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HYPOTHESIS

Hidden biochemical fossils reveal an evolutionary trajectory for glycolysis in the prebiotic era

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Glycolysis is present in nearly all organisms alive today. This article proposes an evolutionary trajectory for the development of glycolysis in the framework of the chemoautotrophic theory for the origin of life. In the proposal, trioses and triose-phosphates were appointed to starting points. The six-carbon and the three-carbon intermediates developed in the direction of gluconeogenesis and glycolysis, respectively, differing from the from-bottom-to-up development of enzymatic glycolysis. The examination of enzymatic reaction mechanisms revealed that the enzymes incorporated chemical mechanisms of the nonenzymatic stage, making possible to identify kinship between glyoxalases and glyceraldehyde 3-phosphate dehydrogenase as well as methylglyoxal synthase and triose-phosphate isomerase. This developmental trajectory may shed light on how glycolysis might have developed in the nonenzymatic era.

Keywords: glyceraldehyde 3-phosphate dehydrogenase; glycolysis; glyoxalase; methylglyoxal; methylglyoxal synthase; phosphate; triose; triose-phosphate isomerase

To put the importance of bioenergetics into a cellular perspective, the initial axiom has to be that living cells are open, non-equilibrium systems [1,2]. Being out-of-equilibrium, the cells need a continuous flow of both matter and energy to maintain their structure and steady state, and to avoid equilibrium, which would mean their homogenization or with other words their death. This sharply stresses the importance of bioenergetics.

There are several ways of getting energy in cells. One of the most fundamental ways of biological energy formation is the widely spread glycolysis [3].

Glycolysis, the breakdown of glucose to two molecules of pyruvate (PYR), is a central metabolic pathway playing a role in both energy production and building other cellular macromolecules [4]. Since it is found with variations in nearly all organisms alive today, being either aerobic or anaerobic, this wide occurrence and the fact that its operation does not require molecular oxygen indicate that it is an ancient metabolic pathway [4–6]. All this makes interesting to examine its development in the course of evolution.

The central question posed by this article is a step-by-step approach to the evolution of nonenzymatic glycolysis, taking into account and stressing the phases important for prebiological evolution. The mechanisms of the development of nonenzymatic glycolysis are compared with some contemporary enzyme-catalyzed reactions as well as to the widely accepted conception of the development of enzymatic glycolysis.

Abbreviations

1,3BPG, 1, 3-bisphosphoglycerate; 3PG, 3 phosphoglycerate; C3, three-carbon; C6, six-carbon; CYS, cysteine; DHAP, dihydroxyacetone-phosphate; FeS, ferrous sulfide; FeS_2, pyrite; fru-1,6BP, fructose 1,6-bisphosphate; GA3PDH, glyceraldehyde 3-phosphate dehydrogenase; GAP, glyceraldehyde 3-phosphate; GLU, glutamic acid; HIS, histidine; HPP, hydroxy-pyruvaldehyde-phosphate; LAC, lactate; MGO, methylglyoxal; PYR, pyruvate; rTCA, reductive citric acid cycle; SH_2, hydrogen sulfide; TE, thioester.

Methods

The majority of reactions used for network construction was already defined and used in earlier communications [7–9]. In summary,

(i) Reduction of the keto-group resulting in the formation of α-hydroxy-carboxylic acid:

$$-CO- + SH_2 \Rightarrow -HC(OH)- + S^o \quad (1)$$

(ii) Reduction of aldehyde group resulting in the formation of hydroxy group on the primary carbon:

$$R-CHO + SH_2 \Rightarrow R-CH_2(OH) + S^o \quad (2)$$

(iii) Oxidation of aldehyde group resulting in the formation of carboxylic acid:

$$R-CHO + S^o + H_2O + FeS \Rightarrow R-COO^- + H^+ + H_2 + FeS_2 \quad (3)$$

In the pyrite (FeS_2)-pulled scenario, the formation of FeS_2 from hydrogen sulfide (SH_2) and ferrous sulfide (FeS) is suggested to be linked to endergonic reactions, making them exergonic [10].

$$SH_2 + FeS \Rightarrow H_2 + FeS_2 \qquad \Delta G_o' = -38.4\ kJ \cdot mol^{-1} \quad (4)$$

It is assumed that SH_2 is the hydrogen donor in hydrogenations

$$SH_2 \Rightarrow HS^- + H^+ \Rightarrow H_2 + S^o$$
$$\Delta G_o' = 28.8\ kJ \cdot mol^{-1} \quad 5$$

and the opposite reaction takes place in hydrogen extraction [11].

SH_2 exists in a 50 : 50 equilibrium with $HS^- + H^+$ at pH 7, while it is approximately 69% ionized at pH 7.4, and the ε_o' values for S^o/HS^- and H^+/H_2 redox couples are −270 mV and −414 mV, respectively [11–13].

Newly considered reactions in this article for the evaluation of the development of glycolysis are as follows.

Keto-enol tautomerism

In a compound containing a carbonyl group, an equilibrium is established between the keto (oxo) form (a ketone or an aldehyde, C=O) and the enol form comprising a double bond between two carbon atoms to one of which a hydroxyl group is attached (C=C-OH). The interconversion of these two forms, called keto-enol tautomerism or prototropic change, involves proton movement and shifting of bonding electrons within the same molecule (Fig. 1A). The reaction is a subject to general catalysis, meaning that it can be carried out either by an acid or by a base. The capacity of carbonyl-containing compounds to form enol, however, differs [14–16]. In general, the oxo form is thermodynamically more stable than the enol form since C=O bond has a greater bond energy than C=C bond. So, keto form predominates over enol form at the equilibrium for most of the ketones, but the latter is important for some reactions of biochemical importance [17]. Particularly, in the presence of a base, both keto and enol forms can be deprotonated, thus forming a strong nucleophile intermediate, referred to as an enolate anion (Fig. 1A). The free electrons on the oxygen act as a base or nucleophile for chemical reactions. But actually, enolate anion is able to add a proton at carbon, too, but this happens much slower than at oxygen [18].

Aldol condensation

Generally speaking, aldol condensation is a nucleophile attack on the electrophilic carbonyl of an aldehyde or ketone molecule to make a β-hydroxyketone or -aldehyde. The nucleophile is generally an enolate anion (Fig. 1B). On its own, the reaction is slow, but an acidic or basic solution can catalyze the reaction, which is often followed by dehydration giving a conjugated enone [15].

Hydration/dehydration

Hydration of aldehydes and ketones is an acid/base-catalyzed reaction [19]. The addition of water to aldehydes and ketones is accelerated by acidic or basic conditions (Fig. 1C), whereas it is unfavorable at neutral pH [20].

