Carbohydrate Research 389 (2014) 18–24

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Chemical and enzymatic methodologies for the synthesis of enantiomerically pure glyceraldehyde 3-phosphates

Dominik Gauss, Bernhard Schoenenberger, Roland Wohlgemuth *

Sigma–Aldrich GmbH, Industriestrasse 25, 9471 Buchs, Switzerland

ARTICLE INFO

Article history:
Received 14 September 2013
Received in revised form 21 December 2013
Accepted 27 December 2013
Available online 10 January 2014

Keywords:
Glycolysis
Glycerokinase
Enzymatic phosphorylation
D-Glyceraldehyde 3-phosphate
L-Glyceraldehyde 3-phosphate

ABSTRACT

Glyceraldehyde 3-phosphates are important intermediates of many central metabolic pathways in a large number of living organisms. D-Glyceraldehyde 3-phosphate (D-GAP) is a key intermediate during glycolysis and can as well be found in a variety of other metabolic pathways. The opposite enantiomer, L-glyceraldehyde 3-phosphate (L-GAP), has been found in a few exciting new pathways. Here, improved syntheses of enantiomerically pure glyceraldehyde 3-phosphates are reported. While D-GAP was synthesized by periodate cleavage of D-fructose 6-phosphate, L-GAP was obtained by enzymatic phosphorylation of L-glyceraldehyde. ^{1}H- and ^{31}P NMR spectroscopy was applied in order to examine pH-dependent behavior of GAP over time and to identify potential degradation products. It was found that GAP is stable in acidic aqueous solution below pH 4. At pH 7, methylglyoxal is formed, whereas under alkaline conditions, the formation of lactic acid could be observed.

1. Introduction

The development of biochemistry is very much related to key discoveries made in the enzymatic reactions with D-glucose in yeast extract by Hans and Eduard Buchner,[1] Arthur Harden, and William Young[2] as well as in muscle extracts by the classical work of Gustav Embden,[3] Otto Meyerhof,[4] Carl Neuberg,[5] Jacob Parnas,[6] Otto Warburg,[7] Gerty and Carl Cori.[8] The sequence of enzymatic reaction steps for the complete anaerobic D-glucose catabolic pathway in eukaryotes, with the corresponding chemical structure of the metabolites, has been established with the Embden–Meyerhof–Parnas pathway (EMP). The natural variety of alternative D-glucose metabolic pathways[9] is of continuing interest in prokaryotes, with the Entner–Doudoroff pathway (ED) as the most common, as well as in archaea, since in a phylogenetic analysis of genomes the genes of 30% of the organisms could not be annotated to either the EMP- or the ED-pathway.[10] In order to elucidate the reaction topologies and protein functions under different conditions, especially when branchpoints are involved, the preparation of the corresponding enzyme substrates is important. The availability of all relevant metabolites from the different glycolytic pathways in pure and stable form as well as comprehensive knowledge about their stabilities has been a challenge up to the present. The central metabolite D-glyceraldehyde 3-phosphate (D-GAP), shared by the EMP and ED pathways in lower glycolysis, plays a role not only in glycolysis and gluconeogenesis, but in a variety of additional metabolic pathways like for example the methylerythritol phosphate (MEP) pathway,[11,12] photosynthetic carbon fixation,[13,14] the shikimate pathway,[15,16] clavulanic acid biosynthesis,[17] and thiamine metabolism.[18] In contrast, the L-glyceraldehyde 3-phosphate (L-GAP) has not been found to play the corresponding role in a L-glucose catabolic pathway in *Paracoccus* sp.[19] and the discovery of YghZ in *Escherichia coli* catalyzing the stereospecific reduction of L-GAP to L-Glycerol 3-phosphate[20] has provided the first evidence of a metabolic bypass for the removal of toxic L-GAP.

Otto Warburg observed already in 1924 that cancer cells metabolize glucose into lactate even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation,[21–23] a finding which has obtained renewed attention.[24,25]

The first synthesis of racemic glyceraldehyde 3-phosphate, later to be known as Fischer–Baer ester, had a considerable effect on the development of the Embden–Meyerhof scheme and enabled studies of its properties.[26–28] Since D- and L-glyceraldehyde 3-phosphates have different biological properties, the synthesis of pure enantiomers is highly important.[29–31] Whereas there are numerous papers dealing with syntheses of racemic GAP, the number of published protocols leading to enantiomerically pure or enriched D- or L-GAP is small and all of them have drawbacks which make them unattractive for lab scale production purposes.

The oldest published preparative formation of D-GAP is a lengthy multistep synthesis using mercury salts in one of the deprotection steps.[29] An attractive one step synthesis[31] starting with D-fructose 6-phosphate does not consider the pH lability of the product and

* Corresponding author. Tel.: +41 81 7552640; fax: +41 81 7552840.
E-mail address: roland.wohlgemuth@sial.com (R. Wohlgemuth).

http://dx.doi.org/10.1016/j.carres.2013.12.023
0008-6215/

gave in our hands varying yields and product purities. Another oxidative degradation of D-fructose 6-phosphate uses toxic and teratogenic lead-tetraacetate and requires extensive and non-scaleable purification at near neutral pH implying partial product degradation.[40,43] Alternatively the product was not purified at all but used directly in a next reaction step.[35] A disadvantage of the enzymatic synthesis of D-GAP using an aldolase-catalyzed cleavage of D-fructose 1,6-diphosphate is the often incomplete conversion due to the thermodynamic equilibrium of this reaction.[42] Therefore a considerable purification effort is necessary in order to obtain a pure product. The degree of purity of the isolated D-GAP as well as the type of aldolase used was not specified.[42]

