corresponding minimum stationary points associated with reactants and products.

Electronic energies, zero-point vibrational energies, thermal correction to enthalpies, and entropies, evaluated at the B3LYP/6-31G(d) level, for all the reactants, transition states, and products, involved in the reactions of MDA with guanine, adenine, and cytosine, are collected in Table 1.

RESULTS AND DISCUSSION

All of the studied reactions have been modeled in the gas phase at 298.15 K and 1 atm. In the addition reactions to the exocyclic amino group of guanine, adenine and cytosine (thymine does not have this amino group) and the corresponding eliminations of water molecules we have considered four-membered cyclic transition states (see Figure 5).

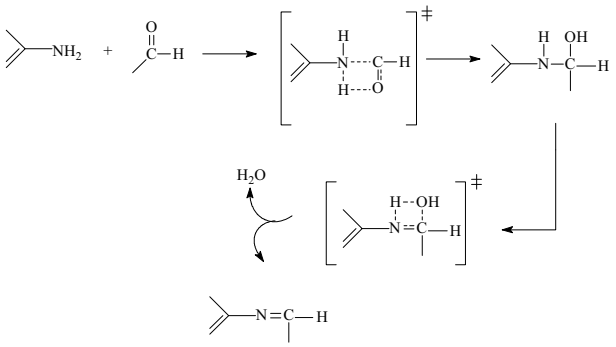

Figure 5. Postulated addition-elimination mechanism.

In the reaction of MDA with guanine, a first intermediate product, GUAPRO1, is formed via the four-membered cyclic transition state, GUATS1. This intermediate can eliminate a water molecule following two different pathways, via the transition states GUATS2 and GUATS3, forming two intermediates, GUAPRO2 and GUAPRO3, with different position of the double bond formed. After this step, two different cyclization processes can occur yielding to GUAPRO2B and GUAPRO3B. At last, the elimination of a water molecule in GUAPRO2B via the cyclic transition state GUATS2C produces an adduct similar to that experimentally obtained, M_1G. The elimination of water in GUAPRO3B is not possible via a four-membered

Table 1. Electronic energies, evaluated at the B3LYP/6-31G(d) level, zero-point vibrational energies, ZPE, and thermal corrections to enthalpies, TCH, in Hartrees, and entropies, S, in cal mol^{-1} K^{-1}, for all the reactants, transition states and products involved in the reactions studied.

Species	*Ee*	*ZPE*	*TCH*	*S*
GUANINE	*-542.55009*	*0.11709*	*0.12623*	*88.63*
MDA	*-267.13973*	*0.06544*	*0.07202*	*76.29*
GUATS1	*-809.62964*	*0.18185*	*0.19622*	*117.19*
GUAPRO1	*-809.71089*	*0.18838*	*0.20282*	*116.80*
GUATS2	*-809.61460*	*0.18089*	*0.19542*	*116.85*
GUAPRO2	*-733.26217*	*0.15884*	*0.17213*	*114.02*
GUATS3	*-809.61674*	*0.18006*	*0.19483*	*117.62*
GUAPRO3	*-733.27399*	*0.15990*	*0.17328*	*112.01*
GUATS2B	*-733.16922*	*0.15352*	*0.16566*	*104.50*
GUAPRO2B	*-733.27318*	*0.16260*	*0.17427*	*102.04*
GUATS3B	*-733.20122*	*0.15960*	*0.17188*	*105.87*
GUAPRO3B	*-733.27846*	*0.16309*	*0.17489*	*102.12*
GUATS2C	*-733.18438*	*0.15484*	*0.16665*	*102.42*
GUATS3C	*-733.17752*	*0.15652*	*0.16773*	*99.16*
ADDUCT-G	*-656.85175*	*0.13443*	*0.14475*	*94.71*
ADENINE	*-467.31817*	*0.11240*	*0.12055*	*83.74*
ADETS1	*-734.39166*	*0.17687*	*0.19045*	*114.41*
ADEPRO1	*-734.47812*	*0.18389*	*0.19721*	*111.81*
ADETS2	*-734.37810*	*0.17707*	*0.19053*	*112.75*
ADEPRO2	*-658.02100*	*0.15464*	*0.16680*	*107.79*
ADETS3	*-734.38346*	*0.17601*	*0.18975*	*114.03*
ADDUCT-A	*-658.05299*	*0.15663*	*0.16826*	*103.35*
CYTOSINE	*-394.92801*	*0.09877*	*0.10633*	*80.36*
CYTOTS1	*-662.00749*	*0.16486*	*0.17737*	*108.09*
CYTOPRO1	*-662.09175*	*0.17048*	*0.18316*	*108.21*
CYTOTS2	*-662.00526*	*0.16420*	*0.17671*	*109.39*
CYTOPRO2	*-585.63487*	*0.14135*	*0.15286*	*103.95*
CYTOTS3	*-662.00808*	*0.16363*	*0.17624*	*107.58*
ADDUCT-C	*-585.66489*	*0.14316*	*0.15417*	*99.90*
H_2O	*-76.40895*	*0.02117*	*0.02495*	*45.14*