Phosphorylation

The phosphate is a poor electrophile due to the fact that the negatively charged oxygen atoms shield the phosphorus atom in center from a nucleophile attack [21]. An important aspect of biological phosphate transfer reactions is that the electrophilicity of the phosphorus atom is enhanced by the effect of magnesium ion(s), pulling electron density away from the phosphorus atom and this way increasing its electrophile nature (Fig. 1D(a)) [21].

While enolates are nucleophiles, the ketones are electrophiles [15]. For the above reason, self-condensation is always a potential problem, but from evolutionary point of view it is understandable why enol form is advantageous in the sense of phosphorylation. As to the mechanism, the nucleophile in the course of phosphoryl transfer approaches the electrophilic center, the phosphorous atom (Fig. 1D(b)).

Herewith, a resonance between 2- and 3-phosphates is postulated through cyclic phosphate compound formation (Fig. 1E).

Thiolation

Thiolation means a reaction of a given residue with thiol. Thiolations relevant to the topic discussed are summarized in Table 1. parallel to phosphorylations.

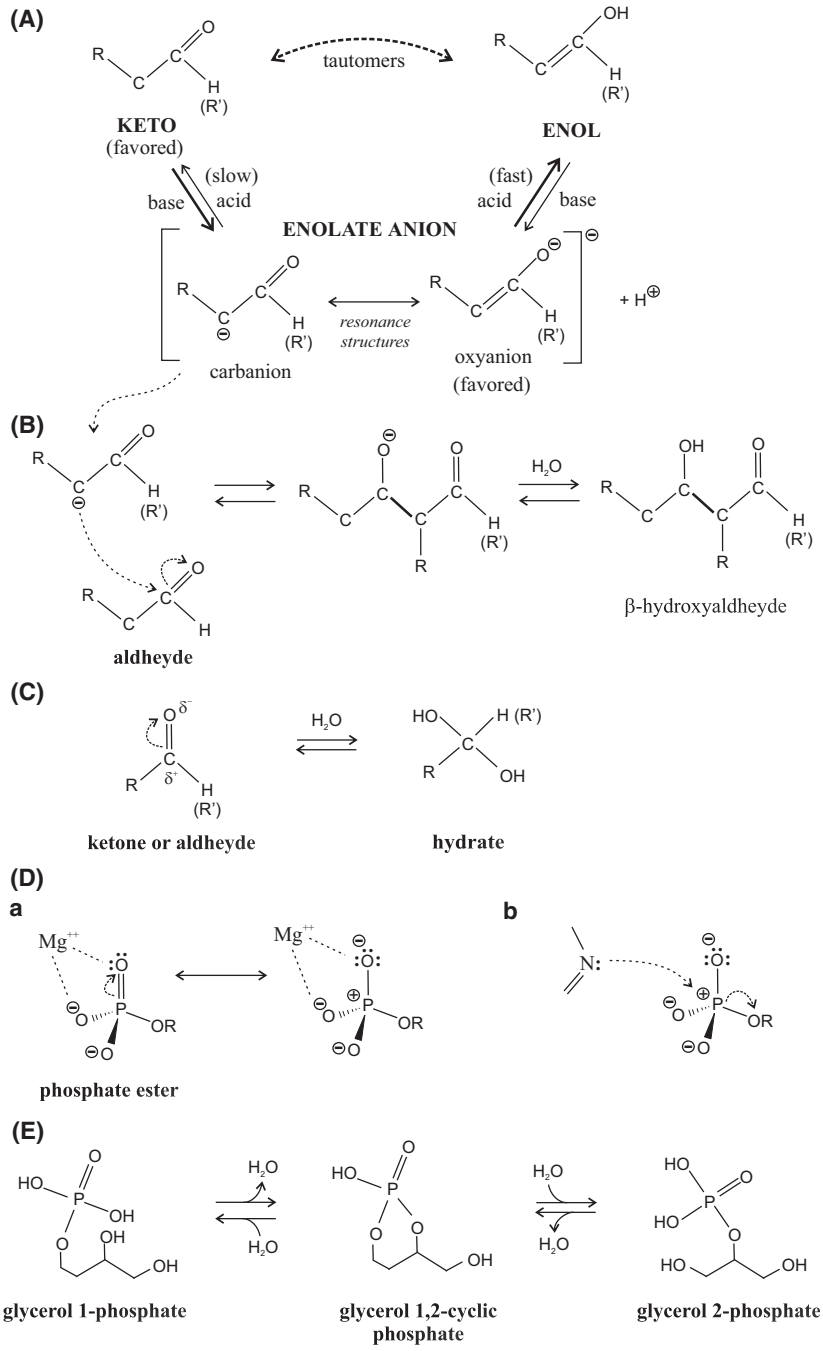


Fig. 1. Considered reactions in network formation. (A) Keto-enol tautomery, (B) aldol condensation, (C) hydration, (D) phosphorylation (a) the effect of magnesium, (b) nucleophilic attack, (E) postulated resonance between 2 and 3-phosphate tri-carbon compounds through cyclic phosphate formation.

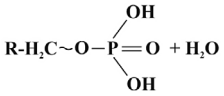
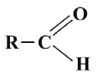
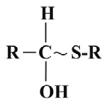
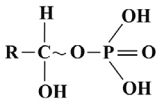
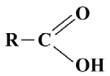
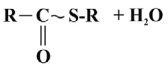
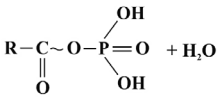
Results

Nonenzymatic stages of the development of the pay-off phase of glycolysis

First stage, the conversion of methylglyoxal into pyruvate

As shown (Fig. 2), methylglyoxal (MGO) as an offshoot of formose cycle is converted through lactate (LAC) to PYR [23,24]. This pathway was already proposed almost a quarter of a century ago as the anaplerotic route for reductive citric acid cycle (rTCA) in surface metabolists [24]. And as such, it is legitimate to assume here that this route was the first primitive ancestor of the three-carbon (C3) part of glycolytic sequence. Later imidazole, metal ions and thiol compounds were incorporated as catalysts in the more sophisticated version of the route [7].

Table 1. Reactions, the name of the products and examples for the release of Gibbs free energy during hydrolysis.

