Orig Life Evol Biosph (2008) 38:279–292
DOI 10.1007/s11084-008-9141-6

PREBIOTIC CHEMISTRY

Sugar-Driven Prebiotic Synthesis of 3,5(6)-Dimethylpyrazin-2-one: A Possible Nucleobase of a Primitive Replication Process

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Received: 19 May 2008 / Accepted: 4 June 2008 /
Published online: 26 June 2008

Abstract Reaction of glyceraldehyde with alanine amide (or ammonia) under anaerobic aqueous conditions yielded 3,5(6)-dimethylpyrazin-2-one that is considered a possible complementary residue of a primitive replicating molecule that preceded RNA. Synthesis of the dimethylpyrazin-2-one isomers under mild aqueous conditions (65°C, pH 5.5) from 100 mM glyceraldehyde and alanine amide (or ammonia) was complete in about 5 days. This synthesis using 25 mM glyceraldehyde and alanine amide gave a total pyrazinone yield of 9.3% consisting of 42% of the 3,5-dimethylprazin-2-one isomer and 58% of the 3,6-dimethylpyrazin-2-one isomer. The related synthesis of the dimethylpyrazin-2-one isomers from glyceraldehyde and ammonia was about 200-fold less efficient than the alanine amide reaction. This synthetic process is considered a reasonable model of origin-of-life chemistry because it uses plausible prebiotic substrates, and resembles modern biosynthesis by employing the energized carbon groups of sugars to drive the synthesis of small organic molecules. Possible sugar-driven pathways for the prebiotic synthesis of polymerizable 2-pyrazinone monomers are discussed.

Keywords Sugar chemistry · Glyceraldehyde · Pyrazinone synthesis · Sugar–amine reaction · Alanine amide · Template replication · Nucleobase synthesis · Prebiotic chemistry · Origin of life

Introduction

Modern life depends upon two types of specialized macromolecules – nucleic acids that store, replicate, and translate cellular information; and proteins that catalytically control the rates and specificity of metabolic reactions. A central question in understanding biogenesis is how these two types of macromolecules were made and functionally integrated yielding the first primitive life on the early Earth 4 billion years ago. This problem has been especially difficult to solve because the nucleic acids (DNA, RNA) are complex, relatively

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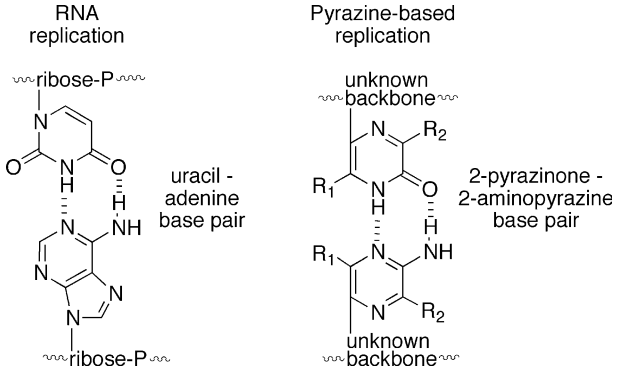

Fig. 1 Hydrogen bonds of the uracil/adenine base pair of RNA compared to the 2-pyrazinone/2-aminopyrazine base pair of a hypothetical pyrazine polymer

unstable molecules that are difficult to synthesize and maintain using only plausible prebiotic chemistry (Orgel 2004; Shapiro 2006 and references therein). In the origin-of-life field this dilemma has spurred a search for alternative self-replicating molecules that preceded nucleic acids as the first replicating molecules (Joyce et al. 1987; Orgel 1995). Most studies of alternative genetic systems have examined polymers that have different backbones than nucleic acids, but use the natural complementary A/U(T) and G/C base pairs (Orgel 2004; Schoning et al. 2002; Nelson et al. 2000). The replacement of the typical A/U and G/C base pairs with alternative base pairs (like those of pyrazines) has also been studied in molecules having the ribose backbone of nucleic acids (Benner 2004). However, the prebiotic synthesis and template replication of the alternative genetic molecules studied so far appear to be almost as problematic as that of the natural nucleic acids (Orgel 2004).

In the past year we began to explore the sugar-driven prebiotic synthesis of 2-pyrazinones and 2-aminopyrazines as possible nucleobases of a primitive replicating polymer, because as shown in Fig. 1, (a) 2-pyrazinones and 2-aminopyrazines contain groups that could form a complementary hydrogen-bonded pair resembling the complementary adenine/uracil hydrogen-bonded pair of nucleic acids (Barlin 1982), and (b) the prebiotic synthesis of the 2-pyrazinones and 2-aminopyrazines seemed feasible, since sugar–amine reactions yield α-ketoaldehydes which are known to react with amino acid amides and amino acid amidines to give substituted 2-pyrazinones and 2-aminopyrazines, respectively (Barlin 1982). Sugars are also known to react with amines and ammonia to yield other types of N-heterocyclic compounds with the potential to act as complementary hydrogen-bonded pairs (imidazoles, pyrroles, and pyridines; Kort 1970; Ledl and Schleicher 1990), and to generate melanoidin polymers (Cammerer et al. 2002; Ellis 1959) that in some cases coalesce into growing semi-solid microspherules (Weber 2005). Cleaves and Miller (2001) previously showed that the biological pyridines, nicotinic acid and its amide, could be synthesized by reacting trioses with aspartic acid and asparagine, respectively.