cyclic transition state. The only possibility is a six-membered cyclic transition state, forming a bicyclic species GUATS3C with a higher energy than that of GUATS2C.

Adenine forms with MDA an intermediate product, ADEPRO1, via a four-membered cyclic transition state, ADETS1, and after loses a water molecule leading to ADEPRO2 and the adduct.

Cytosine reacts with MDA and forms the intermediate, CYTOPRO1, via a four-membered cyclic transition state, CYTOTS1, leading to CYTOPRO2 and the adduct, after the elimination of a water molecule.

Observing the free energy reaction profiles (Figures 6, 7, and 8), obtained at the B3LYP/6-31G(d) level, we can conclude that the most stable products in the reactions of MDA with guanine, adenine and cytosine are the adducts similar to those experimentally obtained (M_1G, M_1A and M_1C).

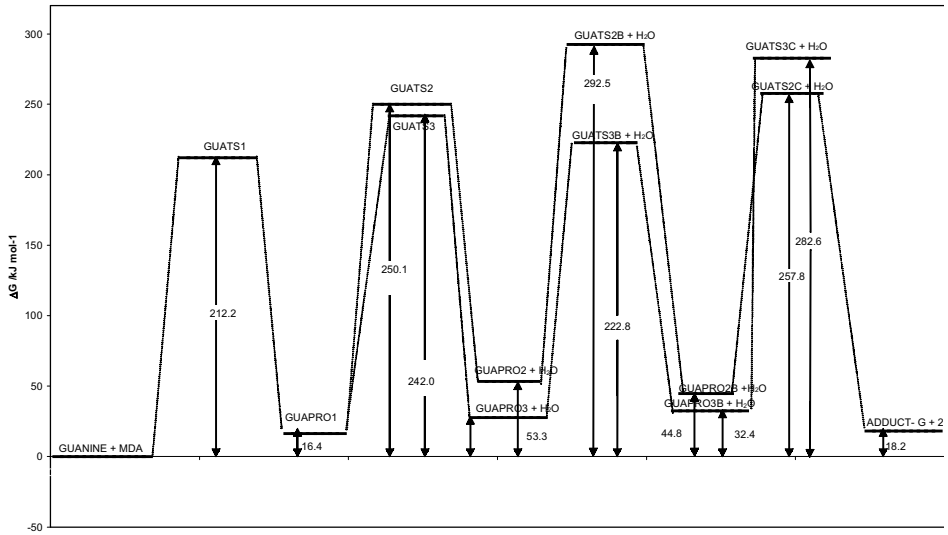

Figure 6. Free energy profile for the reaction of MDA with guanine, obtained at the B3LYP/6-31G(d) level.

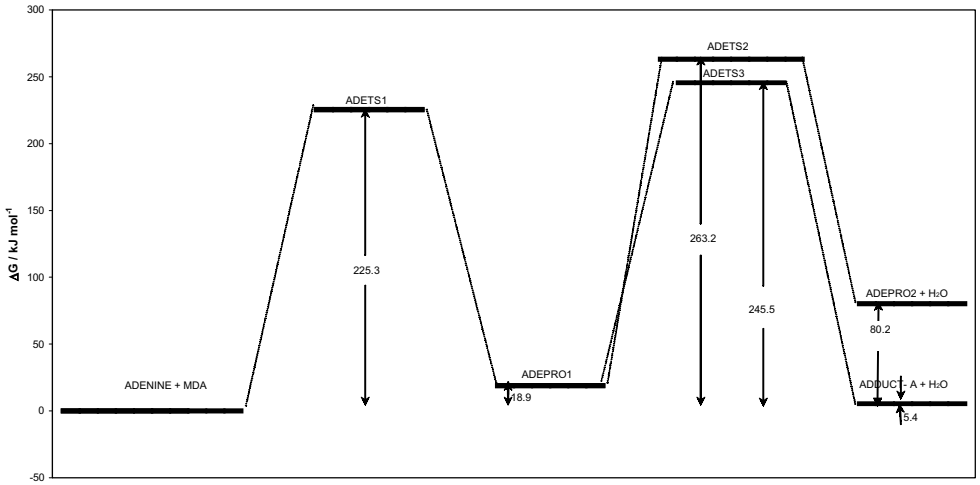

Figure 7. Free energy profile for the reaction of MDA with adenine, obtained at the B3LYP/6-31G(d) level.

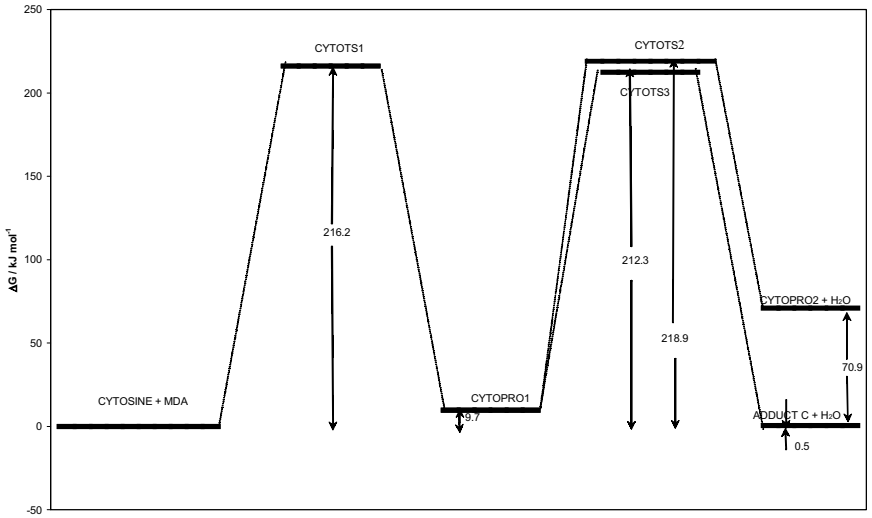

Figure 8. Free energy profile for the reaction of MDA with cytosine, obtained at the B3LYP/6-31G(d) level.

In the reaction of MDA with guanine, the process occurs through the intermediates GUAPRO3 and GUAPRO3B, more stables and with lower activation energies than their alternative processes, although the last elimination of a water molecule to yield the adduct presents a higher energy barrier, via the bicyclic transition state.

Taking into account the free energies of the reactants and the final adducts, it is observed that the three reactions are endergonic (see Table 2). It would be expected that the more abundant adduct will be that with cytosine, and the less abundant the adduct with guanine. However, the first step of the reaction presents a lower barrier in the reaction of MDA with guanine indicating that probably the kinetic factor is more important in the formation of these adducts.

Table 2. Reaction free energies in the formation of the adducts of malondialdehyde to guanine, adenine, and cytosine.