Functional group	Reaction partners	
	thiol component HS-R	phosphoric acid H_3PO_4
$R\text{-}CH_2\text{-}OH$	a $R\text{-}CH_2\sim S\text{-}R + H_2O$	b 
	c 	d 
	e 	f 

(a) physiologically not important
(b) phospho-ester bond,
example: glycerol 3-phosphate + H_2O → glycerol + $H_2PO_4^-$
$\Delta G_o' = -9.7$ kJ·mol^{-1} (textbook knowledge)
(c) hemithioacetal bond,
example: methylglyoxal + HS-glutathione → methylglyoxal hemithioacetal
$\Delta G_o' = -26.75$ kJ·mol^{-1} [22]
(d) physiologically not important
(e) thioester bond,
example: S-D-lactoylglutathione + H_2O → D-lactate + HS-glutathione
$\Delta G_o' = -49.23$ kJ·mol^{-1} [19]
(f) phosphoanhydride bond,
example: 1,3-bisphosphoglycerate + H_2O ⇌ 3-phosphoglycerate + $H_2PO_4^-$
$\Delta G_o' = -49.6$ kJ·mol^{-1} (textbook knowledge)

Second stage, phosphorylated tri-carbons without 1, 3-bisphosphoglycerate

Recently, an oxido-reduction approach fitting to chemoautotrophic origin of life and implying the role of MGO in triose formation was presented [25]. Taking MGO as raw molecule, glycerol, glyceric acid, and tartronic acid were identified as end-products parallel to the role of LAC. A simplified network was deduced involving all trioses playing crucial role in extant metabolism (Fig. 3A) [25]. Intriguing feature of the set of these compounds is that at the present status of knowledge almost all compounds exist in phosphorylated form, too (Fig. 3B). Bold lines show the interactions originating from the oxido-reduction network from which Fig. 3A was extracted, while continuous thin lines represent additional interactions and dashed thin lines show those that are not clearly identified.

A set of reactions with phosphorylated compounds, which may correspond to an ancient form of pay-off phase of contemporary glycolysis sequence, was constructed rooting in Fig. 3A (Fig. 3C).

Third stage, the involvement of 1, 3-bisphosphoglycerate

As described earlier [7], imidazole in cooperation with metals might have played a role in the nonenzymatic conversion of MGO to PYR through LAC under prebiotic conditions. In an analogous manner to MGO—LAC conversion (Fig. 3A), it is suggested that such chemistry might have been involved in the transformation of hydroxy-pyruvaldehyde-phosphate (HPP) into

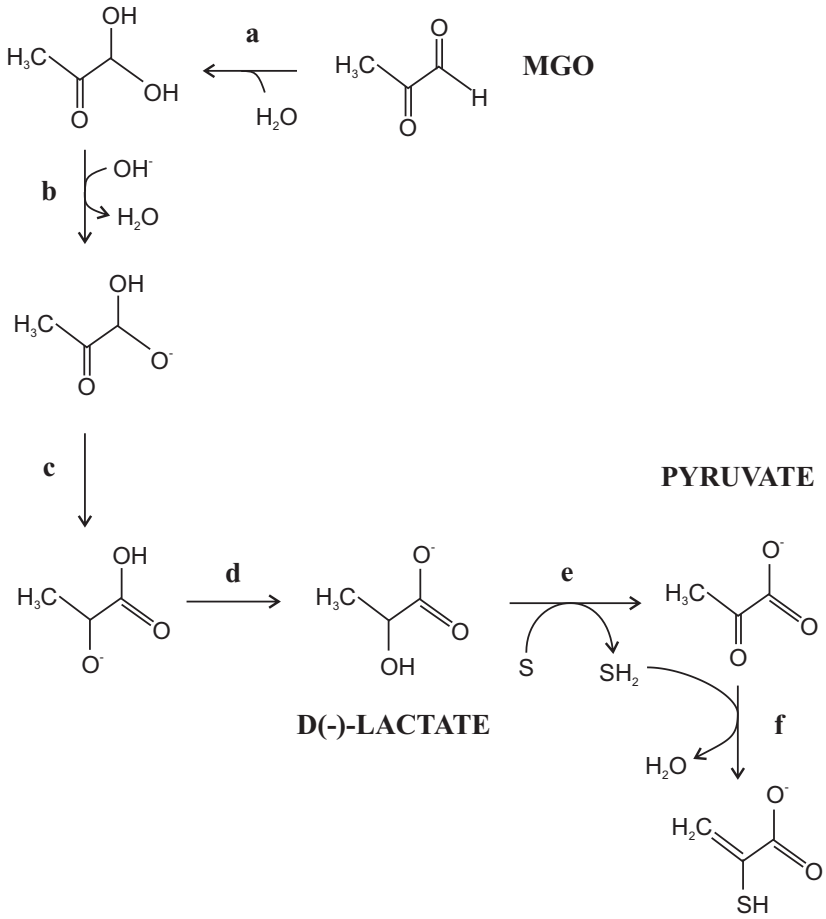


Fig. 2. Conversion of methylglyoxal to pyruvate in the proposed anaplerotic route to reductive citric acid cycle. (a) Hydration; (b–d) suggested disproportionation of methylglyoxal to lactate in which (c) is slow while reaction (d) is fast [23]; (e) reduction linked to SH_2 formation; (f) reaction in the reductive citric acid cycle. MGO, methylglyoxal.

3-phosphoglycerate (3PG, denoted glyceric acid-P in Fig. 3B), too (dashed line). The possible mechanism for the conversion of HPP to 3PG is shown in Fig. 4. However, the formed acyl-imidazole in the presence of phosphates can react with phosphate to yield a new acyl-phosphate, 1, 3-bisphosphoglycerate (1,3BPG), and imidazole is regained (Fig. 4). Since the protonation is necessary for the recovery of the original state of imidazole and the source of the proton has to be the phosphate, this requirement limits the pH range. The second and third pK values for phosphate are 7.2 and 12.3, respectively, thus designating the possible pH range in which the reaction may occur. This range, however, fits well the pH conditions suggested for hydrothermal vents [26,27].

Parallel to the above detailed mechanism for 3PG and 1,3BPG formation, another way for the production of these phosphorylated compounds from glyceraldehyde 3-phosphate (GAP) became possible as shown in Fig. 5. The major difference between the pathways is that Cannizzaro reaction takes place in the former one in which metals, particularly Zn, participate (Fig. 4), while sulfur as hydrogen acceptor plays a role in the latter one (Fig. 5). From energetic point of view, it is obvious that the GAP—1,3BPG pathway is superior to the HPP—1,3BPG route as SH_2 can contribute to pyrite formation, thus increasing Gibbs free energy change for the reaction (see Eqn 4). Accordingly, the comparison of these two pathways in this sense makes it clear why the GAP—1,3BPG pathway might have represented a selection advantage in the course of evolution already under prebiotic nonenzymatic conditions, too.

