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Investigation of DL-glyceraldehyde–dihydroxyacetone interconversion by FTIR spectroscopy

Varoujan A. Yaylayan *, Susan Harty-Majors, Ashraf A. Ismail

Department of Food Science and Agricultural Chemistry, McGill University, 21,111 Lakeshore, Ste. Anne de Bellevue, Quebec H9X 3V9, Canada

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Abstract

Interconversion of dihydroxyacetone and DL-glyceraldehyde was studied in different solvents and temperatures by FTIR spectroscopy. Dissolution in water or triethylamine, and increasing temperatures caused the dissociation of the dimeric forms of both compounds into monomers and subsequently inter-conversion of dihydroxyacetone and DL-glyceraldehyde. Dioxane, on the other hand, did not initiate such inter-conversions. FTIR analysis in different solvents has also indicated that monomeric DL-glyceraldehyde can exist in two distinct intramolecularly H-bonded forms. A five-membered ring form was predominant in aqueous solutions of the dissociated DL-glyceraldehyde dimer, whereas a six-membered ring form was preferred in triethylamine solution or in aqueous solution of dissociated dihydroxyacetone dimer. However, in aqueous solutions of DL-glyceraldehyde dimer, the five-membered ring conformation was slowly transformed into the six-membered ring form under slightly basic pH. In addition, dihydroxyacetone predominantly converted into the six-membered H-bonded conformation of glyceraldehyde when dissolved in water. This was attributed to the preferential formation of the *trans*- or *E*-enediol as an intermediate. Temperature-dependent spectra have also indicated that increasing the temperature favored the formation of glyceraldehyde in the aqueous equilibrium mixtures of dimeric DL-glyceraldehyde and dihydroxyacetone.

Keywords: FTIR analysis; Dissociation; Dimerization and interconversion of DL-glyceraldehyde and 1,3-dihydroxyacetone; *trans*-Enediol

1. Introduction

The enzymatic interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, through the common enediol intermediate, has been studied extensively [1] due to its importance in carbohydrate metabolism through triosephosphate isomerase (EC 5.3.1.1). The kinetic studies [2] have indicated that the enzyme increases the enolization rate of dihydroxyacetone phosphate by a factor of more than 10^9 over that of the uncatalyzed reaction in water. These studies have also indicated that the noncatalyzed rate of enolization of D-glyceraldehyde phosphate was 1.3×10^3 fold faster than that of 1,3-dihydroxyacetone phosphate at 30 °C and pH 7.0. However, the rate of conversion of the common enediol into D-glyceraldehyde phosphate was 3.4 times faster than its conversion into dihydroxyacetone. The equilibrium, however, favored the formation of thermodynamically more stable dihydroxyace-

* Corresponding author. Tel.: +1-514-398-7918; fax: +1-514-398-7977.

E-mail address: yaylayan@agradm.lan.mcgill.ca (V. A. Yaylayan)