In the L-GAP synthesis, the disadvantages of using racemic glyceraldehyde as starting material for the glycerokinase-catalyzed phosphorylation with the phospho-enolpyruvate/pyruvate kinase system for ATP recycling[35] will be outlined later in this paper. Scale-up of this procedure resulted in diminished product yields. A multi-enzyme procedure directed toward kinetic resolution of racemic GAP with D-glyceraldehyde-3-phosphate dehydrogenase suffers from the same drawback.[32]

A lengthy approach for the preparation of both enantiomers of GAP was the enzymatic resolution of racemic glycidaldehyde diethylacetal via a series of reaction steps. As the resulting (*R*)- or (*S*)-glycidaldehyde diethylacetal respectively was subjected to a nucleophilic phosphorylation in a pH range which is unfavorable for the stability of GAP, no further purification was attempted and the material was used directly for the following reaction step instead.[38,39]

2. Results and discussion

2.1. Stability of DL-GAP

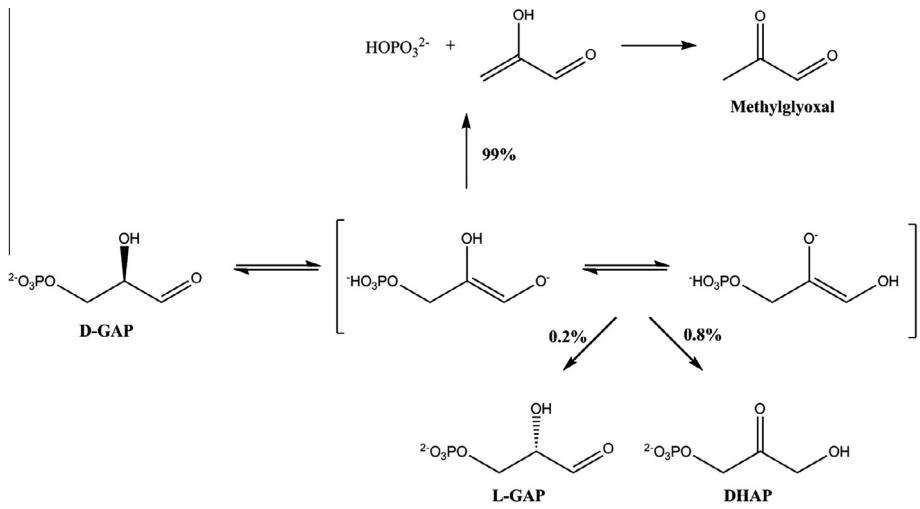
The instability of GAP under alkaline or neutral pH-conditions has been known for a very long time. Nevertheless, aqueous conditions in the neutral pH-range are used in nearly all published procedures dealing with synthesis, purification, or application of the compound. Here, ^{1}H and ^{31}P NMR experiments were used in order to identify degradation products and to examine pH-dependent behavior of GAP over time. Furthermore, these results could provide a closer insight in terms of stability and degradation mechanisms of GAP.

GAP is transformed to methylglyoxal via an enediolate phosphate transition product in water at pH 7 (Scheme 1).[32,33,41] In alkaline conditions, lactic acid is formed.[26,28,34] Figure 1 shows the comparison of ^{1}H NMR spectra of lactic acid and DL-GAP at strongly alkaline conditions in D_2O. The chemical shift values are exactly matching confirming the formation of lactic acid which has not been described in a previous kinetic analysis.[33] However, the signal at 4.1 ppm, which can be assigned to the C*H*OH proton, shows a different coupling pattern. While the signal is split up into a quartet in case of lactic acid resulting from coupling to the adjacent methyl group, it appears as a triplet in the spectrum of the DL-GAP degradation product under alkaline conditions.

Although the mechanism of the conversion from GAP to lactic acid is not definitely known, a possible explanation for the different coupling patterns is the deuteration of the newly formed methyl group by the deuterated solvent. This assumption is confirmed by the ratio of the observed integral values (only two protons in the case of the deuterated methyl group) and the weak H/D-coupling of the deuterated lactic acid methyl group (upper left in Fig. 1). There are two more interesting observations worth to note regarding the degradation of DL-GAP: (1) if the compound is left in neutral, aqueous solution for several days, methylglyoxal signals are appearing in the ^{1}H NMR spectrum, but no formation of lactic acid can be observed and (2) methylglyoxal itself does not show any formation of lactic acid even after 15 h under alkaline conditions. Therefore, the decomposition mechanism of GAP under alkaline conditions is different from the one under neutral conditions and methylglyoxal does not seem to be an intermediate in the non-enzymatic degradation of GAP at high pH-values.

In order to identify the pH-range where GAP is stable and to get first insights into degradation velocities, NMR spectra of aqueous solutions of DL-GAP at different pH values were recorded. To quantify the amount of remaining DL-GAP, the percentage of the integrals in the ^{31}P NMR spectra were plotted against time in solution (Fig. 2). As discussed earlier, in both routes of non-enzymatic GAP-degradation, the compound is dephosphorylated and inorganic phosphate is released.

It can be concluded that the product is stable at least for days under strongly acidic conditions. After 4 days at pH 4.2, degradation can be observed at a low level. Former experiments indicated full stability at pH 3.5. At pH 5.6 more than 50% of the compound is



Scheme 1. Non-enzymatic degradation of D-GAP to methylglyoxal and inorganic phosphate in water at pH 7.[33] The formation mechanism of the enediolate phosphate intermediate is analogous to L-GAP.