An important feature of these routes is that because of the pK values for phosphate they must have operated between pH 7.2 and 12.3, creating a situation that enhances the dissociation of thiol proton resulting in a more reactive thiolate formation as the pK for unperturbed cysteine (CYS) is about 8.25 [28].

Nonenzymatic formation of six-carbon intermediates of glycolysis

Trioses and their phosphorylated forms by interacting with each other are able to condensate to six-carbon (C6) intermediates. Specifically, dihydroxyacetone-phosphate (DHAP) and GAP can be aldol-condensated

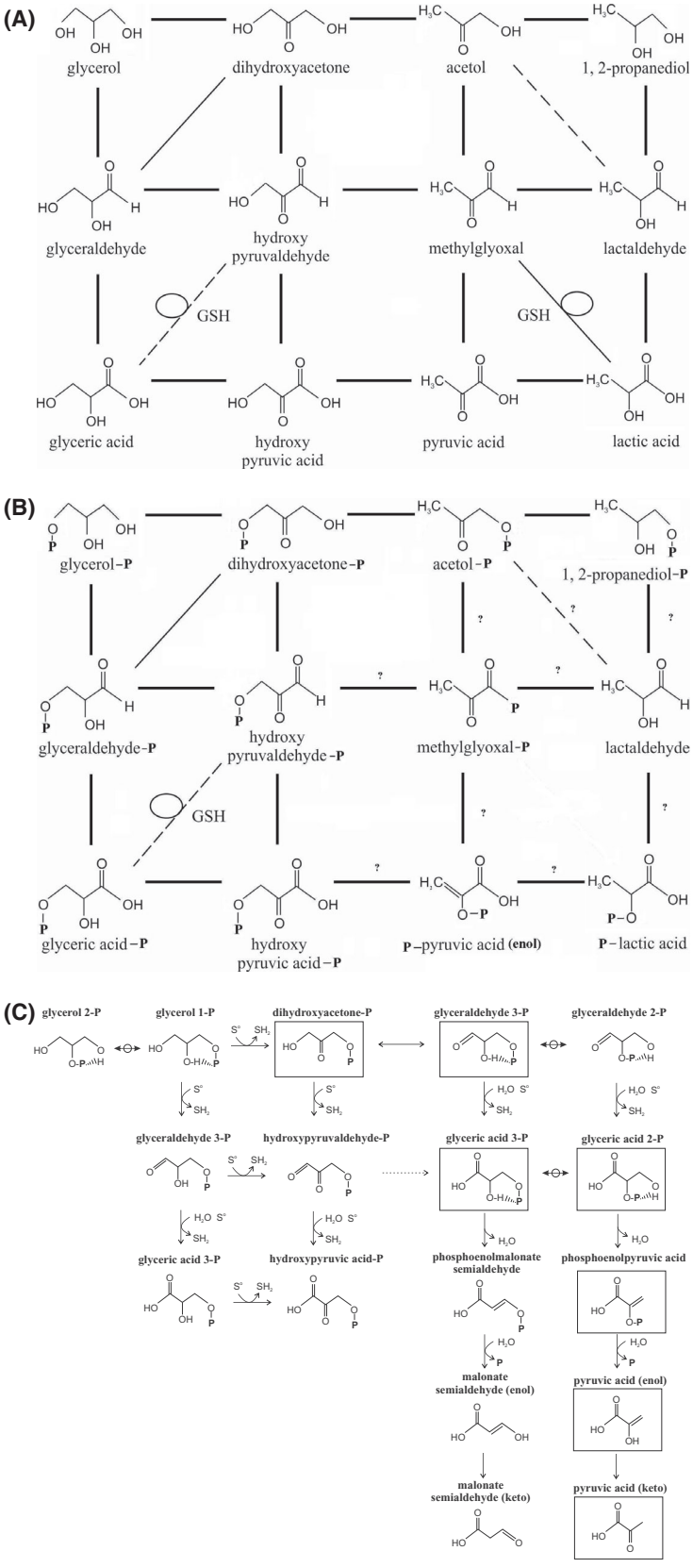


Fig. 3. Phosphorylated tri-carbons mimicking the pay-off phase of glycolysis without 1, 3-bisphosphoglycerate. Panel A. The simplified network of trioses deduced from the proposed oxido-reductive network of methylglyoxal [25] (with the permission of the Publisher). Panel B. The known phosphorylated forms of network members. Taking into consideration the fact that these C3 compounds possess several functional groups having the ability of interacting with phosphate (Table 1), theoretically mono-, di-, and triphosphate derivatives may have occurred regardless their existence in present-day biochemistry. Question marks mean that those conversions and compounds have not been identified yet. Panel C. The network of phosphorylated tri-carbons mimicking the pay-off phase of glycolysis without 1, 3-bisphosphoglycerate. The set of these phosphorylated compounds roots in Panel A.