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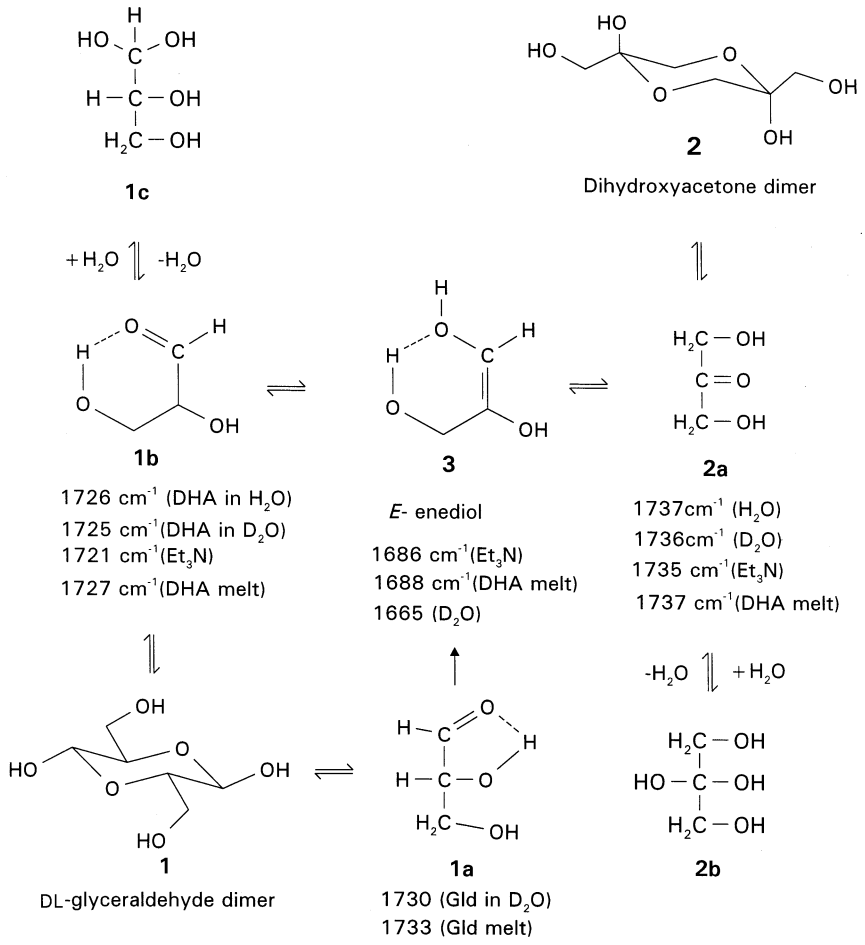
tone phosphate ($K_{eq} = 22$). Dihydroxyacetone and D-glyceraldehyde are the simplest keto and aldo sugars and are considered to be the cornerstone of carbohydrate chemistry. In addition, dihydroxyacetone is commercially important as the main component of sunless body-tanning preparations [3]. Fourier-transform infrared spectroscopy (FTIR) has been used to study the effect of temperature [4] on acyclic forms of D-fructose, mutarotation of D-glucose and D-fructose [5] and enolization and carbonyl group migration in selected sugars [6]. Kobayashi et al. [7] studied dimeric structures of DL-glyceraldehyde and dihydroxyacetone by infrared and Raman spectroscopy. Recently, the mechansim of dissociation of glycolaldehyde dimer was elucidated by FTIR analysis [8]. In this study the interconversion of dihydroxyacetone and glyceraldehyde was monitored by FTIR spectroscopy, and the effect of temperature and solvent was investigated.

2. Results and discussion

The study of the interconversion of DL-glyceraldehyde (Gld) and dihydroxyacetone (DHA) is complicated due to the presence of different species in the equilibrium mixture, generated through dimerization and enolization (see Scheme 1). Further complication of the composition of aqueous mixtures arises due to the ability of aldehydes and ketones to undergo rapid hydration, thus reducing drastically the intensity of the carbonyl absorption peaks during IR spectroscopic analysis. DL-Glyceraldehyde exists as cyclic hemiacetal (**1**) and dihydroxyacetone exists as cyclic hemiketal (**2**) in the solid state; however, they dissoci-



Scheme 1. Molecular transformations of dihydroxyacetone and DL-glyceraldehyde in aqueous solution. DHA = dihydroxyacetone, Gld = glyceraldehyde.

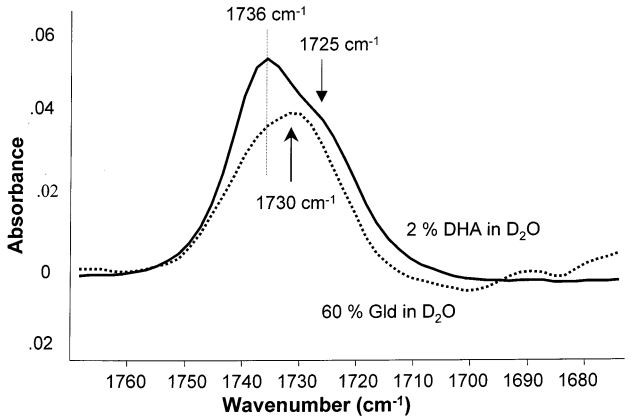


Fig. 1. Relative intensities of the carbonyl bands (1710–1760 cm^{-1}) of a 2% solution of dihydroxyacetone dimer in D_2O (solid line) and a 60% solution of DL-glyceraldehyde dimer (dotted line) in D_2O at 40 °C.