Materials and Methods

Materials

DL-Glyceraldehyde dimer (95%), dihydroxyacetone (97%), ammonium chloride (99.998%), glacial acetic acid (99.8%), L-alaninamide hydrochloride, L-leucinamide hydrochloride, pyruvaldehyde, ethanol, diethyl ether, sodium hydroxide (99.99%), potassium hydroxide,

37% hydrochloric acid (99.999%), and magnesium sulfate were obtained from Aldrich; sodium acetate trihydrate, DL-lithium lactate, acetonitrile (HPLC grade), 28% ammonium hydroxide, 3-chloro-2,5-dimethylpyrazine from Sigma; succinic acid, chloroform, ethyl acetate from Mallinckrodt; benzene from Fisher; 1 ml vacules from Thomas Scientific; and Silica Gel-RP18 Machery-Nagel TLC plates from Alltech (no. 811073). Previously we showed that the Aldrich DL-glyceraldehyde contained about 64% glyceraldehyde and 36% dihydroxyacetone (Weber 2007).

Synthesis of Chemical Standards

3,5-Dimethylpyrazin-2-one was synthesized from pyruvaldehyde and L-alaninamide as described in Helliwell et al. (2006) that was an adaption of the method of Karmas and Spoerri (1952); m.p. 148–150°C; lit. m.p. 146–147°C [Karmas and Spoerri 1952] and 149.5–152°C [Klein et al. 1964]. 3,6-Dimethylpyrazin-2-one was synthesized from 3-chloro-2,5-dimethylpyrazine by refluxing in 25% potassium as described in Micetich and MacDonald (1964); m.p. 210–212°C; lit. 210–211°C [Karmas and Spoerri 1952] and 215°C [Micetich and MacDonald 1964].

Reaction Method

Sugar–amine reactions were carried out in heat-sterilized 1 ml vacules using the reagents and conditions described in the figures. The vacules containing the reaction solutions were sealed in vacuo after being deaerated in a frozen state by cycling four times between a vacuum and nitrogen gas, then heated at the desired temperature in a Labnet (Model 611D) incubator, and finally stored at −80°C until analyzed.

Analysis of Pyrazine Products

Aliquots of the reaction solutions were diluted with water and analyzed by HPLC using a Beckman Model 126 HPLC system equipped with a JASCO fluorescence detector. The pyrazine products were measured by their fluorescence (excitation 320 nm, emission 400 nm) after separation on an Alltech Alltima-C18 column (5 μm, 150×3.2 mm) eluted at 0.7 ml/min with eluents (A) 20 mM Na_2HPO_4 (pH 8.7) and acetonitrile (97:3 *v*/*v*), and (B) 20 mM Na_2HPO_4 (pH 8.7) and acetonitrile (3:1 *v*/*v*). The column was initially equilibrated with 100% eluent (A). At 7 min the solvent mixture was changed linearly to 16% eluent (B) over 1 min and maintained for 11 min, then at 19 min the solvent mixture was changed linearly to 80% eluent (B) over 10 min and maintained for 4 min. Elution times of pyrazines were 2-aminopyrazine (5.0 min), 3,5-dimethylpyrazin-2-one (6.4 min), 3,6-dimethylpyrazin-2-one (7.1 min). TLC analysis used Silica Gel-RP18 Machery-Nagel TLC plates (0.25 mm, 5× 20 cm, UV-254) eluted with hexane/chloroform/methanol (15:15:2 *v*/*v*).

Results

Synthesis of 2-pyrazinones from Sugars and Amino Acid Amides

We began our investigation of the prebiotic synthesis of 2-pyrazinones by preparing crystalline 3,5-dimethylpyrazin-2-one and 3,6-dimethylpyrazin-2-one using established methods (Helliwell et al. 2006; Micetich and MacDonald 1964). After considerable effort,