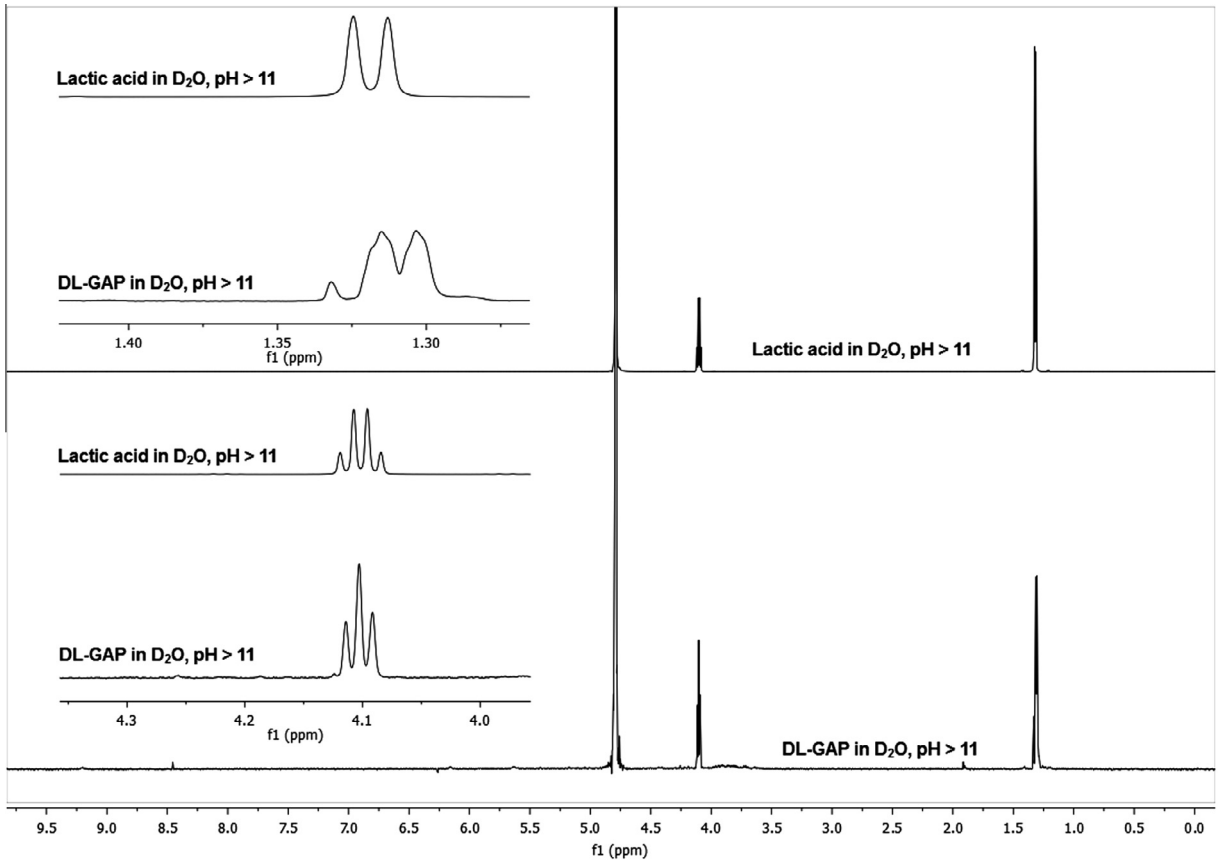


Figure 1. ^{1}H NMR spectra of lactic acid (top) and DL-GAP (bottom) in D_2O under alkaline conditions. DL-GAP is obviously converted into lactic acid although the signal at 4.1 ppm shows a different coupling pattern (lower left part of the spectra). The *CH*OH-signal, split up to a quartet by the methyl group, appears as a triplet in the DL-GAP spectrum, probably because of the presence of a CH_2D-functionality. This is confirmed by the extract on the upper left, where, in contrast to the non-deuterated lactic acid, a small H/D-coupling can be seen in the methyl group signal of the deuterated lactic acid.

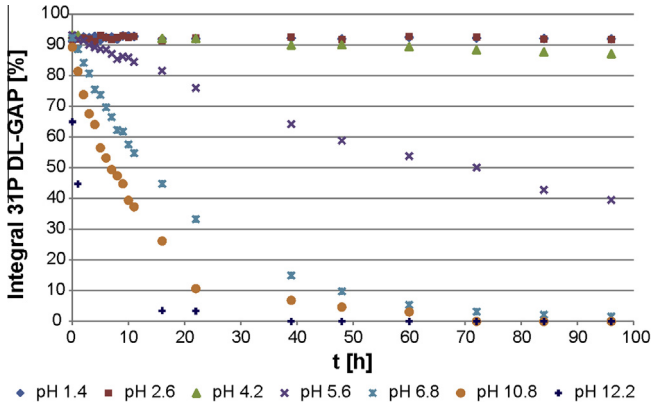


Figure 2. ^{31}P NMR integral values of DL-GAP are plotted as a function of time at different pH-values. The compound is stable under acidic conditions only and decomposes above a pH-value of 4. The pH 1.4-curve is nearly completely covered by the pH 2.6-curve.

degraded after 4 days. Under alkaline conditions (pH 12.2), less than half of the DL-GAP amount is remaining after 1 h.

2.2. Chemical synthesis of D-GAP

Synthesis of enantiomerically pure D-GAP was achieved using a modified procedure published by Szewczuk et al.,[31] where D-fructose 6-phosphate was cleaved with periodic acid to obtain the calcium salt of D-GAP (Scheme 2). As discussed earlier, the compound is degrading at pH-values above 4. Therefore, the pH-value was always kept below 4 during the whole procedure. Another modification compared to the published procedure was the use of acetone instead of ethanol for precipitation of the calcium salt because the latter could not be removed completely under reduced pressure.

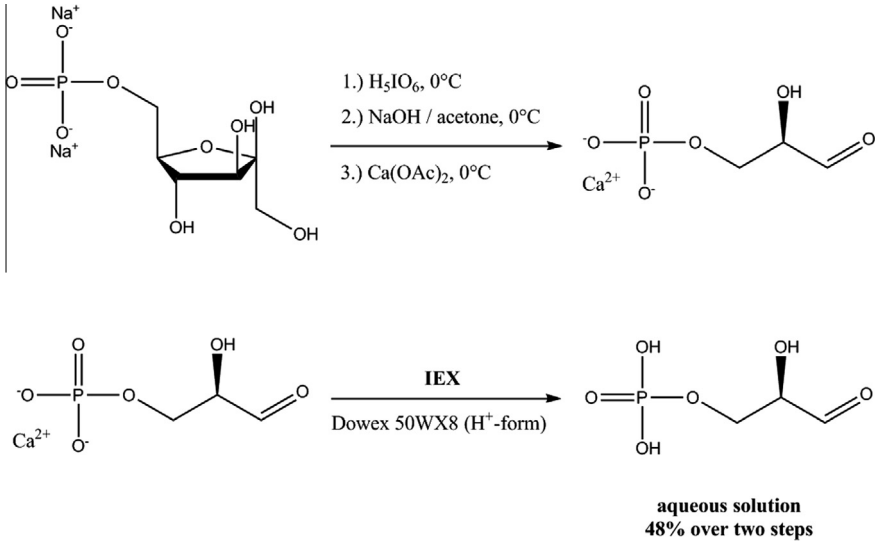
The D-GAP calcium salt is only soluble under strongly acidic conditions. Enzymatic determination of the content resulted in values of about 50% (w/w). The calcium salt was transferred to a solution of its free acid using cation exchange chromatography.

2.3. Enzymatic synthesis of L-GAP

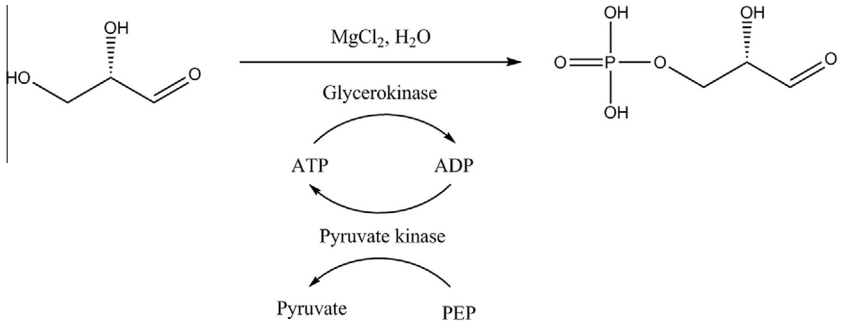
Since no chemical synthesis of L-GAP is known, an enzymatic approach for the phosphorylation of L-glyceraldehyde (L-GA) has been followed to yield enantiomerically pure L-GAP. It was described[35] that glycerokinase from *Cellulomonas* sp. (EC 2.7.1.30) has the ability to phosphorylate selectively only the L-isomer of DL-glyceraldehyde with adenosine 5′-triphosphate (ATP) as phosphorylation reagent. ATP was continuously rephosphorylated with phosphoenolpyruvic acid (PEP) and pyruvate kinase from rabbit muscle (EC 2.7.1.40) (Scheme 3) and used in a molar ratio of 1:100 compared to L-GA and PEP.

2.3.1. Evaluation of the substrate

D-Glyceraldehyde (D-GA) is described to catalyze the dephosphorylation of ATP in the presence of glycerokinase.[36] We therefore



Scheme 2. Chemical synthesis of D-glyceraldehyde 3-phosphate. Step one is a modification of an already published method[31] to obtain the calcium salt of D-GAP. In a second step, the calcium salt was converted into the corresponding free acid of D-GAP by ion exchange.



Scheme 3. Phosphorylation of L-glyceraldehyde to enantiomerically pure L-glyceraldehyde 3-phosphate using a coupled enzymatic system.

investigated the use of pure D- and L-enantiomers as starting materials for the enzymatic phosphorylation with glycerokinase by ^{31}P NMR spectroscopy.

Using enantiomerically pure D-GA as starting material, no phosphorylation can be observed in the ^{31}P NMR (Fig. 3C). However, there was a significant formation of inorganic phosphate, in agreement with the observations of Hayashi.[36] A significant formation of inorganic phosphate as a result of the dephosphorylation of L-GAP in neutral solution is unlikely in view of the degradation kinetics of DL-GAP in neutral solution (see Fig. 2).

This is confirmed by the experiment where pure L-GA is used as starting material (Fig. 3B): L-GAP is formed in a significant amount with a much slower generation of inorganic phosphate. The latter can be explained by the instability of the compound in neutral solution (see Section 2.1).