ate into monomeric forms upon dissolution in water or by heating. The rate of dimer to monomer conversion depends on the solvent and temperature. In Me_2SO and dioxane at room temperature, the conversions are very slow [9]. In addition, due to the stability of hemiacetal bonds relative to hemiketal, the extent of dissocation of DHA is much higher than that of Gld under the same conditions [7]. Monitoring the interconversion of DL-glyceraldehyde and dihydroxyacetone through the common enediol intermediate (**3**) also involves dimer-to-monomer conversions. NMR spectroscopic studies [9] have suggested that dimeric 1,3-dihydroxyacetone in solution has the structure of 2,5-dihydroxymethyl-2,5-dihydroxy-1,4-dioxane (**2**), and an X-ray diffraction study [10] indicated that dimeric DL-glyceraldehyde has the structure of 3,6-dihydroxymethyl-2,5-dihydroxy-1,4-dioxane (**1**).

Band assignments.—Freshly prepared solutions of dimeric DL-glyceraldehyde in D_2O show very weak carbonyl absorption bands, unlike dihydroxyacetone dimer solutions that show strong absorption in the carbonyl region even at room temperature. However, in glyceraldehyde solution—over time or through heating—carbonyl bands appear and increase in intensity indicating dissociation of dimeric forms. Fig. 1 compares the intensities of the carbonyl absorption band of a 60% DL-glyceraldehyde solution with that of a 2% dihydroxyacetone solution. Comparing the areas under the carbonyl region of both solutions indicates that an equivalent concentration of DHA dimer generates 45-fold more monomers than Gld under similar conditions. This is mainly attributed to the relative strengths of hemiketal versus hemiacetal bonds and also to the extensive hydration of the aldehyde groups relative to ketone. It has been estimated that 42% of dihydroxyacetone phosphate and 96.2% of glyceraldehyde-3-phosphate exist in hydrated forms at room temperature [11,12]. According to Davis [9] only 20% of dihydroxyacetone exists in hydrated form at room temperature. FTIR analysis of carbonyl absorption bands (see Table 1) of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate [1] also indicated that the former exhibits a carbonyl band centered at 1733 cm^{-1}, and the latter shows a carbonyl band centered at 1730 cm^{-1}. In addition, the carbonyl absorption band of dihydroxyacetone phosphate consisted of a two component peak, with maxima at about 1738 and 1730 cm^{-1} due to cisoid and transoid conformations of the phosphate group relative to the carbonyl. No such effect was observed for glyceraldehyde-3-phosphate. In addition, Reynolds et al. [12] reported a value of 1735 cm^{-1} for the carbonyl absorption band of dihydroxyacetone phosphate. It is expected that the unphosphorylated analogs should exhibit similar absorption frequencies for the carbonyl absorption bands.

The interconversion of DHA and Gld and assignment of the resulting carbonyl bands due to enolization can be best verified under basic conditions. Gld in triethylamine (TEA) showed two carbonyl peaks one centered at 1721 and the other at 1735 cm^{-1}. DHA, on the other hand, showed the same two peaks in different relative intensities. Tentatively, the peak at 1721 cm^{-1} was assigned to the alde-

Table 1
Comparison of carbonyl absorption bands (cm^{-1}) of phosphorylated and unphosphorylated glyceraldehyde (Gld) and dihydroxyacetone (DHA) in D_2O

	Phosphorylated	Unphosphorylated
DHA	1733 [a], 1735 [b]	1736 [c]
Gld	1730 [a]	1730 [c]

[a] Ref. [1].
[b] Ref. [12].
[c] Present work.

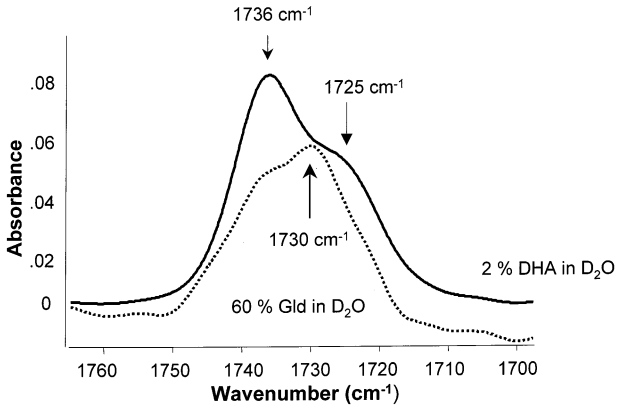
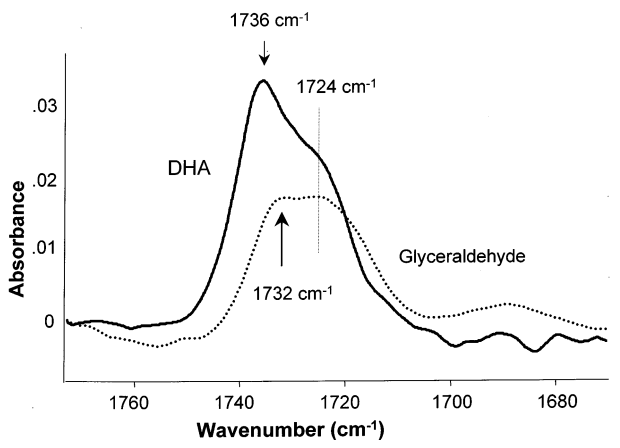


Fig. 2. Fourier self-deconvoluted (bandwidth 9.6, enhancement factor 1.4) carbonyl bands (1710–1750 cm^{-1}) of a 2% solution of dihydroxyacetone dimer in D_2O (solid line) and a 60% solution of DL-glyceraldehyde dimer (dotted line) in D_2O at 40 °C.

hyde band of glyceraldehyde, and the peak at 1735 cm^{-1} was assigned to the ketone band of DHA based on the presence of two α-hydroxyl groups in DHA, since the introduction of electron-withdrawing groups such as hydroxyl at the α-carbons is known to lead to a shift of the carbonyl stretching frequency to higher values relative to simple alkyl-substituted carbonyl compounds, provided that the hydroxyl group can rotate to eclipse the carbonyl group. The magnitude of this shift depends on the torsional angle. This effect was demonstrated in monosaccharides [6] by observing a shift to a higher frequency in the carbonyl absorption bands of different sugars relative to their α-deoxy derivatives. At equilibrium in TEA, glyceraldehyde dimer dissociated into 60% DHA and 40% monomeric glyceraldehyde based on peak areas. On the other hand, DHA dimer dissociated into 65% free DHA and 35% Gld. This observation confirms the fact that thermodynamically more stable DHA predominates in the equilibrium mixture of both solutions at room temperature. Visual inspection of the carbonyl absorption band of Gld equilibrated in D_2O indicates the presence of a wide peak centered at 1730 cm^{-1} and a shoulder at 1736 cm^{-1}. In DHA, the carbonyl band was centered at 1736 cm^{-1}, but the shoulder was shifted from the expected value of 1730 cm^{-1} observed in Gld solution to 1725 cm^{-1}. The presence of the two peaks were verified by second-derivative analysis and by Fourier self-deconvolution studies (Fig. 2). When both compounds were analyzed in the absence of water (as a melt), DHA exhibited a wide band whose second-derivative spectrum showed a major peak at 1737 and a shoulder at 1727 cm^{-1}. On the other hand, the Gld melt exhibited a single symmetrical peak at 1730 cm^{-1}. Based on the above observations, the band at 1736 cm^{-1} was assigned to the DHA, and the band at 1730 cm^{-1} was assigned to the Gld in D_2O. The shift in the absorption of the glyceraldehyde band observed in the equilibrated DHA solution was first attributed to the incorporation of deuterium at C-1 during the conversion of DHA to Gld through the enediol intermediate. However, analysis of the spectra of both compounds in H_2O eliminated this possibility. The shift to lower frequency of the Gld band observed in the DHA solution can be rationalized by the existence of two distinct conformations of glyceraldehyde, each stabilized by intramolecular H-bonding: one in which the carbonyl group is in staggered conformation with respect to the α-hydroxyl group and the other in which it is in eclipsed or near-eclipsed conformation, thus causing a shift to a higher frequency for the carbonyl absorption band. In a similar fashion, the existence of two distinct cisoid and transoid conformations of dihydroxyacetone phosphate has been reported, thus changing the orientation of the carbonyl group relative to the phosphate with subsequent shift in the carbonyl stretching frequencies [1]. Such two conformations of DL-glyceraldehyde are shown in Scheme 1 (structures **1a** and **1b**). Structure **1a** is a five-membered ring formed through intramolecular H-bonding between the C-2 hydroxyl hydrogen and the carbonyl carbon, and structure **1b** is a six-membered ring formed through intramolecular H-bonding between C-3 hydroxyl hydrogen and the carbonyl carbon. The absorption band of glyceraldehyde at 1730 cm^{-1} was assigned to structure **1a**, since the α-hydroxyl group is in near-eclipsed orientation. This assignment is consistent with the reported value of 1730 cm^{-1} [1] for the absorption of glyceraldehyde-3-phosphate, which is unable to attain a conformation similar to **1b**. The fact that DHA is predominantly converted into glyceraldehyde

1b indicates that the intermediate enediol somehow directs the formation of the six-membered ring structure **1b**. This could be achieved through the formation of more stable *trans*-enediol (**3**) or *E*-enediol versus *cis*-enediol (or *Z*-enediol) as shown in Scheme 1. To provide further evidence for this hypothesis, a solution of DL-glyceraldehyde dimer was equilibrated after the addition of one drop of TEA. FTIR analysis indicated the presence of two glyceraldehyde bands, one centered at 1732 and the other at 1724 cm^{-1} (see Fig. 3). This indicates that under the experimental conditions and in D_2O alone, the equilibrium was shifted continuously towards the formation of DHA with negligible amount of reversal. However, when TEA was added to this aqueous solution, faster rates of enolization caused an appreciable extent of equilibrium reversal and formation of **1b**. Similarly, when the enolization of DHA was accelerated by the addition of TEA, only one glyceraldehyde peak centered at 1722 cm^{-1} was observed, strongly suggesting that *trans*-enediol preferentially ketonizes into conformation **1b**.

Effect of temperature on the interconversion in aqueous solution.—To study the effect of temperature on the interconversion of dihydroxyacetone and DL-glyceraldehyde, the sample solutions were subjected to heating and cooling cycles between 30 and 80 °C. The initial temperature (30 °C) of the cell was raised by 1 °C per min, and every 5 min the temperature was kept constant for 15 min to record the spectra. During the heating cycle, the carbonyl bands in both dihydroxyacetone and DL-glyceraldehyde solutions increased and decreased during the cooling cycle, indicating the reversibility of dimerization (see Fig. 4). Analysis of the temperature-dependent second-derivative spectra of DL-glyceraldehyde indicated that at higher temperatures, the intensities of both DHA and Gld peaks increased; however, the increase of the intensity of dihydroxyacetone peak at 1736 cm^{-1} relative to the glyceraldehyde peak at 1730 cm^{-1} was much slower. This could be explained by the fact that at lower temperatures, most of the glyceraldehyde dimer still remained in dimeric form, that increasing the temperature accelerated dissociation of the dimer, and that the rate of dissociation of the dimer in aqueous solution was faster than the rate of enolization of Gld. On the other hand, in dihydroxyacetone solution, again, increasing the temperature increased the intensities of both DHA and Gld carbonyl peaks; however, the intensity of the Gld peak increased at a faster rate relative to DHA, especially after 50 °C. This indicates that the dimeric DHA is almost completely dissociated even at lower temperatures. Increasing the temperature, therefore, will not appreciably increase the DHA band relative to Gld.