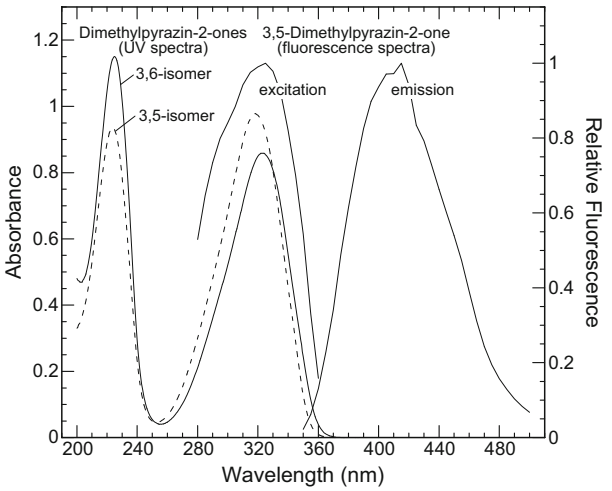

Fig. 2 Aqueous UV spectra of 3,5- and 3,6-dimethylpyrazin-2-one (0.0143 mg/ml), and fluorescence spectra of 3,5-dimethylpyrazin-2-one

we developed an HPLC method using an eluent containing dibasic sodium phosphate at pH 8.7 that gave a baseline separation of the 3,5- and 3,6-dimethylpyrazin-2-one isomers. A pH near the 2-pyrazinone p*K*a of 8.5 was required to separate the isomers. As shown in Fig. 2 we next measured the absorbance spectra of 3,5- and 3,6-dimethylpyrazin-2-one, and the fluorescence excitation and emission and spectra of 3,5-dimethylpyrazin-2-one. These absorbance and fluorescence spectra enabled the development of a sensitive HPLC detection method for the 2-pyrazinone products.

With our new HPLC method, we examined the time course of the synthesis of 3,5- and 3,6-dimethylpyrazin-2-one isomers at 65°C from 100 mM DL-glyceraldehyde and 100 mM L-alanine amide in a 500 mM acetate buffer (pH 5.5). As shown in Fig. 3a, under these

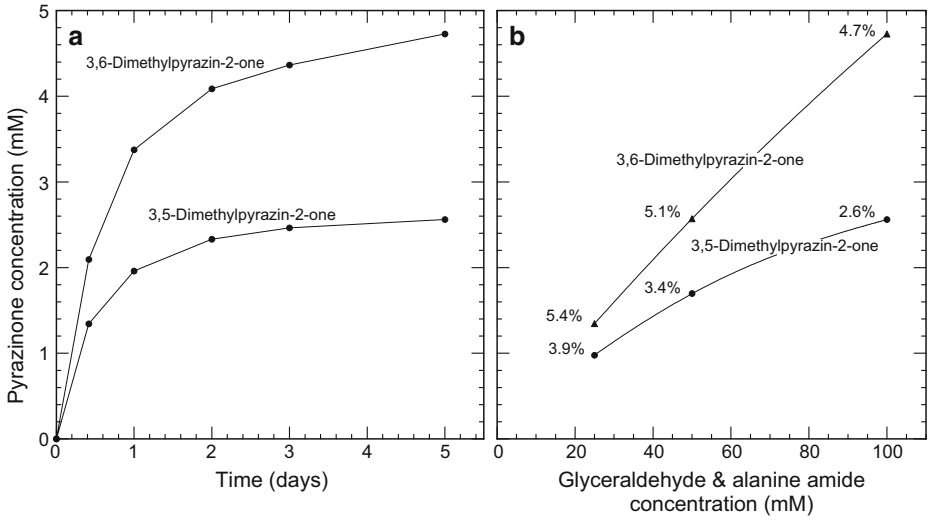

Fig. 3 Synthesis of 3,5-dimethylpyrazin-2-one and 3,6-dimethylpyrazin-2-one from DL-glyceraldehyde and L-alanine amide in a 500 mM sodium acetate at pH 5.5 and 65°C as a function of **a** reaction time using 100 mM glyceraldehyde and 100 mM alanine amide, and **b** reactant concentration (yields measured at 5 days). The percentages shown are percent yields based on glyceraldehyde

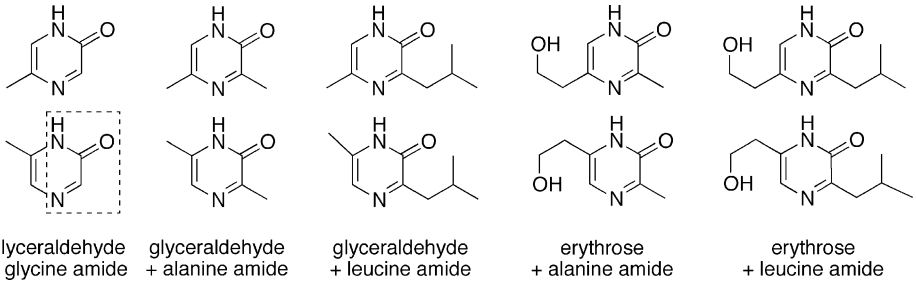

Fig. 4 Structure of 3,5- and 3,6-disubstituted 2-pyrazinone isomers derived from different sugar and amino acid amide reactants

conditions pyrazinone synthesis is nearly complete by 5 days and yields roughly twice as much 3,6-isomer as 3,5-isomer. