Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 98 (2006) 395–402
Food Chemistry
www.elsevier.com/locate/foodchem

Formation of methyl glyoxal in dihydroxyacetone and glycine Maillard reaction: A computational study

Md. Abul Haider Shipar *

Hiroshi Morita Laboratory, Building No. 08, Room No. 310, Department of Information and Image Sciences, Faculty of Engineering, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Received 23 March 2005; accepted 23 March 2005

Abstract

By considering the formation of methyl glyoxal as one of the possible intermediates, mechanisms for the intermediate stage of the Maillard reaction of dihydroxyacetone and glycine under different pH conditions have been proposed, following the Hodge-scheme. Density functional theory calculations have been performed at the standard state on the proposed mechanisms to calculated the Gibb's free energy changes for the formation of different compounds in different steps of the reaction. Thus, the possibility for the formation of different compounds in the proposed mechanisms has been evaluated. Electronic energy changes for the formation of different compounds in the proposed mechanisms have been calculated to observe the internal energy changes of the reaction. The total mass balance has been followed during the calculation of electronic and Gibb's free energies. The result reveals that methyl glyoxal is one of the most likely intermediates in the reaction, and dihydroxyacetone + deprotonated glycine and dihydroxyacetone + unprotonated glycine reactions are assumed favorable for the production of methyl glyoxal. The gaseous phase reaction is supposed to be more feasible than the aqueous phase reaction for the production of methyl glyoxal. Glyceraldehyde + protonated glycine and glyceraldehyde + glycine zwitterion reactions are postulated as less feasible for the formation of methyl glyoxal.

Keywords: Methyl glyoxal; Dihydroxyacetone; Glycine; Maillard reaction; Density functional theory calculations

1. Introduction

α-Dicarbonyl compounds, such as methyl glyoxal (**MG**), glyoxal (**Gox**), etc., have received intense interest due to their link to various pathologies. **MG**, which is an alkyl derivative of **Gox**, has been reported as more reactive than **Gox** in the Maillard reaction (Hollnagel & Kroh, 1998; Maillard, 1912; Meade, Miller, & Gerrard, 2003; Namiki & Hayashi, 1983). **MG** is a mutagenic compound, and is one of the most common intermediates in the Maillard reaction in vivo as well as vitro (Davídek, Velíšek, & Pokorný, 1990; Eskin, 1990; Macrane, Robinson, & Saadler, 1993; Reber et al., 2002; Uchida et al., 1997). It has been detected in many foods and beverages (Hayashi & Shibamoto, 1985; Hirayama, Yamada, Nohara, & Fukui, 1984), and increased concentrations of **MG** have been identified during uremia, hyperglycemia, diabetes, atherosclerosis, oxidative stress, aging, etc. (Odani et al., 1998; Thorpe & Baynes, 2003; Uchida et al., 1997). **MG** has been postulated predominantly in the Szent-Gyorgyi's theory of cancer (Nitzsche & Davidson, 1978; Szent-Györgyi, Együd, & McLaughlin, 1967). It inhibits the growth of a wide range of organisms and mammalian cells, and reduces the synthesis of proteins, RNA and DNA (Fravel & McBrien, 1980; Riley, 1980; Uchida et al., 1997; White & Rees, 1982). **MG** undergoes a rapid reaction with proteins, even under physiological conditions, and results in

* Tel.: +81 043 290 3471; fax: +81 043 290 3490.
E-mail addresses: shipar47@graduate.chiba-u.jp, shipar7@yahoo.com.

0308-8146/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2005.03.042

the formation of advanced glycation end products (**AGE**s) (Katarína et al., 2001; Meade et al., 2003; Thorpe & Baynes, 2003). Due to the electrophilic nature of $-CH_3$ group and O-atoms, the positive charge on the carbonyl carbon of **MG** is assumed to be increased. Therefore, it may become more reactive in nucleophilic addition reactions than other dicarbonyl compounds, such as **Gox**. The browning activity of **MG** has been reported as 2.77 liters/min, whereas only 0.515 liters/min has been reported for **Gox**, measured as the change in absorbance at 420 nm by using β-alanine and sugar or carbonyl systems (Namiki & Hayashi, 1983).

Computational studies on glyceraldehyde (**Gald**) and glycine (**Gly**) Maillard reactions have revealed that **MG** is one of the most likely intermediates in the reaction (Shipar, 2004). **Gald** can exist in equilibrium with its keto form, i.e., 1,3-dihydroxyacetone or dihydroxyacetone (**DHA**) (Harrold, 1991; Holum, 1996; Lozynski, Rusinska-Roszak, & Mack, 1997; Thornalley, Wolff, Crabbe, & Stern, 1984; Yaylan, Majors, & Ismail, 1999). Due to the commercial and biological importance (Brown, 2001; Feige, Ried, & Bachmann, 1996; Kobayashi & Takahashi, 1979; Kobayashi, Igarashi, Takahashi, & Higashi, 1976; Kurz, 1994; Marnett et al., 1985; Mersch-Sundermann, Schneider, Klopman, & Rosencrantz, 1994; Morita, 1991; Navrátil, Tkáč, Švitel, Danielsson, & Šturdik, 2001; Petersen, Wulf, Gniadecki, & Gajkowska, 2004; Pham, DeMarini, & Brockmann, 1980; Stanko & Adibi, 1986; Yamaguchi, 1982; Zhu et al., 2003), widespread investigation on the role of **DHA** in the Maillard reaction is necessary. The **DHA + Gly** Maillard reaction has not been well studied, and therefore the mechanism is still obscure. However, computational study on the Heyns rearrangement (**HR**) in the initial stage of **DHA + Gly** Maillard reaction under different pH conditions has revealed that **DHA + DGly** reaction is the most favorable for the formation of the Heyns rearrangement products [**HRP(DGly)**s] (Shipar, 2006). The gaseous phase reaction has been found to be more favorable than the aqueous phase reaction in producing **HRP(DGly)**s. Production of both of the enol and keto forms of the Heyns rearrangement products (**HRPE**(**DGly**) and **HRPK**(**DGly**), respectively) has been supposed to be feasible in the **DHA + DGly** reaction. The **DHA + UGly** reaction has been reported to be the second most favorable for the formation of the Heyns rearrangement products (**HRP**s), and the gaseous phase reaction has been supposed to be more feasible than that of the aqueous phase reaction (Shipar, 2006). **DHA + UGly** reaction has been reported not to be feasible for the formation of the enol form of the Heyns rearrangement product **(HRPE)** in the aqueous state, and therefore, the rate of browning under these conditions has been assumed to be lower than that of the gaseous phase **DHA + UGly** reaction. Formation of **HRP**s and **HRP(DGly)**s has been reported as unfavorable in **DHA + PGly** and **DHA + GlyZ** reactions, respectively, and therefore, the reaction under these conditions has been assumed to be hindered, resulting in the lower browning rate than **DHA + UGly** and **DHA + DGly** reactions. Interconversion of **DHA** to **Gald** has been reported to be unfeasible, whereas interconversion of **Gald** to **DHA** has been stated to be more favorable in the gaseous state than in the aqueous state. Production of hydroxyacetaldehyde **(Hald)** from **DHA**, as a C2-fragmentation product, has been reported as more favorable in the aqueous phase reaction than that of the gaseous phase (Shipar, 2006).

The Maillard reaction can follow various pathways to produce melanoidines with different characteristics (Davídek et al., 1990; Eskin, 1990; Macrane et al., 1993). **HRP**s and/or **HRP**(**DGly**)s, produced in the initial stage of **DHA + Gly** Maillard reaction (Shipar, 2006), can be decomposed to deoxyosones in the intermediate stage of the reaction. In this paper, by following the Hodge-scheme (Hodge, 1953), and considering the formation of **MG** as one of the possible intermediates, mechanisms for the intermediate stage of **DHA + Gly** Maillard reaction under different pH conditions are proposed. Density Functional computations have been performed to evaluate the possibility of the formation of different compounds in the proposed mechanisms through calculation of the Gibb's free energy changes (ΔG^0). The total mass balance for different steps of the reaction has been maintained during the calculation of ΔG^0. To observe the possible internal energy changes, electronic energy changes (ΔE^0) for the formation of different compounds in different steps of the reaction have also been calculated by following the total mass balance of the reaction.

In the intermediate stage of **DHA + Gly** reaction, **HRPE** and/or **HRPE**(**DGly**) can be decomposed into 3-deoxyosones via 1,2-enolization, and **HRPK** and/or **HRPK**(**DGly**) can be decomposed into 1-deoxyosones via 2,3-enolization. In glucose–amino compound systems, the l,2-enolization or 3-deoxyosone route is appeared more important for the formation of brown colors, whereas the 2,3-enolization or 1-deoxyosone route results in the formation of flavor products (Hurrell, 1984). The 3-deoxyosone route in glucose–amino compound systems is not only related to the production of brown compounds, but also related to the formation of off-flavors, which may be a factor in stored, overheated, or dehydrated food products (Davídek et al., 1990; Hurrell, 1984; Macrane et al., 1993). In **DHA + UGly** reaction, the 3-deoxyosone route (Fig. 1) may involve the elimination of **UGly** from **HRPE**, leading to the formation of the enol form of 3-deoxyosone, **3DE** (2-hydroxy-prop-l-al-3-ene). Through keto-enolic tautomerization (**KET**), **3DE** can be rearranged to its more stable keto form, **3DK**, or methyl glyoxal, **MG**

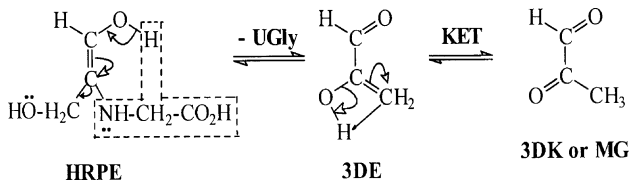

Fig. 1. Proposed mechanism for the formation of methyl glyoxal through the 3-deoxyosone route in the intermediate stage of **DHA + UGly** reaction. *Abbreviations*: **DHA**, dihydroxyacetone (1,3-dihydroxy-prop-2-one); **UGly**, unionized or unprotonated glycine; **PGly**, protonated glycine; **HRPE**, enol form of the Heyns rearrangement product (2-glycino-1,3-dihydroxy-prop-1-ene); **3DE**, enol form of 3-deoxyosone (2-hydroxy-prop-1-al-3-ene); **3DK**, keto form of 3-deoxyosone (2-keto-propanal-1 or prop-1-al-2-one); **MG**, methyl glyoxal (2-keto-propanal-1 or prop-1-al-2-one) = **3DK**; **KET**, keto-enolic tautomerization. The same mechanism is followed for the 3-deoxyosone route in the intermediate stage of **DHA + PGly** reaction in which the eliminated **UGly** is instantly protonated to **PGly,** and mass–energy balance is maintained accordingly during the calculation of ΔG^0 and ΔE^0.

(2-keto-propanal-l or prop-l-al-2-one or pyruvaldehyde). **HRPE** can also be formed in the initial stage of the **DHA + PGly** reaction (Shipar, 2006), Therefore, it is possible that the 3-deoxyosone route in the intermediate stage of the **DHA + PGly** reaction follows the same mechanism proposed for the 3-deoxyosone route of the **DHA + UGly** reaction (Fig. 1). The only difference is that the eliminated **UGly** will instantly be protonated to **PGly** in the **DHA + PGly** reaction. As the reactants and products in **DHA + UGly** and **DHA + PGly** reactions are different, the total mass balances, related to the energy changes, will differ from each other. This difference in the total mass balance has been maintained accordingly during the calculation of ΔG^0 and ΔE^0.

In the 3-deoxyosone route of the **DHA + DGly** reaction (Fig. 2), elimination of **DGly** from **HRPE(DGly)** can lead to the production of **3DE**, which can subsequently be rearranged to **Mg** through **KET**. As **HRPE(DGly)** can be formed in the initial stage of the **DHA + GlyZ** reaction (Shipar, 2006), the 3-deoxyosone route in the intermediate stage of the **DHA + GlyZ** reaction can follow the same mechanism proposed for the 3-deoxyosone route of the **DHA + DGly** reaction (Fig. 2). However, the eliminated **DGly** will immediately be protonated to **GlyZ** in the **DHA + GlyZ** reaction, and due to the difference in reactants and products in **DHA + DGly** and **DHA + GlyZ** reactions, the total mass balances, related to the energy changes, will differ from each other, which have been maintained consequently during the calculation of ΔG^0 and ΔE^0.

The 1-deoxyosone route in the intermediate stage of **DHA + UGly** reaction (Fig. 3) may involve the elimination of **UGly** from **HRPK** to form the enol form of 1-deoxyosone, **IDE**. Interestingly, **IDE** is same as **3DE**, produced through the 3-deoxyosone route (Fig. 1). Through KET, **IDE** can be rearranged to its more stable keto form, **1DK** (Fig. 3). Notably, **1DK** is same as **3DK** or **MG**, produced through the 3-deoxyosone route (Fig. 1). **HRPK** can be produced in the initial stage of **DHA + PGly** reaction (Shipar, 2006), and therefore the 1-deoxyosone route in the intermediate stage of **DHA + PGly** reaction can follow the same mechanism for the 1-deoxyosone route of **DHA + UGly** reaction (Fig. 3). However, the eliminated **UGly** will instantly be protonated to **PGly** in the **DHA + PGly** reaction. Due to the different reactants and products, the total mass balances, related to the energy changes, will be different for **DHA + UGly** and **DHA + UGly** reactions, which have been maintained accordingly during the calculation of ΔG^0 and ΔE^0.

In the 1-deoxyosone route of **DHA + DGly** reaction (Fig. 4), **DGly** can be eliminated from **HRPK(DGly)**, resulting in the formation of **IDE**, which can consequently be rearranged to **MG** through **KET**. The mechanism of the 1-deoxyosone route of **DHA + DGly** reaction (Fig. 4) can also be applicable to the **DHA + GlyZ** reaction as **HRPK(DGly)** is produced in the initial stage of the **DHA + GlyZ** reaction (Shipar, 2006). However, the eliminated **DGly** will instantly be protonated to

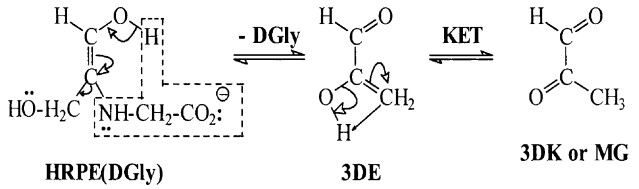

Fig. 2. Proposed mechanism for the formation of methyl glyoxal through the 3-deoxyosone route in the intermediate stage of **DHA + DGly** reaction. *Abbreviations*: **DGly**, deprotonated glycine; **GlyZ**, glycine zwitterion; **HRPE(DGly)**, enol form of the Heyns rearrangement product (2-deprotonated glycino-1,3-dihydroxy-prop-l-ene). For other abbreviations, see the caption of Fig. 1. The same mechanism is followed for the 3-deoxyosone route in the intermediate stage of **DHA + GlyZ** reaction in which the eliminated **DGly** is instantly protonated to **GlyZ**, and mass–energy balance is maintained accordingly during the calculation of ΔG^0 and ΔE^0.

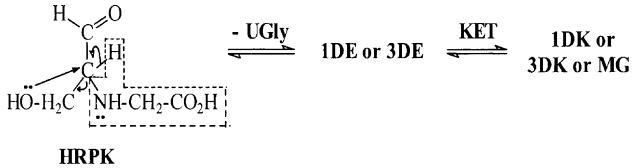

Fig. 3. Proposed mechanism for the formation of methyl glyoxal through the 1-deoxyosone route in the intermediate stage of **DHA + UGly** reaction. *Abbreviations*: **HRPK**, keto form of the Heyns rearrangement product (2-glycino-3-hydroxy-prop-l-al); **IDE**, enol form of 1-deoxyosone (2-hydroxy-prop-l-al-3-ene) = **3DE**; **1DK**, keto form of 1-deoxyosone (2-keto-propanal-1 or prop-1-al-2-one) = **3DK**. For other abbreviations, see the caption of **Fig. 1.** The same mechanism is followed for the 1-deoxyosone route in the intermediate stage of **DHA + PGly** reaction in which the eliminated **UGly** is instantly protonated to **PGly,** and mass–energy balance is maintained accordingly during the calculation of ΔG^0 and ΔE^0.

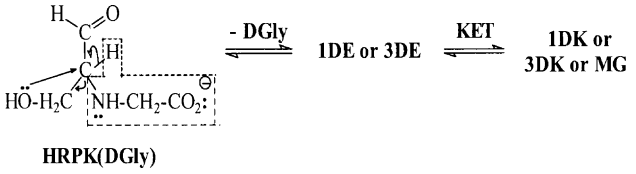

Fig. 4. Proposed mechanism for the formation of methyl glyoxal through the 1-deoxyosone route in the intermediate stage of **DHA + UGly** reaction. *Abbreviations*: **HRPK(DGly)**, keto form of the Heyns rearrangement product (2-deprotonated glycino-3-hydroxy-prop-1-al). For other abbreviations, see the captions of Figs. 1 and 2. The same mechanism is followed for the 3-deoxyosone route in the intermediate stage of **DHA + GlyZ** reaction in which the eliminated **DGly** is instantly protonated to **GlyZ**, and mass–energy balance is maintained accordingly during the calculation of ΔG^0 and ΔE^0.

GlyZ in the **DHA + GlyZ** reaction. The difference in the reactants and products for **DHA + DGly** and **DHA + GlyZ** reactions will follow different total mass balances, which have been maintained accordingly during the calculation of ΔG^0 and ΔE^0.

2. Methodology

At the standard state, all compounds in the proposed mechanisms (Figs. 1–4) have been studied in their gaseous and aqueous phases by using a Gaussian 98 program (Becke, 1992a, 1992b, 1993, 1996; Foresman & Frisch, 1996; Frisch & Foresman, 1998; Hehre, Radom, Schleyer, & Pople, 1986; Larid, Ross, & Ziegler, 1996; Miertus & Tomasi, 1982; Miertus, Scrocco, & Tomasi, 1981; Salahub et al., 1991; Springborg, 1995; Young, 2001). Only general optimized structures of the compounds at a specific method (RB3LYP) [Becke, 1992a, 1992b, 1993, 1996; Foresman and Frisch, 1996; Frisch and Foresman, 1998; Hehre et al., 1986] have been used in both of the gaseous and aqueous phases to avoid complexities and simplify the calculation (Shipar, 2006). A 6-31G(d) polarized basis set (Foresman & Frisch, 1996; Frisch & Foresman, 1998; Hehre et al., 1986; Young, 2001) has been used for all calculations. During the optimization, all structural parameters, e.g., bond-lengths, bond-angles and dihedral angles, have been used as variables. The term "Stationary point found" has been verified in the geometry output to confirm the ground state (Foresman & Frisch, 1996; Frisch & Foresman, 1998). During the optimization, special care has been taken to avoid the possible intramolecular cyclization of the compounds (Shipar, 2006). All RB3LYP/6-31G(d) optimized structures have been taken for RB3LYP/6-31G(d) and PCM/RB3LYP/6-31G(d) frequency calculations to achieve the relevant zero point energy (ZPE) and the Gibb's free energy (G^0) in the gaseous and aqueous states, respectively (Foresman & Frisch, 1996; Frisch & Foresman, 1998). Opt. = *Z*-matrix and/or Freq. = NoRaman keywords have been used when necessary (Foresman & Frisch, 1996; Frisch & Foresman, 1998). Single point energy calculations in the gaseous and aqueous states have been performed on the RB3LYP/6-31G(d) optimized structures by using RB3LYP/6-31G(d) and PCM/RB3LYP/6-31G(d), respectively (Foresman & Frisch, 1996; Frisch & Foresman, 1998). The dielectric constant, $\varepsilon = 78.39$ has been used to represent the aqueous solution (Foresman & Frisch, 1996; Frisch & Foresman, 1998). Relevant ZPE have been added to the single point energies to get the total electronic energies (E^0). Finally, electronic and free energy changes ($\Delta E^0 = E^0_{\text{Product(s)}} - E^0_{\text{Reactant(s)}}$, and $\Delta G^0 = G^0_{\text{Product(s)}} - G^0_{\text{Reactant(s)}}$, respectively) for different compounds in the proposed mechanisms (Figs. 1–4) have been calculated by following the total mass balance of the reaction. Thus, the possibility and internal energy changes for the formation of different compounds in the proposed mechanisms have been investigated.

3. Results and discussion

ΔE and ΔG of any reaction are important as they provide for the prediction of the internal energy changes and spontaneity of the reaction at constant temperatures and pressures, respectively. ΔG^0 for the formation of different compounds in the proposed mechanisms for the intermediate stage of the **DHA + Gly** reaction under different pH conditions (Figs. 1–4) are presented in Table 1, calculated by using **DHA + Gly** total free energies ($G^0_{\text{DHA}} + G^0_{\text{UGly/PGly/DGly/Glyz}}$) as the standard in the equation $\Delta G^0 = G^0_{\text{Product(s)}} - G^0_{\text{Reactant(s)}}$ (Shipar, 2006). ΔE^0 for the formation of different compounds in the proposed mechanisms are presented in Table 2, calculated by using **DHA + Gly** total electronic energies ($E^0_{\text{DHA}} + E^0_{\text{UGly/pGly/DGly/Glyz}}$) as the standard in the equation $\Delta E^0 = E^0_{\text{Product(s)}} - E^0_{\text{Reactant(s)}}$ (Shipar, 2006). The total mass balance of any reaction is important as it is related to the energy changes. The total mass balance of the intermediate stage of **DHA + Gly** reaction under different pH conditions has been maintained during the calculation of ΔE^0 and ΔG^0. The main problem in balancing the total mass arises for protonation ($+H^+$) and/or deprotonation ($-H^+$), particularly during the protonation of **UGly** and **DGly** to **PGly** and **GlyZ**, respectively. Therefore, the following equation has been used during balancing the total mass:

$$\underset{\text{Proton}}{H^+} + \underset{\text{water}}{H_2O} \leftrightarrow \underset{\text{hydroxoniumion}}{H_3O^+}$$

(protonation and deprotonation)

Based on ΔG^0, formation of deoxyosones, i.e., **3DE** or **1DE** (will be indicated as **DE** in advance), and **3DK** or **1DK** or **MG** (will be indicated as only **MG** in advance) is plausible in both of the gaseous and aqueous

Table 1
ΔG^0 (in kJ/mol) for the formation of different compounds in the intermediate stage of **DHA + Gly** Maillard reaction under different conditions, calculated by using **DHA + Gly** total free energies ($G^0_{DHA} + G^0_{UGly/PGly/DGly/GlyZ}$) as the standard in the equation $\Delta G^0 = G^0_{product(s)} - G^0_{Reactant(s)}$

Reactions →	**DHA + UGly**		**DHA + PGly**	
	Gaseous	Aqueous	Gaseous	Aqueous
HRPE (Shipar, 2006)	−0.54	10.0	192.5	64.4
HRPK (Shipar, 2006)	−48.1	−36.0	145.0	82.4
1DE or **3DE**	−17.7	−11.1	−17.4	−11.0
1DK or **3DK** or **MG**	−55.5	−45.3	−55.2	−45.2
	DHA + DGly		**DHA + GlyZ**	
HRPE(DGly) (Shipar, 2006)	−127.8	−44.7	597.8	132.8
HRPK(DGly) (Shipar, 2006)	−130.0	−82.5	595.7	95.2
1DE or **3DE**	−17.8	−11.2	−17.2	−10.8
1DK or **3Dk** or **MG**	−55.8	−45.5	−55.0	−45.0

For abbreviations and details of the compounds, see the captions and mechanisms of Figs. 1–4.

Table 2
ΔE^0 (in kJ/mol) for the formation of different compounds in the intermediate stage of **DHA + Gly** Maillard reaction under different conditions, calculated by using **DHA + Gly** total electronic energies ($E^0_{DHA} + E^0_{UGly/PGly/DGly/GlyZ}$) as the standard in the equation $\Delta E^0 = E^0_{product(s)} - E^0_{Reactant(s)}$

Reactions →	**DHA + UGly**		**DHA + PGly**	
	Gaseous	Aqueous	Gaseous	Aqueous
HRPE (Shipar, 2006)	−10.8	23.7	203.6	78.6
HRPK (Shipar, 2006)	−55.6	−24.1	158.8	30.8
1DE or **3DE**	20.8	27.4	25.1	18.2
1DK or **3DK** or **MG**	−13.3	−3.58	−10.1	−0.8
	DHA + DGly		**DHA + GlyZ**	
HRPE(DGly) (Shipar, 2006)	−83.0	16.6	585.6	120.7
HRPK(DGIy) (Shipar, 2006)	−80.6	−16.4	587.9	87.5
1DE or **3DE**	21.1	25.3	24.9	20.4
1DK or **3DK** or **MG**	−13.5	−3.6	−8.8	−2.4

For abbreviations and details of the compounds, see the captions and mechanisms of Figs. 1–4.

phase **DHA + Gly** reactions under all pH conditions (Table 1). This is consistent with the previous computational result for **Gald + Gly** reaction (Shipar, 2004). The possibility of the formation of **DE** and **MG** is assumed almost identical in the aqueous phase **DHA + Gly** and **Gald + Gly** reactions under all pH conditions, whereas this varies slightly in the gaseous phase **DHA + Gly** and **Gald + Gly** reactions. **DHA + Gly** gaseous phase reaction is assumed to be less favorable for the formation of **DE** and **MG** than **Gald + Gly** gaseous phase reaction. In producing **DE** and **MG**, the gaseous phase **DHA + Gly** reaction has been found to be more feasible than the aqueous phase **DHA + Gly** reaction under all pH conditions (Table 1), which is in agreement with the prior computational finding for the **Gald + Gly** reaction (Shipar, 2004). In both of the gaseous and aqueous states, **DHA + DGly** reaction has been found to be the most favourable for the formation of **DE** and **MG** (Table 1). This is also consistent with the previous report for **Gald + Gly** reaction that **Gald + DGly** reaction is the most favourable for the formation of **DE** and **MG** (Shipar, 2004). **DHA + UGly** reaction is assumed to be the second most favorable for the production of **DE** and **MG** (Table 1), which is also in favor with the early finding for **Gald + UGly** reaction (Shipar, 2004). However, the gaseous phase **DHA + UGly** reaction has been reported not to be favorable for the production of **HRPE** in the initial stage (Shipar, 2006), and therefore, formation of **DE** and **MG** under this condition is thought to be impeded. Formation of **HRP**s and **HRP(DGly)**s in the initial stage of **DHA + PGIy** and **DHA + GlyZ** reactions, respectively, has been stated as unfavorable in both of the gaseous and aqueous states (Shipar, 2006). Therefore, it can be postulated that **DHA + PGIy** and **DHA + GlyZ** reactions are less favorable for the formation of **DE** and **MG** than **DHA + UGly** and **DHA + DGly** reactions.

In both of the gaseous and aqueous states, formation of **MG** appears to be more favourable than the formation of **DE** (Table 1). According to ΔE^0 (Table 2), **DE** is electronically more stable in the gaseous state than the aqueous state **DHA + UGly** and **DHA + DGly** reactions, whereas more stable in the aqueous state than the gaseous state **DHA + PGly** and **DHA + GlyZ** reactions. **DE** may have an extreme role in the Maillard reaction. Adequate information on **DE** is still not available, and

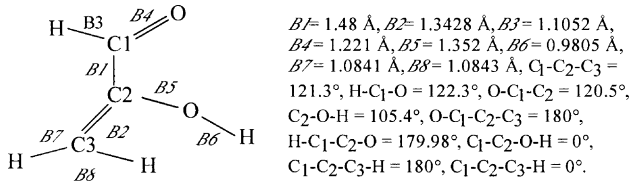

Fig. 5. RB3LYP/6-31G(d) geometric parameters of the enol form of 3- or 1-deoxyosone [**3DE** or **1DE** (2-hydroxy-prop-l-al-3-ene)].

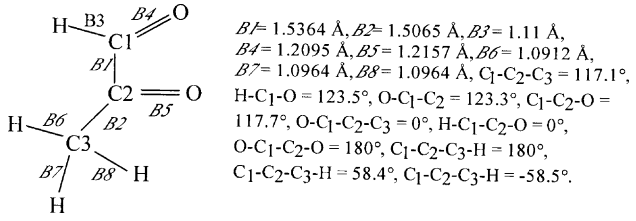

Fig. 6. RB3LYP/6-31G(d) geometric parameters of the keto form of 3- or 1-deoxyosone or methyl glyoxal [**3DK** or **1DK** or **MG** (2-keto-propanal-1 or prop-1-al-2-one or pyruvaldehyde)].

therefore extensive further theoretical and experimental investigations are necessary. On the other hand, **MG** is electronically more stable in the gaseous state reaction than in the aqueous state under all pH conditions (Table 2). In both of the gaseous and aqueous states, **MG** is electronically more stable than **DE** (Table 2). Formation of **MG** is assumed more favorable than the formation of **DE** (Table 1). Therefore, it can be proposed that **MG** is the most likely intermediate in the reaction. This is consistent with the previous computational result for the formation of **MG** in **Gald + GIy** reaction (Shipar, 2004). It is also consistent with other literature statements that **MG** is one of the most common intermediates in the Maillard reaction (Davídek et al., 1990; Eskin, 1990; Macrane et al., 1993; Reber et al., 2002; Uchida et al., 1997). RB3LYP/6-31G(d) geometric parameters of **HRP**s and **HRP**(**DGly**)s have been reported in the previous computational study on the initial stage of the **DHA** + **Gly** Maillard reaction (Shipar, 2006). Some important RB3LYP/6-31G(d) geometric parameters of **DE** and **MG**, used in the present study, are presented in Figs. 5 and 6, respectively. The dipole moments (μ) and heats of formation (ΔH_f^0) are presented in Table 3.

According to the total mass balance of the reaction, water is produced in excess during the formation of **DE** and/or **MG** under all pH conditions. One molecule of water has been found to be produced in excess during the initiation step of the reaction (Shipar, 2006). Actually, this water molecule has been reported as produced during the formation of **HRP**s and/or **HRP**(**DGly**)s in the initial stage (Shipar, 2006), and remains unchanged during the formation of **DE** and/or **MG** in the intermediate stage. From this finding, it can be assumed that though water is necessary for the initiation and production of **HRP**s and/or **HRP**(**DGly**)s in the initial stage of **DHA** + **Gly** reaction under different pH conditions, this requirement is not applicable for the production of **DE** and/or **MG** from **HRP**s and/or **HRP**(**DGly**)s in the intermediate stage.

4. Conclusion

By following the total mass balance for different steps of the reaction, density functional computations at the standard state have been performed on the proposed mechanisms for the intermediate stage of **DHA** + **Gly** reaction under different pH conditions to evaluate the possibility of the formation of different compounds by calculating ΔG^0. Internal energy changes for different compounds of the reaction have also been investigated by calculating ΔE^0 through following the total mass balance of the reaction. **MG** has been found to be the most likely intermediate in the reaction. The possibility for the formation of **MG** under different pH conditions is assumed almost identical in both of the gaseous and aqueous states. This assumption is also applicable to the formation of **DE**. However, adequate information on **DE** is still unavailable. In both of the gaseous and aqueous states, formation of **MG** is assumed more plausible than **DE**. In producing **MG**, the **DHA** + **DGly** reaction is supposed to be the most feasible. The **DHA** + **DGly** reaction has been reported to be favorable for the production of **HRPE**(**DGly**) and **HRPK**(**DGly**)s

Table 3
Dipole moments (μ, in Debye) and heats of formation (ΔH_f^0, in kJ/mol) of the Heyns rearrangement products and deoxyosones

Compounds	μ		ΔH_f^0 [AM1]
	Gaseous [RB3LYP/ 6-31G(d)]	Aqueous [PCM/RB3LYP/6-31G(d)]	
HRPE	2.98	4.134	−688.6
HRPE(**DGly**)	9.197	11.24	−805.247
HRPK	5.044	6.823	−692.4
HRPK(**DGly**)	6.61	8.659	−818.218
1DE or **3DE**	1.832	2.407	−1028.846
1DK or **3DK** or **MG**	0.953	1.118	−1132.191

For abbreviations and details of the compounds, see the captions and mechanisms of Figs. 1–4.

in the initial stage (Shipar, 2006). Therefore, possibility of the formation of **MG** in **DHA + DGly** reaction is assumed more plausible, resulting in higher rate of browning than that of the others. The **DHA + UGly** gaseous phase reaction has been reported not to be feasible for the production of **HRPE** in the initial stage (Shipar, 2006). Hence, formation of **MG** under this condition is assumed to be impeded. In the initial stage of the reaction, **DHA + PGly** and **DHA + GlyZ** reactions have been found not to be feasible for the formation of **HRP**s and **HRP**(**DGly**)s, respectively (Shipar, 2006). Therefore, the consequent formation of **MG** is assumed to be inhibited under these conditions, resulting in lower browning rate than in **DHA + UGly** and **DHA + DGly** reactions. However, adequate information is still not available, and therefore further widespread investigations are required. The global minima, maxima and transition states on the potential energy surfaces of **MG**, **DE** as well as other compounds are also necessary. **MG** possesses a great importance in the Maillard reaction in vivo as well as vitro (Davídek et al., 1990; Eskin, 1990; Fravel & McBrien, 1980; Hollnagel & Kroh, 1998; Macrane et al., 1993; Meade et al., 2003; Odani et al., 1998; Reber et al., 2002; Riley, 1980; Uchida et al., 1997; White & Rees, 1982). Therefore, appropriate mechanisms for the formation of **MG** in **DHA + Gly** and other Maillard reaction systems need to be evaluated, which will be helpful to find out proper techniques for controlling the production of **MG** through the Maillard reaction in vivo as well as vitro. Moreover, computational studies on the final stage of **DHA + Gly** reaction are also necessary to evaluate the possibility for the formation of melanoidines.

References

Becke, A. D. (1992a). Density-functional thermochemistry I, the effect of the exchange-only gradient correction. *Journal of Chemical Physics, 96*, 2155–2160.

Becke, A. D. (1992b). Density-functional thermochemistry II, the effect of the Perdew–Wang generalized-gradient correlation correction. *Journal of Chemical Physics, 97*, 9173–9177.

Becke, A. D. (1993). Density-functional thermochemistry III, the role of exact exchange. *Journal of Chemical Physics, 98*, 5648–5652.

Becke, A. D. (1996). Density-functional thermochemistry IV, a new dynamical correlation functional and implications for exact-exchange mixing. *Journal of Chemical Physics, 104*, 1040–1046.

Brown, D. A. (2001). Skin pigmentation enhancers. *Journal of Photochemistry and Photobiology B: Biology, 63*, 148–161.

Davídek, J., Velíšek, J., & Pokorný, J. (1990). *Development in food science: chemical changes during food processing* (Vol. 21). Amsterdam: Elsevier Science Publishers (pp. 117–152).

Eskin, N. A. E. (1990). *Biochemistry of foods* (pp. 239–296) (2nd ed.). San Diego: Academic Press Inc..

Feige, K., Ried, T., & Bachmann, K. (1996). Determination of formaldehyde by capillary electrophoresis in the presence of a dihydroxyacetone matrix. *Journal of Chromatography A, 730*, 333–336.

Foresman, J. B., & Frisch, A. (1996). *Exploring chemistry with electronic structure methods* (2nd ed.). Pittsburgh, USA: Gaussian, Inc..

Fravel, H. N. A., & McBrien, D. C. H. (1980). The effect of methyl glyoxal on cell division and the synthesis of protein and DNA in synchronous and asynchronous cultures of Escherichia coli B/r. *Journal of General Microbiology, 117*, 127–134.

Frisch, Æ, & Foresman, J. B. (1998). *Gaussian 98 user's reference*. Pittsburg, USA: Gaussian, Inc..

Harrold, H. (1991). *Organic chemistry. A short course* (8th ed.). Boston: Houghton Mifelin Company (pp. 425–467).

Hayashi, T., & Shibamoto, T. (1985). Analysis of methyl glyoxal in foods and beverages. *Agricultural and Biological Chemistry, 33*, 1090–1093.

Hehre, W. J., Radom, L., Schleyer, P. V. R., & Pople, J. A. (1986). *Ab initio molecular orbital theory*. New York: John Wiley & Sons.

Hirayama, T., Yamada, N., Nohara, M., & Fukui, S. (1984). The existence of the 1,2-dicarbonyl compounds glyoxal, methyl glyoxal and diacetyl in autoxidized edible oils. *Journal of the Science of Food and Agriculture, 37*, 1357–1362.

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry, 1*, 928–943.

Hollnagel, A., & Kroh, L. W. (1998). Formation of α-dicarbonyl fragments from mono- and disaccharides under caramelization and Maillard reaction conditions. *Zeitschrift fur Lebnsmittel-Untersuchung und-Forschung, 207*, 50–54.

Holum, J. R. (1996). *Introduction to organic and biological chemistry*. New York: John Wiley & Sons, Inc. (pp. 196–204).

Hurrell, R. F. (1984). Reactions of food proteins during processing and storage, and their nutritional consequences. In B. J. F. Hudson (Ed.). *Development in food proteins* (Vol. 3, pp. 213–244). London: Elsevier Applied Science.

Katarína, Š, Marica, K.-K., Reinhard, S., Veronika, F., Jana, K., & August, H. (2001). Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. *European Journal of Nutrition, 40*, 275–281.

Kobayashi, Y., & Takahashi, H. (1979). Conformational studies of glycolaldehyde and 1,3-dihydroxyacetone in solution by ^{1}H-NMR. *Specrochimica Acta A, 35*, 307–314.

Kobayashi, Y., Igarashi, T., Takahashi, H., & Higashi, K. (1976). Infrared and raman studies of 1,3-dihydroxyacetone, D(+)- and DL-glyceraldehyde. *Journal of Molecular Structure, 35*, 85–99.

Kurz, T. (1994). Formulating effective self-tanners with DFIA. *Cosmetics and Toiletries, 109*, 55–61.

Larid, B. B., Ross, R. B., & Ziegler, T. (1996). Density-functional methods in chemistry: an overview. In B. B. Larid, R. B. Ross, & T. Ziegler (Eds.), *Chemical applications of density-functional theory. American Chemical Society Symposium Series* (Vol. 629, pp. 1–19). Washington, DC: American Chemical Society.

Lozynski, M., Rusinska-Roszak, D., & Mack, H. G. (1997). MP2 and density functional studies of hydrogen bonding in model trioses: D-(+)-glyceraldehyde and dihydroxyacetone. *Journal of Physical Chemistry A, 101*, 1542–1548.

Macrane, R., Robinson, R. K., & Saadler, M. J. (1993). *Encyclopedia of food science, food technology and nutrition* (Vol. 1, pp. 146–166). London: Academic Press Limited.

Maillard, L. C. (1912). Action des acides aminés sur les sucres: Formation des mélanoides par voi méthodique. *Comptes rendus de l Academie des Sciences Serie I, 145*, 66–68.

Marnett, L. J., Kurd, H. K., Hollstein, M. C., Levin, D. E., Wsterbauer, H., & Ames, B. N. (1985). Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. *Mutation Research, 148*, 25–34.

Meade, S. J., Miller, A. G., & Gerrard, J. A. (2003). The role of dicarbonyl compounds in non-enzymatic crosslinking: a structure activity study. *Bioorganic and Medicinal Chemistry, II*, 853–862.

Mersch-Sundermann, V., Schneider, U., Klopman, G., & Rosencrantz, H. S. (1994). SOS induction in *Escherichia coli* and *Salmonella* mutagenicity: a comparison using 330 compounds. *Mutagenesis, 9*, 205–224.

Miertus, S., Scrocco, E., & Tomasi, J. (1981). Electrostatic interaction of a solute with a continuum: a direct utilization of ab initio molecular potentials for the prevision of solvent effects. *Chemical Physics, 55*, 117–129.

Miertus, S., & Tomasi, J. (1982). Approximate evaluations of the electrostatic free energy and internal energy changes in solution processes. *Chemical Physics, 65*, 239–245.

Morita, J. (1991). Induction of strand breakage in DNA by hexose derivatives and carbonyl compounds. *Agricultural and Biological Chemistry, 55*, 2407–2408.

Namiki, M., & Hayashi, T. (1983). A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In G. R. Waller & M. S. Feather (Eds.), *The Maillard reaction in foods and nutrition. American Chemical Society Symposium Series* (Vol. 215, pp. 1–46). Washington, DC: American Chemical Society.

Navrátil, M., Tkáč, J., Švitel, J., Danielsson, B., & Šturdik, E. (2001). Monitoring of the bioconversion of glycerol to dihydroxyacetone with immobilized *Gluconobacter oxydans* cell using thermometric flow injection analysis. *Process Biochemistry, 36*, 1045–1052.

Nitzsche, L. E., & Davidson, E. R. (1978). Ab initio studies of the $3n\pi^*$ states of glyoxal and methyl glyoxal. *Chemical Physics Letters, 58*, 171–174.

Odani, H., Shinzato, T., Usami, J., Matsumoto, Y., Frye, E. B., Baynes, J. W., et al. (1998). Imidazolium crosslinks derived from reaction of lysine with glyoxal and methyl glyoxal are increased in serum proteins of uremic patients: evidence for increased oxidative stress in uremia. *Federation of European Biochemical Societies Letters, 427*, 381–385.

Petersen, A. B., Wulf, H. C., Gniadecki, R., & Gajkowska, B. (2004). Dihydroxyacetone, the active browning ingredient in sunless tanning lotions, induces DNA damage, cell-cycle block and apoptosis in cultured HaCaT keratinocytes. *Mutation Research, 560*, 173–186.

Pham, H. N., DeMarini, D. M., & Brockmann, H. E. (1980). Mutagenicity of skin tanning lotions. *Journal of Environmental Pathology and Toxicology, 3*, 227–231.

Reber, F., Kasper, M., Siegner, A., Kniep, E., Seigel, G., & Funk, R. H. W. (2002). Alteration of the intracellular pH and apoptosis induction in a retinal cell line by the AGE-inducing agent glyoxal. *Graefe's Archive for Clinical and Experimental Ophthalmology, 240*, 1022–1032.

Riley, P. A. (1980). Inhibition of protein and DNA synthesis in tissue culture by a derivative of methyl glyoxal and ascorbate. *Chemico-Biological Interactions, 31*, 73–80.

Salahub, D. R., Fournier, R., Mlynarski, P., Papai, I., St-Amant, A., & Ushio, J. (1991). Gaussian-based density functional methodology, software, and applications. In J. K. Labanowski & J. W. Andzelm (Eds.), *Density functional methods in chemistry* (pp. 77–100). New York: Springer.

Shipar, M. A. H. (2004). Computational studies on glyceraldehyde and glycine Maillard reaction – II. *Journal of Molecular Structure – Theochem, 710*, 65–71.

Shipar, M. A. H. (2006). Formation of the Heyns rearrangement products in dihydroxyacetone and glycine Maillard reaction: A computational study. *Food Chemistry, 97*, 231–243.

Springborg, M. (1995). *Density-functional methods in chemistry and materials science*. New York: John Willy & Sons.

Stanko, R. T., & Adibi, S. A. (1986). Inhibition of lipid accumulation and enhancement of energy expenditure by the addition of pyruvate and dihydroxyacetone to a rat diet. *Metabolism, 35*, 182–186.

Szent-Györgyi, A., Együd, L. G., & McLaughlin, J. A. (1967). Keto-aldehydes and cell division: glyoxal derivatives may be regulators of cell division and open a new approach to cancer. *Science, 155*, 539–541.

Thornalley, P., Wolff, S., Crabbe, J., & Stern, A. (1984). The autoxidation of glyceraldehyde and other simple monosaccharides under physiological conditions catalysed by buffer ions. *Biochimica et Biophysica Acta, 797*, 276–287.

Thorpe, S. R. & Baynes, J. W. (2003). Maillard reaction products in tissue proteins: new products and new perspectives. *Amino acids*, Review Article.

Uchida, K., Khor, O. T., Oya, T., Osawa, T., Yasuda, Y., & Miyata, T. (1997). Proteien modification by a Maillard reaction intermediate methyl glyoxal immunochemical detection of fluorescent 5-methylimidazolone derivatives in vivo. *Federation of European Biochemical Societies Letters, 410*, 313–318.

White, J. S., & Rees, K. R. (1982). Inhibitory effects of methyl glyoxal on DNA, RNA and protein synthesis in cultured guinea pig keratocytes. *Chemico-Biological Interactions, 38*, 339–347.

Yamaguchi, T. (1982). Mutagenicity of trioses and methyl glyoxan on *Salmonella typhimurium*. *Agricultural and Biological Chemistry, 46*, 849–851.

Yaylan, V. A., Majors, S. H., & Ismail, A. A. (1999). Investigation of DL-glyceraldehyde–dihydroxyacetone interconversion by FTIR spectroscopy. *Carbohydrate Research, 378*, 20–25.

Young, D. C. (2001). *Computational chemistry: a practical guide for applying techniques to real world problems*. New York: John Wiley & Sons.

Zhu, Y., Youssef, D., Porte, C., Rannou, A., Ogletree, D., & Somarriba, B. L. M. L. (2003). Study of the solubility and the metastable zone of 1,3-dihydroxyacetone for the drowning-out process. *Journal of Crystal Growth, 257*, 370–377.