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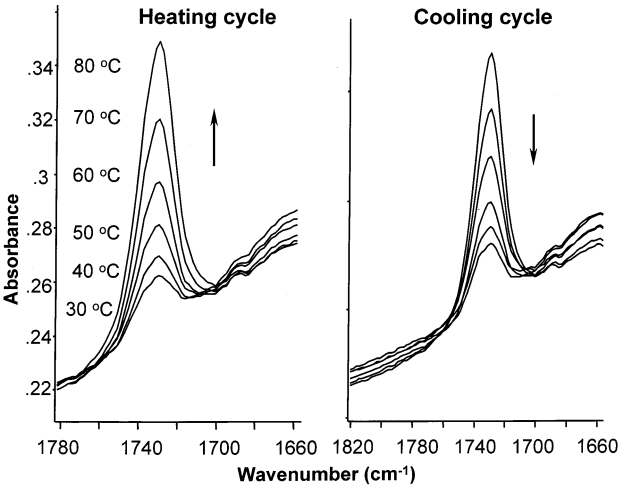

Fig. 4. Effect of heating (30–80 °C) and cooling (80–30 °C) cycles on the intensity of carbonyl absorption bands (1700–1780 cm^{-1}) of a 20% solution of DL-glyceraldehyde dimer in D_2O.

3. Experimental

All reagents and chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI) and used without further purification. All solvents used were of HPLC grade. D_2O was purchased from MSD Isotopes (Montreal, Canada).

FTIR analysis.—Infrared spectra were recorded on a Nicolet 8210 Fourier-transform infrared spectrometer purged with dry air and equipped with a deuterated triglycine sulfate (DTGS) detector. The spectra were acquired on a CaF_2 IR cell with a 25-μm Teflon spacer at room temperature unless otherwise specified. A total of 128 scans at 4 cm^{-1} resolution were co-added. The samples were equilibrated for 1 h before recording the spectra. Processing of the FTIR data was performed using GRAMS/386 version 3.01. Second-order derivatization was performed using the Savitsky–Golay function (9 points).

Temperature studies.—Sample solutions (10%) were placed in a CaF_2 IR cell with a 25-μm Teflon spacer. The temperature of the sample was regulated by placing the IR cell in a temperature-controlled cell holder. Infrared spectra were recorded as described above. The initial temperature of the cell was raised by 1 °C per min, and every 5 min the temperature was kept constant for 15 min to record the spectra. A total of 128 scans at 4-cm^{-1} resolution were co-added.

Acknowledgements

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References

[1] J.G. Belasco, J.R. Knowles, *Biochemistry*, 19 (1980) 472–477.
[2] A. Hall, J.R. Knowles, *Biochemistry*, 14 (1975) 4348–4352.
[3] J.A. Johnson, R.M. Fusaro, in T.B. Labuza, G. Reineccius, V.M. Monnier, J.W. Baynes (Eds.), *Maillard Reactions in Chemistry, Food and Health*, Royal Society of Chemistry, Cambridge. 1994, pp. 114–119.
[4] V.A. Yaylayan, A.A. Ismail, *J. Carbohydr. Chem.*, 11 (1992) 149–158.
[5] D.M. Back, D.F. Michalska, P.L. Polavarpu, *Appl. Spectrosc.*, 38 (1984) 173–178.
[6] V.A. Yaylayan, A.A. Ismail, *Carbohydr. Res.*, 276 (1995) 253–265.
[7] Y. Kobayashi, T. Igarashi, H. Takahashi, K. Higasi, *J. Mol. Struct.*, 35 (1976) 85–99.
[8] V.A. Yaylayan, S. Harty-Majors, A.A. Ismail, *Carbohydr. Res.*, 309 (1998) 31–38.
[9] L. Davis, *Bioorg. Chem.*, 2 (1973) 197–201.
[10] M. Senma, Z. Taira, K. Osaki, T. Taga, *J. Chem. Soc., Chem. Commun.*, (1973) 880–881.
[11] G.R. Gray, R. Barker, *Biochemistry*, 9 (1970) 2454–2462.
[12] S.J. Reynolds, D.W. Yates, C.I. Pogson, *Biochem. J.*, 122 (1971) 285–295.