Base	Adduct	ΔG^0/kJ mol^{-1}
G	Adduct-G	+18.2
A	Adduct-A	+5.4
C	Adduct-C	+0.5

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BIBLIOGRAPHICAL REFERENCES

1. Llanillo,M., Sánchez-Yagüe,J., Martín-Valmaseda,E.M. and Hernández-Hernández,A. (1994) Erythrocyte storage as a model for lipid peroxidation studies. An experimental assay for students of biochemistry and molecular biology. Biochem. Educ. 22, 158-161.
2. Gutteridge,J.M.C. and Halliwell,B. (1990) The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci. 15, 129-135.
3. Halliwell,B. and Gutteridge,J.M.C. (1985) Free Radicals in Biology and Medicine. Clarendon Press, Oxford.
4. Hakala,K., Auriola,S., Koivisto,A. and Lönnberg,H. (1999) Mass spectrometric (HPLC/ESI-MS/MS) quantification of pyrimido[1,2-a]purin-10(3H)-one, a guanine adduct formed by reaction of malondialdehyde with DNA. J. Pharm. Biomed. Anal., 21, 1053-1061.
5. Marnett,L.J. (1999) Lipid peroxidation-DNA damage by malondialdehyde. Mutat. Res. 424, 83-95.
6. Marnett,L.J. (2000) Oxyradicals and DNA damage. Carcinogenesis 21, 361-370.
7. Marnett,L.J. (1994) Generation of mutagens during arachidonic acid metabolism. Cancer Metastasis Rev. 13, 303-308.