With a racemic mixture of D- and L-GA, the desired product is formed in a considerably lower amount compared to the use of the pure L-enantiomer, whereas a higher amount of inorganic phosphate formation can be observed (Fig. 3A). If the inorganic phosphate would solely originate from the dephosphorylation of the L-GAP formed, only half of the amount would be expected using racemic GA compared to pure L-GA. This is in contrast to our experimental results. We therefore conclude that glycerokinase from *Cellulomonas* sp. is selectively phosphorylating the L-GA to form L-GAP while D-GA is catalyzing the dephosphorylation of ATP.

2.3.2. Reaction optimization and scale-up

L-Glyceraldehyde is the starting material of choice for the straight forward preparation of enantiomerically pure L-GAP. However, yields of around 30% are not satisfying for converting the reaction into a preparative scale. Modifying the reaction conditions like temperature, pH-value, or concentration of the reaction partners is often a simple way to speed up an enzymatic reaction or to stabilize the desired product.

The optimum pH-values of the used enzymes are pH 7.5 for the pyruvate kinase from rabbit muscle[37] and pH 7.3–9.8 for the glycerokinase from *Cellulomonas* sp.[36] respectively. Experiments at slightly acidic conditions (pH 4.8) resulted in no formation of the desired product. Regarding the instability of GAP at higher pH-values, neutral conditions seemed to be a good compromise between activities of the enzymes and the stability of the desired product.

As shown in Figure 3 the formation of L-GAP from L-GA is diminished to a large extent by its degradation due to the low enzyme concentrations used. Therefore, higher concentrations of pyruvate kinase and glycerokinase were used and the reaction was again monitored by ^{31}P NMR spectroscopy. A tenfold increase in enzyme concentration already leads to >90% conversion to L-GAP after a reaction time of about two hours.

In a larger scale preparation, the reaction was quenched by adding aqueous HCl. A pH decrease to pH 2–3 resulted in a reaction stop and the product is kept under stable conditions. After removal of the enzymes using ultracentrifugal filter units, the L-GAP was

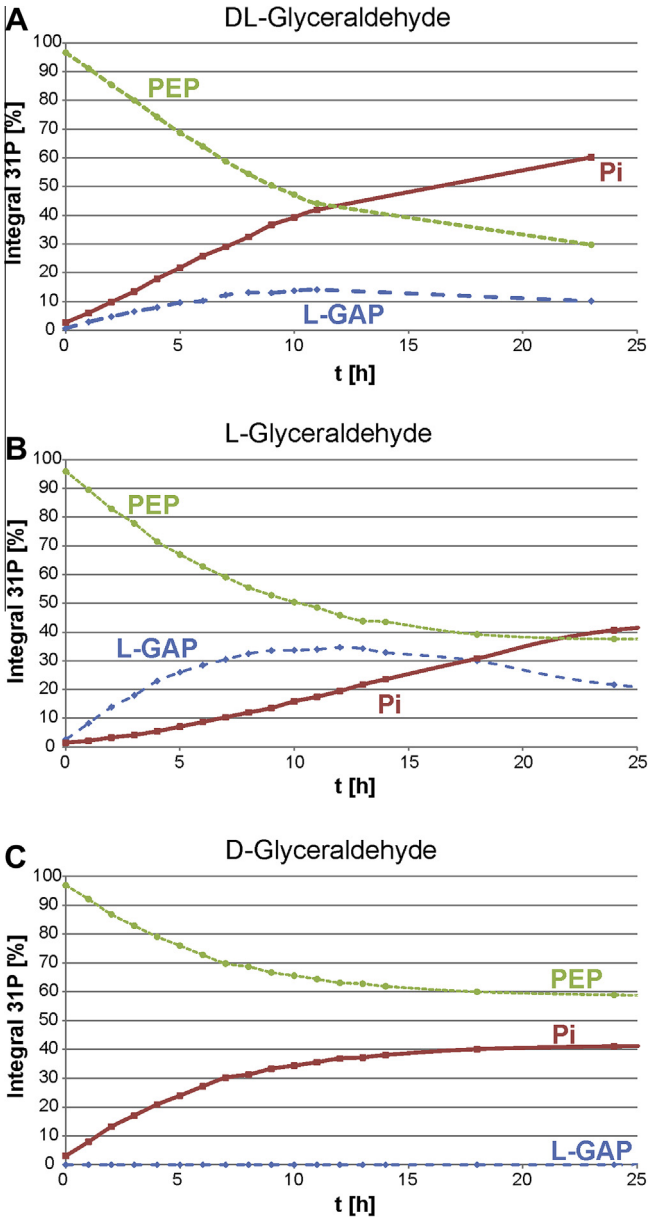


Figure 3. ^{31}P NMR monitoring of the enzymatic phosphorylation of different enantiomers of glyceraldehyde. Only the L-isomer is phosphorylated by glycerokinase from *Cellulomonas* sp., whereas D-glyceraldehyde catalyzes the dephosphorylation of ATP.

precipitated as its calcium salt, while non-phosphorylated compounds remained in solution. The white solid was washed with acetone and hydrochloric acid, dried under reduced pressure, and converted into an aqueous solution of its free acid using cation exchange chromatography.

3. Conclusions

The pH-dependent stability of glyceraldehyde 3-phosphate (GAP) and the syntheses of the enantiomerically pure D-GAP and L-GAP are reported. The stability in aqueous solution was examined using ^{1}H- and ^{31}P NMR spectroscopy. GAP has been shown to be stable below pH 4, whereas two different degradation products appeared at higher pH-values: under neutral conditions, methylglyoxal is formed, whereas under alkaline conditions, formation of lactic acid could be observed. Methylglyoxal does not seem to be an intermediate in non-enzymatic lactic acid formation.

To obtain the two enantiomerically pure isomers of GAP, two completely different routes for synthesis have been applied: While D-GAP has been synthesized using periodate cleavage of D-fructose 6-phosphate, L-GAP has been obtained by enzymatic phosphorylation of L-glyceraldehyde with glycerokinase from *Cellulomonas* sp. The enzyme has been shown to phosphorylate specifically the L-isomer, whereas the D-isomer remained not just nonphosphorylated, but moreover catalyzed the dephosphorylation of ATP. Therefore, we have established the use of L-glyceraldehyde instead of racemic DL-glyceraldehyde for the enzymatic synthesis of L-GAP as the preferred route regarding yields and purity.

4. Experimental part

4.1. General

All reagents and solvents were from Sigma–Aldrich GmbH without any further purification. NMR-spectra were recorded on a Bruker Avance III 600 MHz spectrometer equipped with a BBO probe head with z-gradient using 600.2 MHz for ^{1}H, 150.9 MHz for ^{13}C, and 243 MHz for ^{31}P. Spectra were recorded in D_2O or H_2O/D_2O at 298.2 K. Since common ^{31}P NMR spectra are not suitable for quantification, a delay of 10 s between each pulse, a pulse angle of 90° and inverse gated decoupling has been applied for the ^{31}P NMR measurements conducted herein. Differentiation between organic and inorganic phosphate was achieved using non proton decoupled ^{31}P NMR spectra. Chemical shifts were referenced to 3-(trimethyl-silyl)-propionic-2,2,3,3-d_4 acid sodium salt (TSP) in case of ^{1}H and ^{13}C NMR spectra (0.00 ppm) and phosphoric acid in case of ^{31}P NMR spectra (0.00 ppm), respectively. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter with the sodium D line at 20 °C and 1 cm path length.

4.2. Stability of DL-GAP at different pH-conditions

Preparation of the NMR-samples was performed as follows: 50 μL of aqueous DL-GAP solution (50 mg/mL) was solved in 600 μL D_2O and the particular pH value was adjusted by adding NaOD in D_2O. ^{1}H- and ^{31}P NMR spectra were recorded in intervals of 1 h for the first 11 h and after 16, 22, 39, 48, 60, 72, 84, and 96 h, respectively. For quantification of remaining DL-GAP and formed inorganic phosphate, the sum of the two integral values was normalized to 100 and the fraction of the DL-GAP signal was plotted against time in solution.

4.3. D-Glyceraldehyde 3-phosphate calcium salt

An aqueous solution of D-fructose 6-phosphate disodium salt hydrate (5 g, 15.5 mmol in 155 mL of water) was cooled with ice and a cold solution of periodic acid (12.6 g, 108.8 mmol in 56 mL of water) was added dropwise. After stirring for 1 h, ethylene glycol (5 mL, 89.7 mmol) was added and the solution was stirred for another 30 min. The pH was adjusted to 3.5 with 2 N NaOH and 150 mL of acetone was added, resulting in the formation of a white precipitate. The suspension was stirred for 90 min and the precipitate was removed by filtration and washed twice with acetone. To the clear filtrate, $Ca(OAc)_2$ (7.9 g, 43.5 mmol in 45 mL of water) was added. After stirring the formed suspension in an ice bath for 90 min, the white solid was filtrated and washed with small portions of acetone (2×), 10 μM HCl (1×), 1 mM HCl (1×), and acetone (2×). After drying overnight under reduced pressure, 1.97 g of a white solid was obtained which contained only a low amount of D-GAP.

To the clear filtrate, acetone was added and the white suspension was left at +4 °C overnight. The precipitate was filtrated and washed with small portions of acetone (2×), 10 μM HCl (1×), 1 mM HCl (1×), and acetone (2×). After drying overnight under reduced pressure, 2.26 g (10.9 mmol, 70%) of a white solid was obtained and converted into the free acid without any further purification.

4.4. D-Glyceraldehyde 3-phosphate free acid solution

D-Glyceraldehyde 3-phosphate calcium salt (33 mg, 0.16 mmol) was stirred with excess Dowex 50WX8 resin (hydrogen form) in 2 mL H_2O for 10 min until the white solid was dissolved completely. The mixture was filtered over some more resin, the latter washed with distilled water until the pH of the eluent was >4 and then concentrated under reduced pressure to yield 1.7 mL of a 10.9 mg/mL aqueous solution of D-GAP (18.5 mg, 0.11 mol, 69%). The low yield presumably results from a less than 100% D-GAP content in the starting calcium salt. $[\alpha]_D^{20}$ +15.5° (*c* 1.1, H_2O); ^{1}H NMR (600 MHz, D_2O, pH 1.7): δ ppm 3.70 (m, 1H, CHOH), 3.93 (m, 1H, CH_2), 4.02 (m, 1H, CH_2), 4.99 (d, *J* = 5.89 Hz, 1H, CHO); ^{13}C NMR (151 MHz, D_2O, pH 1.7): δ ppm 68.87 (d, *J* = 5.7 Hz, CH_2), 75.95 (d, *J* = 7.8 Hz, CHOH), 92.31 (s, CHO); ^{31}P NMR (243 MHz, D_2O, pH 1.7, decoupled): δ ppm 0.58 (s, H_2PO_4); ^{31}P NMR (243 MHz, D_2O, pH 1.7, non-decoupled): δ ppm 0.58 (t, *J* = 5.8 Hz, H_2PO_4).