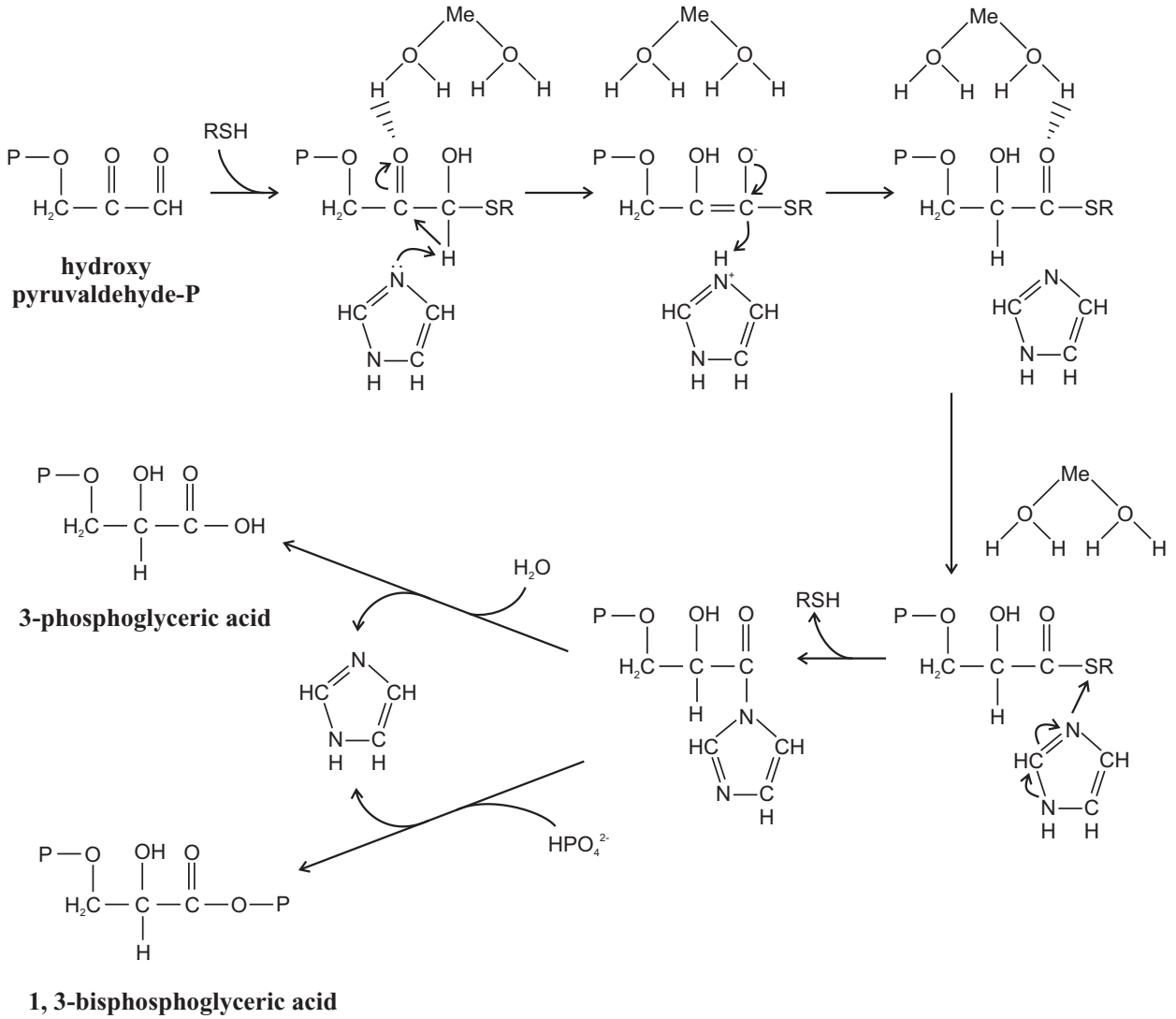


Fig. 4. Proposed mechanism for the conversion of hydroxy-pyruvaldehyde-phosphate to 3-phosphoglycerate and 1, 3-bisphosphoglycerate. Me, metal (Zn, Fe); RSH, thiol compound.

and fructose 1,6-bisphosphate (fru-1, 6BP) is formed, thus creating the first C6 element from triose-phosphates in the nonenzymatic system, which fits to the development of from-bottom-to-up direction and this way it is in a harmony with contemporary gluconeogenesis [29]. Other C3s can also participate in aldolization and produce different carbohydrates with five or six carbons [30,31].

It may be assumed that aldol condensations might have occurred parallel to the second and third stages reactions (Figs 3 and 4), when the amounts of trioses and their phosphate derivatives might have been present in a sufficiently enough concentration for such condensations.

Emergence of enzymatic catalysis at triose branching in the evolution of glycolysis

The glyoxalase path and the glyceraldehyde 3-phosphate dehydrogenase

Apparently, the glyoxalase route and glyceraldehyde 3-phosphate dehydrogenase (GA3PDH)-catalyzed reaction are very far from each other. This impression roots in the consideration of present-day function of these enzymes based on having different substrates and displaying seemingly different mechanisms. Yet, from evolutionary point of view these enzymes are in close neighborhood as to have quite similar catalytic mechanisms and they may have shared their substrates

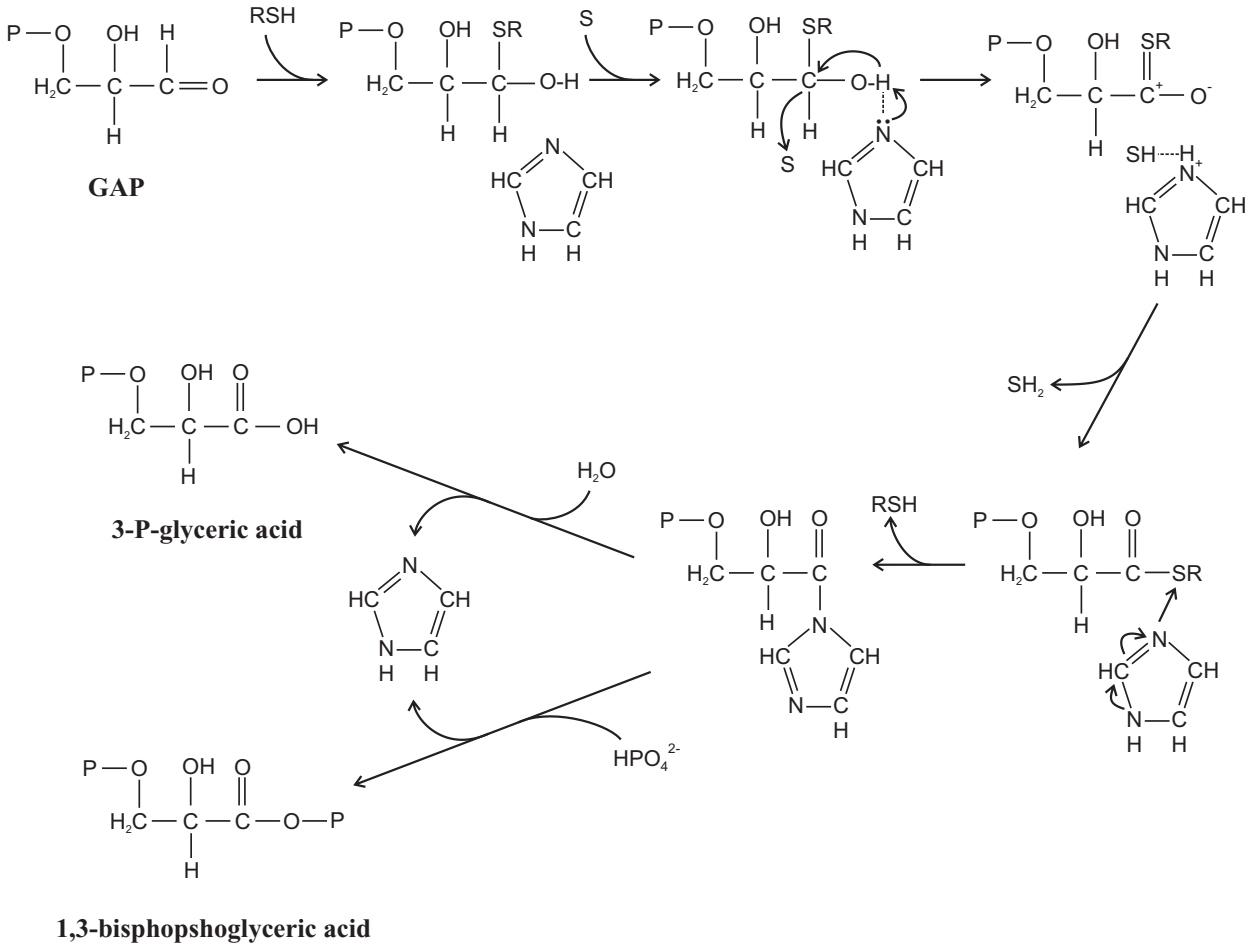


Fig. 5. Proposed mechanism for the conversion of glyceraldehyde 3-phosphate (GAP) to 3-phosphoglycerate and 1,3-bisphosphoglycerate. RSH, thiol compound.

in the early stage of evolution. This note is strongly supported by essential observations upon enzymatic mechanism. In both cases, a glutamic acid (GLU) residue orientates substrate, histidine (HIS) residue plays a critical role in hydride transfers, and thioester (TE) bond formation is a part of transformation, mostly already present in the prebiotic era (Figs 4 and 5) [32]. In the case of the last item, there is, however, a difference regarding the residue taking place in TE formation, because while the thiol belongs to a CYS residue in GA3PDH, the reduced glutathione provides the thiol component for the reaction of glyoxalases [33,34]. Of course, the enzymes differ in proton transfer mechanism and the capability for phosphorylation, too.

Triose-phosphate isomerase and methylglyoxal synthase

The reaction mechanisms of triose-phosphate isomerase (Fig. 6A) and methylglyoxal synthase (Fig. 6B) are highly related, which suggests a common root for those. Of course, while there are numerous common elements in the reactions, they mismatch in several aspects. HIS as an actor in proton movement as well as asparagine/GLU as general base are crucial in enzymatic mechanism [35,36].

Discussion

Glycolysis, Embden–Meyerhof–Parnas pathway, participating in both bioenergetics and building block syntheses in cells, is a universal, but not uniform, widely spread and highly conserved pathway in all known domains of contemporary living beings. This is particularly true to the lower reaction chain, named trunk pathway, in which very small number of variations are only observed, indicating its ancient origin [32,37,38]. The ancient origin of trunk pathway is further emphasized by the fact that it is a part of Entner–Doudoroff

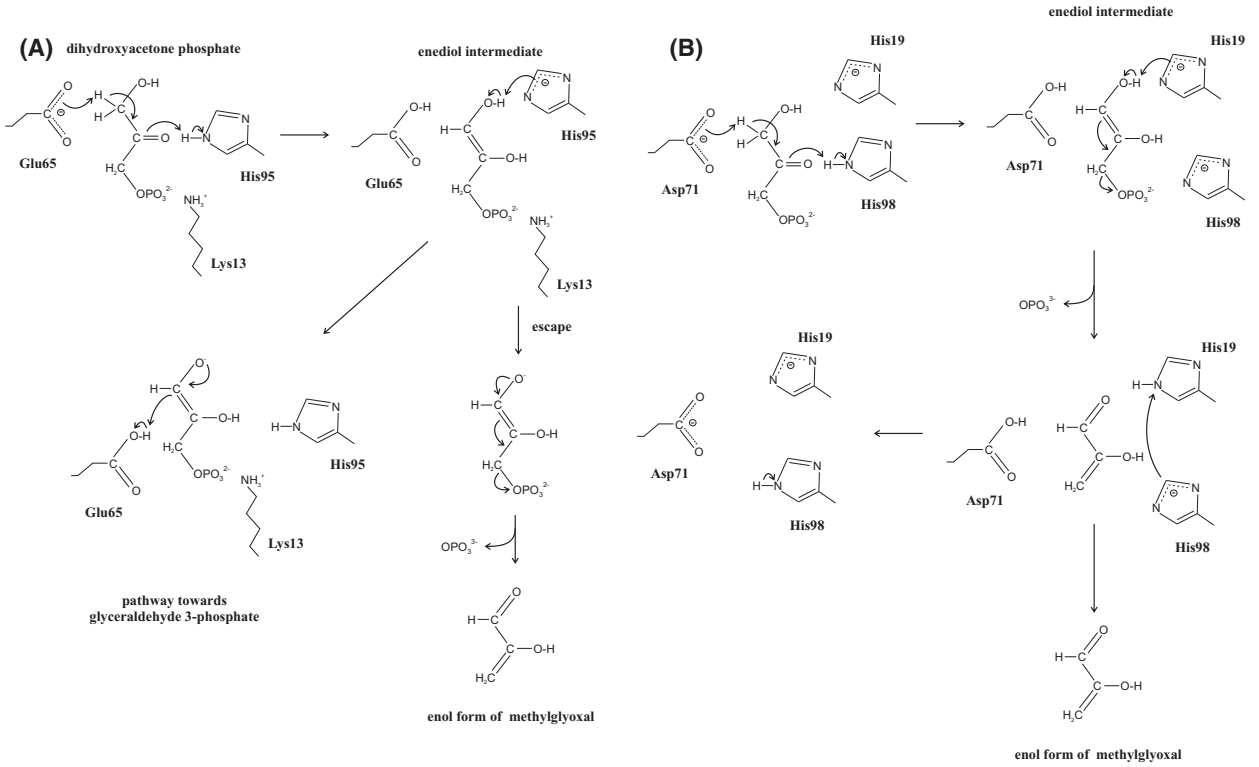


Fig. 6. Reaction mechanisms of triose-phosphate isomerase and methylglyoxal synthase. Panel A. Triose-phosphate isomerase-catalyzed reaction. Panel B. Methylglyoxal synthase-catalyzed reaction.

and the pentose phosphate pathways, alternatives for glucose breakdown, and overlaps Calvin cycle, too [39–42].

According to the generally accepted view upon the evolution of enzyme-catalyzed glycolysis, it may have essentially assembled as a reversal of gluconeogenesis [43,44]. This view is eventually held upon sequence analytic research, a method widely used for the evaluation of relationships and diagrammatic depiction of biological entities that are connected through common descent [45,46]. In contrast, the above-mentioned approach is obviously inapplicable in early evolution as then genes were not present yet; thus, the development of glycolysis, or better to say glycolysis-like oxidative processes, in the pre-enzymatic era is less clear in part due to methodological difficulties as well as uncertainties regarding prebiological conditions.

This article attempted to frame the issue of feasible developmental stages of nonenzymatic trunk pathway of glycolysis-like route, and three grades were depicted. As starting point, the first grade was a simple connection between trioses originating from the formose cycle and PYR in the rTCA (Figs 2 and 7). The proposed route was suggested as the anaplerotic pathway to the rTCA in the conjunction with the surface metabolism theory, which in its larval form was already an initiative for the later developed glycolysis without phosphorylated intermediates [24]. In the second stage, a simplified network deduced from the oxido-reduction network of MGO was used (Fig. 3A, [25]). If other reactions, such as aldolization, hydration, and keto-enol tautomerism, are also taken into account, it becomes obvious how large set of compounds can be created. Almost all constituents are presently known in phosphorylated form (Fig. 3B) and using simple reactions a network resembling the trunk pathway was built up (Fig. 3C). Much of the reactions depicted can, however, be found in the extant metabolic machinery of several organisms as enzyme-catalyzed conversions partly in the pay-off phase of contemporary glycolysis sequence (Fig. 3C).

To check how valid the prebiological evolutionary theories are, the science nowadays offers two approaches. On the one hand, intensive collection of environmental data (present-day hydrothermal vents and rock sediments) and, on the other hand, more extensive experimental investigations under conditions suggested for the hydrothermal vents.

Recently, experimental works that investigated the emergence of glycolysis under ancient circumstances

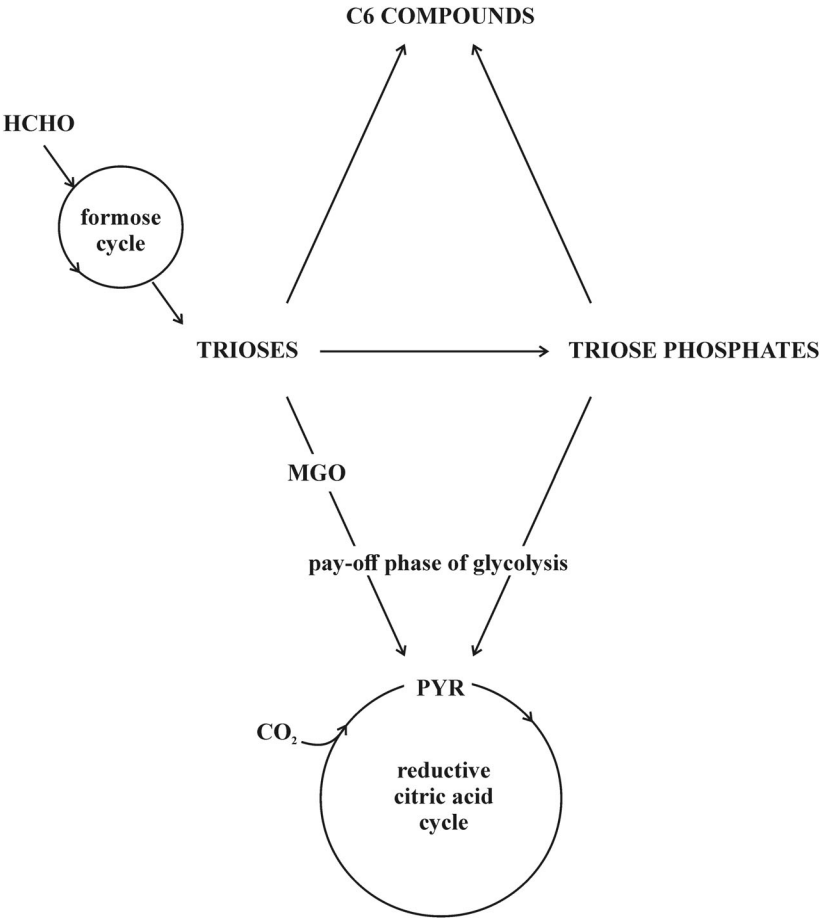


Fig. 7. Schematic integrated network for glycolysis development in the prebiotic era. MGO, methylglyoxal; PYR, pyruvate.

have come to light. Similar to present work, numerous metabolism-like chemical networks were assembled from nonenzymatic reactions under different experimental circumstances resulting in ancient glycolysis and antedating reaction sequences analogous to those used in contemporary pathways [47–49]. It turned also out that huge amount of chemicals might have been present and the observed large number of reactions included the formation and/or interconversion of glycolytic intermediates [47–49]. Moreover, the reconstructed ocean milieu increased the stability of phosphorylated intermediates as well as accelerated the rate of intermediate reactions as a result of its metal content. Particularly, ferrous iron and pH proved to be important factors in selecting out reaction routes [47,48].

In the line with experimental results, this research also showed how many compounds may have been considered in the early stage of chemical network formation from which contemporary reactions had finally been selected out (Fig. 3 and [25]). However, in contrast to the works mentioned, this study also put forward reaction mechanisms that might have played a role in the development of present-day enzyme catalysis and can be recognized as biochemical fossils while investigating enzymatic reactions (Figs 4–6).

From evolutionary perspective, this article stresses an arguably important difference between the development of nonenzymatic and enzymatic glycolysis as to the direction of their building processes. While it "seems plausible that glycolysis may have assembled from the bottom up, essentially as a reversal of gluconeogenesis" in the enzymatic era [43], based on the steps described here, there is a flat suspicion that in the nonenzymatic era the triose level was the starting point for the formation of both the C6 and C3 intermediates (Fig. 7). According to this work, while C3 intermediates evolved in the direction of glycolysis, C6 intermediates evolved in the direction of gluconeogenesis, thus documenting the central roles of trioses and triose-phosphates.

The analysis of reaction mechanisms using retrodiction method revealed a kinship between glyoxalases and GA3PDH as well as between triose-phosphate

isomerase and methylglyoxal synthase (Figs 4–6). The method of back to roots, with other words from the top-to-the-bottom or retrodiction, was proposed as an evolutionary aspect helping in data processing and understanding the development of diseases, thus indicating that the complex tends to arise from the simple [50]. The paradigm that the early chemistry already prefigured some of the key processes by which life constructs itself thus stresses that biochemical mechanisms are, at their depth, very similar in today's living organisms, since once a mechanism proved useful, Nature used it several times, so pointing to the common ancestor for life and being the basis for the unfolding of phylogenetic trees [50–52].

The transition between one developmental stage to the other in the nonenzymatic era itself and between nonenzymatic and enzymatic phases raises the versatile issue of sulfur phosphorus relationship, particularly in the regard of the emergence of high-energy bonding. Both sulfur and phosphorus are participating in high-energy bonds that are characterized by a significant change in free energy while hydrolyzing, thus making possible attached endergonic reactions to occur. On the chemical basis, thiol-moiety may theoretically react with hydroxyl-, carbonyl-, and carboxyl groups to form thioether, hemithioacetal, and TE, respectively, of which the last two are of high biological significance (Table 1.). Phosphoric acid is similarly able to interact with such functional groups, stipulated that in the case of aldehydes the enol form is the target for interaction (Table 1.).

In contemporary biochemistry, high-energy bonds are exclusively seen in bonds formed through either sulfur or phosphorus. However, the generation of such a high-energy bond is endergonic; thus, a link to an exergonic reaction, which performs an activation task, is needed for making the reaction foregoing. Experimental results are not yet available for such activation process under prebiological circumstances, but in the lack of experimental data theoretical assumptions already used and analogous reactions in the contemporary biochemistry may provide a key to the understanding of the events.

TEs are in part formed via oxidative thioesterification in present-day biochemistry as it can be recognized, for example, in the case of acetyl-coA formation in the acetaldehyde dehydrogenase (EC 1.2.1.10)-catalyzed reaction, where the aldehyde is oxidized [53].

$$\begin{aligned} &\text{Acetaldehyde} + \text{NAD}^+ + \text{CoA} \\ &\leftrightarrow \text{Acetyl}-\text{CoA} + \text{NADH} + \text{H}^+ \end{aligned} \tag{6}$$

Eventually, a similar reaction logic can be seen in action in the case of GA3PDH (EC 1.2.1.12) with the exception that thiol belongs to a CYS of the enzyme [54]. This is the mechanism essentially seen in Figs 4 and 5, with the difference that instead of NAD^+, either Zn or S^o are the hydrogen acceptors. Comparing corresponding reactions, it can be hypothesized that S^o could have been the ancestor in the lineage line for NAD^+. Important in this context is that the opposite reaction of Eqn (5), in combination with Eqn (4), could have provided enough driving force for the reactions to forego.

According to *in vitro* experiments, TE formation was enhanced at neutral pH by ultraviolet irradiation or phosphate-imidazole catalysts [55,56]. From these observations, it may be delineated that photochemical activation and/or imidazole-phosphate catalyst may have somehow enhanced TE formation under primitive, abiotic conditions in the early evolution, too. The latter was used in Figs 4 and 5. The possible mechanism for the conversions shown revealed that the formed acyl-imidazole in the presence of phosphates, by reacting with phosphate yielded a new acyl-phosphate, 1,3BPG, and imidazole was regained. The deacylation of this form is, however, conserved in contemporary phosphorylating GA3PDHs, but not in glyoxalases [32,34], stressing that glyoxalases and GA3PDHs existing today might have been in close neighborhood as to have quite similar catalytic mechanisms. And going one step further back in the timeline of evolution, they might have arisen from a common ancestor. At the same time, it is to be noted that the difference between acyl acceptors distinguishes phosphorylating and non-phosphorylating GA3PDHs from each other, too. In the second deacylation step in phosphorylating GA3PDH, inorganic phosphate functions as acyl acceptor resulting in 1,3BPG formation, while in non-phosphorylating GA3PDH, the thioacyl enzyme intermediate is attacked by an activated water molecule, leading eventually to the formation of non-activated 3PG [32].

In the contemporary evolutionary literature, a generally accepted view is that life, whatever is meant by this concept, evolved in hydrothermal vents of the archaic oceans, which were of alkaline pH [26,27]. Alkaline conditions favor phosphate chemistry since due to pK values (pKs = 2.2, 7.2, 12.3) phosphates are negatively charged, thus being rendered hydrophylic [57,58]. Importantly, at the early stage of evolution, phosphate concentrations were relatively constant with the exception of Precambrian oceans, where it was elevated [59]. Therefore, this was perhaps the time when, under such a constraint, phosphorylation as a mechanism became common. Alkaline pH is advantageous for two other reasons, too, as it promotes, on the one hand, proton

dissociation from the SH group, while on the other hand, leads to an increase in enol amount, thus rendering those more reactive [13,17,20,28]. This latter is the factor in the high phosphate transfer potential of phosphoenolpyruvate since the phosphorylated compound is "trapped" in a thermodynamically less favorable enol form, whereas after dephosphorylation it appears in the keto form (Fig. 3C).

The selection role of even simple environmental factors in the formation of a given reaction pathway is evident as indicated by the experimental results. The formation of fru-1,6BP and trunk pathway reactions requires different temperatures, probably due to the heat instability of triose-phosphates [29,44,47–49]. All these strongly suggest that the prebiological evolution of different reaction routes, even though they are tightly interrelated to each other in the contemporary biochemistry, may have happened at different places in dissimilar times and under different conditions. This way the order of pathways in which they appeared is specified, in both nonenzymatic and enzymatic era, meaning a kind of adaptation to environmental conditions [60,61]. If so, adaptation of nonenzymatic routes to environmental challenges is to be considered as a primitive evolution.

In the course of the evolutionary evaluation of the trunk part of glycolysis, this work built on the paradigm of pyrite-pulled theory [10], in which trioses were already appointed to one of the central factors [25]. Since environmental conditions are decisive, the edge conditions under which the system may operate need to be considered. The reductive power of the FeS-H_2S/FeS_2 redox couple depends on H_2S activity and is less reducing at elevated temperatures (>250 °C), while its pressure dependence in the range of 0.1–1 kbar is moderate [62]. For H_2 and SH_2, the gas solubility is more than twice at 200 °C than at 50 °C, and eight times higher at 0 °C than 200 °C, respectively [63]. And, the production of both carboxylic acids and carbonyls is thermodynamically possible in the temperature range of 50–250 °C in vents [64]. These parameters supplemented with the previously mentioned pH range (from 7.2 to 12.3) are within the assumed values for hydrothermal vent environmental factors [65] as well as fit to some extent the conditions under which the experiments were undertaken [47–49]. This posits that the mechanisms delineated here have some experimental support.

To sum up, this article highlights critical steps of nonenzymatic evolution of trunk part of glycolysis and proposes that on the one hand, triose-phosphate level might have been the starting point for the formation of both the C6 and the C3 intermediates in glucose metabolism, with the difference that the former developed in the direction of gluconeogenesis, while the latter in the direction of glycolysis. This mode of evolution differs from the direction of development of the enzymatic glycolytic sequence, stressing that the nonenzymatic and enzymatic development of glycolysis, and of other metabolic routes, as well, may have had different directions. Additionally, in attempting to address the questions of transition from nonenzymatic stage to enzymatic stage, in the course of examination of reaction mechanisms, it is not arguable that the enzymes incorporate reaction mechanisms of nonenzymatic stage and that both glyoxalases and GA3PDH, as well as methylglyoxal synthase and triose-phosphate isomerase, had common ancestors.

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Data accessibility

The data that support the findings of this study are available in the figures, table, and text of the article as well as in articles cited and indicated at the proper place.

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