8. Basu,A.K. and Marnett,L.J. (1983) Unequivocal demonstration that malondialdehyde is a mutagen. Carcinogenesis 4, 331-333.
9. Otteneder,M., Daniels,J.S., Voehler,M. and Marnett,L.J. (2003) Development of a method for determination of the malondialdehyde.deoxyguanosine adduct in urine using liquid chromatography-tandem mass spectrometry. Anal. Biochem. 315, 147-151.
10. Seto,H., Akiyama,K., Okuda,T., Hashimoto,T., Takesue,T. and Ikemura,T. (1981) Structure of a new modified nucleoside formed by guanosine-malonaldehyde reaction. Chem. Lett. 707-708.
11. Nair,V., Turner,G.A. and Offerman,R.J. (1984) Novel adducts from the modification of nucleic acid bases by malonaldialdehyde. J. Am. Chem. Soc. 106, 3370-3371.
12. Marnett,L.J., Basu,A.K., O'Hara,S.M., Weller,P.E., Rahman,A.F.M.M. and Oliver, J.P. (1986) Reaction of malondialdehyde with guanine nucleosides: formation of adducts containing oxadiazabicyclononene residues in the base-pairing region. J. Am. Chem. Soc. 108, 1348-1350.
13. Stone,K., Ksebati,M. and Marnett,L.J. (1990) Investigation of the adducts formed by reaction of malondialdehyde with adenosine. Chem. Res. Toxicol. 3, 33-38.
14. Chaudhary,A.K., Nokubo,M., Reddy,G.R., Yeola,S.N., Morrow,J.D., Blair,I.A. and Marnett,L.J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science, 265, 1580-1582.
15. Vaca,C.E., Fang,J.L., Mutanen,M. and Valsta,L. (1995) ^{32}P-post-labeling determination of DNA adducts of malondialdehyde in humans: Total white blood cells and breast tissue. Carcinogenesis, 16, 1847-1851.
16. Wang,M., Dhingra,K., Hittleman,W.N., Liehr,J.G., de Andrade,M. and Li,D. (1996) Lipid peroxidation- induced putative malondialdehyde-DNA adducts in human breast tissues. Cancer Epidemiol. Biomarkers Prev., 5, 705-710.
17. Benamira,M., Jonson,K., Chaudhary,A., Bruner,K., Tibbetts,C. and Marnett,L.J. (1995) Induction of mutations by replication of malondialdehyde-modified M13 DNA in Escherichia coli: determination of the extent of DNA modification, genetic requirements for mutagenesis and types of mutations induced. Carcinogenesis, 16, 93-99.
18. Fink,S.P., Reddy,G.R. and Marnett,L.J. (1997) Mutagenicity in Escherichia coli of the major DNA adduct derived from the endogenous mutagen, malondialdehyde. Proc. Natl Acad. Sci. USA, 94, 8652-8657.
19. Johnson,K.A., Fink,S.P. and Marnett,L.J. (1997) Repair of propanodeoxyguanosine by nucleotide excision repair in vivo and in vitro. J. Biol. Chem., 272, 11434-11438.
20. Johnson,K.A., Mierzwa,M.L., Fink,S.P. and Marnett,L.J. (1999) MutS recognition of exocyclic DNA adducts that are endogenous products of lipid oxidation. J. Biol. Chem., 274, 27112-27118.
21. Niedernhofer,L.J., Daniels,J.S., Rouzer,C.A., Greene,R.E. and Marnett,L.J. (2003) Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J. Biol. Chem., 278, 31426-31433.
22. VanderVeen,L.A., Hashim,M.F., Shyr,Y. and Marnett,L.J. (2003) Induction of frameshift and base pair substitution mutations by the major DNA adduct of the endogenous carcinogen malondialdehyde. Proc. Natl Acad. Sci. USA, 100, 14247-14252.
23. Hoberg,A.-M., Otteneder,M., Marnett,L.J. and Poulsen,H.E. (2004) Measurement of the malondialdehyde-2´-deoxyguanosine adduct in human urine by immuno-extraction and liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. J. Mass Spectrom., 39, 38-42.
24. Wang,H., Marnett,L.J., Harris,T.M. and Rizzo,C.J. (2004) A novel synthesis of malondialdehyde adducts of deoxyguanosine, deoxyadenosine, and deoxycytidine. Chem. Res. Toxicol., 17, 144-149.
25. Gaussian 98 (Revision A.11.3), Frisch,M.J., Trucks,G.W., Schlegel,H.B., Scuseria,G.E., Robb,M.A., Cheeseman,J.R., Zakrzewski,V.G., Montgomery Jr.,J.A., Stratmann,R.E., Burant,J.C., Dapprich,S., Millam,J.M., Daniels,A.D., Kudin,K.N., Strain,M.C., Farkas,O., Tomasi,J., Barone,V., Cossi,M., Cammi,R., Mennucci,B., Pomelli,C., Adamo,C., Clifford,S., Ochterski,J., Petersson,G.A., Ayala,P.Y., Cui,Q., Morokuma,K., Salvador,P., Dannenberg,J.J., Malick,D.K., Rabuck,A.D., Raghavachari,K., Foresman,J.B., Cioslowski,J., Ortiz,J.V., Baboul,A.G., Stefanov,B.B., Liu,G., Liashenko,A., Piskorz,P., Komaromi,I., Gomperts,R., Martin,R.L., Fox,D.J., Keith,T., Al-Laham,M.A., Peng,C.Y., Nanayakkara,A., Challacombe,M., Gill,P.M.W., Johnson,B., Chen,W., Wong,M.W., Andrés,J.L., González,C., Head-Gordon.M., Replogle,E.S. and Pople,J.A., Gaussian, Inc., Pittsburgh PA, 2001.
26. Becke,A.D. (1993) Density-functional thermochemistry. III. The role of exact exchange. J. Chem. Phys. 98, 5648-5652.
27. Lee,C., Yang,W. and Parr,R.G. (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B 37, 785-789.
28. Stephens,P.J., Devlin,F.J., Chabalowski,C.F. and Frisch,M.J. (1994) Ab initio calculation of vibrational absorption and circular dichroism spectra using density functional force fields. J. Phys. Chem. 98, 11623-11627.
29. Ditchfield,R., Hehre,W.J. and Pople,J.A. (1971) Self-consistent molecular-orbital methods. IX. An extended Gaussian-type basis for molecular-orbital studies of organic molecules. J. Chem. Phys. 54, 724-728.
30. Scott,P.A. and Radom, L. (1996) Harmonic vibrational frequencies: an evaluation of Hartree-Fock, Møller-Plesset, quadratic configuration interaction, density functional theory, and semiempirical scale factors. J. Phys. Chem. 100, 16502-16513.
31. McQuarrie,D.A. and Simon,J.D. (1999) Molecular Thermodynamics, University Science Books: Sausalito, CA.
32. Fukui,K. (1970) Formulation of the reaction coordinate. J. Phys. Chem. 74, 4161-4163.

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