The product content in solution was determined using the following enzymatic procedure:

The aqueous solution was diluted in 0.1 M KH_2PO_4 buffer (pH 5.5) and 20 μL thereof was added to 1 mL of a 0.2 M KH_2PO_4 buffer (pH 6.5) containing EDTA (20 mM) and β-NADH (0.15 mM). Absorption at 340 nm was measured and after addition of 3 μL of α-glycerophosphate dehydrogenase (75–200 U/mg) and triosephosphate isomerase (750–2000 U/mg) from rabbit muscle (suspension in 3.2 M ammonium sulfate, pH = 6, EC 1.1.1.8), the absorption at 340 nm was measured again.

The concentration was calculated according to c [mg/mL] = $(A_* V_* F_* M)/(\varepsilon_* v)$ with A = Difference of the two absorption values at 340 nm, V = total Volume [μL], F = dilution factor, M = molecular weight of D-GAP [mg/μmol], ε = extinction coefficient (6.22 cm^2/μmol), and v = volume of added D-GAP-solution.

4.5. Comparison of DL-, L- and D-Glyceraldehyde as starting materials for the enzymatic synthesis of L-GAP

To prepare the NMR-samples for the quantitative examination of the enzymatic phosphorylation of glyceraldehyde at the 3-position, aqueous solutions of the appropriate enantiomer of glyceraldehyde (100 mM final concentration) were mixed with aqueous solutions of phospho(enol)pyruvic acid monopotassium salt (100 mM final concentration), adenosine 5′-triphosphate disodium salt hydrate (1 mM final concentration), $MgCl_2$ (1 mM final concentration), and triethylphosphine oxide as ^{31}P NMR reference (20 mM final concentration) in a mixture of H_2O/D_2O (final ratio: 9:1). The pH was adjusted to 7.0 and glycerokinase from *Cellulomonas* sp. (1U) and pyruvate kinase from rabbit muscle (2U) were added. ^{31}P NMR-spectra were recorded in hourly intervals for the first twelve hours and after 24 h. For quantification, the sum of the integrals of the arising glyceraldehyde 3-phosphate, phospho(enol) pyruvic acid, and inorganic phosphate were normalized to 100 and the particular values were plotted against the reaction time.

4.6. L-Glyceraldehyde 3-phosphate free acid solution

To a solution of L-glyceraldehyde (0.6 g, 6.65 mmol), adenosine 5′-triphosphate disodium salt hydrate (0.04 g, 66.5 μmol), phospho(enol)pyruvic acid monopotassium salt (1.34 g, 6.5 mmol), and $MgCl_2 \cdot 6H_2O$ (13.5 mg, 66.5 μmol) in 50 mL H_2O/D_2O (95:5) at pH = 6.9, 2kU pyruvate kinase from rabbit muscle and 1kU glycerokinase from *Cellulomonas* sp. were added. The solution was stirred at room temperature and monitored with ^{31}P NMR spectroscopy. After 130 min and a product content of >90%, the reaction was stopped by adding 15 mL of 1 M aqueous HCl. After 2 h at +4 °C, the white precipitate was separated with Amicon ultracentrifugal filter units (molecular weight cutoff mass: 10 kDa). The clear solution was cooled to 0 °C in an ice bath and 25 mL of a 0.8 M aqueous solution of calcium acetate monohydrate (3.5 g, 19.87 mmol) was added. Afterward, 100 mL of acetone was added dropwise at 0 °C to the stirred solution and a white solid precipitated.

The mixture was stirred for an additional hour under cooling in an ice bath and the white precipitate was filtered with suction and washed with small portions of acetone (2×), 10 μM HCl (1×), 1 mM HCl (1×) and acetone (2×). After drying overnight under reduced pressure, 1.35 g of a white solid was obtained.

The white solid was stirred with 50 meq Dowex 50WX8 (hydrogen form) in 80 mL for 1 h and filtered over another 120 meq of ion exchange resin, the latter washed with distilled water until the pH of the eluent was >4 and concentrated under reduced pressure to yield 21.7 mL of a 39 mg/mL aqueous solution of L-GAP (0.85 g, 5 mmol, 77%). For storage and analysis, the solution was diluted to a concentration of 8.6 mg/mL. $[\alpha]_D^{20}$ −12.8° (*c* 0.86, H_2O); ^{1}H NMR (600 MHz, D_2O, pH 1.75): δ ppm 3.70 (m, 1H, CHOH), 3.92 (m, 1H, CH_2), 4.02 (m, 1H, CH_2), 4.99 (d, *J* = 5.66 Hz, 1H, CHO); ^{13}C NMR (151 MHz, D_2O, pH 1.75): δ ppm 68.84 (d, *J* = 5.7 Hz, CH_2), 75.95 (d, *J* = 7.88 Hz, CHOH), 92.30 (s, CHO); ^{31}P NMR (243 MHz, D_2O, pH 1.75, decoupled): δ ppm 0.06 (s, H_2PO_4); ^{31}P NMR (243 MHz, D_2O, pH 1.75, non-decoupled): δ ppm 0.06 (t, *J* = 5.8 Hz, H_2PO_4).

Acknowledgements

We thank Wolfgang Hass for the improvement in the enzymatic assay of D-GAP. The support of the BMBF Project Biokatalyse2021 P28 and Sigma–Aldrich is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.12.023.

References

1. Buchner, E.; Rapp, R. *Ber. Dtsch. Chem. Ges.* **1897**, *30*, 2668–2678.
2. Harden, A.; Young, W. J. *Proc. R. Soc. (London)* **1906**, *77*, 405–420.
3. Embden, G.; Deuticke, H. J.; Kraft, G. *J. Mol. Med.* **1933**, *12*, 213–215.
4. Meyerhof, O. *Experientia* **1948**, *4*, 169–176.
5. Neuberg, C.; Kobel, M. *J. Bacteriol.* **1934**, *28*, 461–471.
6. Parnas, J. K. *Nature* **1943**, *151*, 577–580.
7. Warburg, O.; Walter, C. *Naturwissenschaften* **1941**, *29*, 589–590.
8. Cori, C. T.; Cori, G. T. *Ann. Rev. Biochem.* **1941**, *10*, 151–180.
9. Bar-Even, A.; Flamholz, A.; Noor, E.; Milo, R. *Nat. Chem. Biol.* **2012**, *8*, 509–517.
10. Flamholz, A.; Noor, E.; Bar-Even, A.; Liebermeister, W.; Milo, R. *Proc. Natl. Acad. Sci.* **2013**, *110*, 10039–10044.
11. Rohmer, M. *Pure Appl. Chem.* **2007**, *79*, 739–751.
12. Zhao, L.; Chang, W.; Xiao, Y.; Liu, H. W.; Liu, P. *Ann. Rev. Biochem.* **2013**, *82*, 497–530.
13. Dijkhuisen, L.; Harder, W. *Antonie van Leeuwenhoek* **1984**, *50*, 473–487.
14. Hasunuma, T.; Harada, K.; Miyazawa, S.-I.; Kondo, A.; Fukusaki, E.; Miyake, C. *J. Exp. Bot.* **2010**, *61*, 1041–1051.
15. Floss, H. G. *Nat. Prod. Rep.* **1997**, *14*, 433–452.
16. Herrmann, K. M.; Weaver, L. M. *Ann. Rev. Plant Physiol. Plant. Mol. Biol.* **1999**, *50*, 473–503.
17. Kershaw, N. J.; Caines, M. E. C.; Sleeman, M. C.; Schofield, C. J. *Chem. Commun.* **2005**, 4251–4263.
18. Begley, T. P.; Ealick, S. E.; McLafferty, F. W. *Biochem. Soc. Trans.* **2012**, *40*, 555–560.

19. Shimizu, T.; Takaya, N.; Nakamura, A. *J. Biol. Chem.* **2012**, *287*, 40448–40456.
20. Desai, K. K.; Miller, B. G. *Biochemistry* **2008**, *47*, 7983–7985.
21. Warburg, O.; Posener, K.; Negelein, F. *Biochem. Z.* **1924**, *152*, 319–344.
22. Warburg, O. *The Metabolism of Tumors*; Costable: London, 1930.
23. Warburg, O. *Biochem. Z.* **1956**, *123*, 309–314.
24. Vander Heiden, M. G.; Cantley, L. C.; Thompson, C. B. *Science* **2009**, *324*, 1029–1033.
25. Cairns, R. A.; Harris, I. S.; Mak, T. W. *Nat. Rev. Cancer* **2011**, *11*, 85–95.
26. Barrenscheen, H.; Beneschovsky, H. *Biochem. Z.* **1933**, *265*, 159–168.
27. Fischer, H. O. L.; Baer, E. *Berichte* **1932**, *65*, 1040.
28. Kiessling, W. *Berichte* **1934**, *67*, 869–874.
29. Ballou, C. E.; Fischer, H. O. L. *J. Am. Chem. Soc.* **1955**, *77*, 3329–3331.
30. Oesper, P. *Arch. Biochem. Biophys.* **1961**, *95*, 237–241.
31. Szewczuk, A.; Wolny, E.; Wolny, M.; Baranowsky, T. *Acta Biochim. Pol.* **1961**, *8*, 201–207.
32. Richard, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 4926–4936.
33. Richard, J. P. *Chem. Biol.* **2008**, *3*, 605–607.
34. Trigalo, F.; Jachymczyk, W.; Young, J. C.; Szabo, L. *J. Chem. Soc., Perkin Trans. 1* **1975**, 593–598.
35. Wong, C. H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199–3205.
36. Hayashi, S.-I.; Lin, E. C. C. *J. Biol. Chem.* **1967**, *242*, 1030–1035.
37. Bucher, T.; Pfleiderer, G. *Methods Enzymol.* **1955**, *1*, 435–440.
38. Pedersen, R. L.; Liu, K. K. C.; Rutan, J. F.; Chen, L.; Wong, C. H. *J. Am. Chem. Soc.* **1990**, *55*, 4897–4901.
39. Barbas, C. F., III; Wang, Y. F.; Wong, C. H. *J. Am. Chem. Soc.* **1990**, *112*, 2013–2014.
40. Li, H.; Tian, J.; Wang, H.; Yang, S. Q.; Gao, W. Y. *Helv. Chim. Acta* **2010**, *93*, 1745–1750.
41. Phillips, S. A.; Thornalley, P. J. *Eur. J. Biochem.* **1993**, *212*, 101–105.
42. Rosen, O. M.; Hoffee, P.; Horecker, B. L. *J. Biol. Chem.* **1965**, *240*, 1517–1524.
43. Serianni, A. S.; Pierce, J.; Barker, R. *Biochemistry* **1979**, *18*, 1192–1199.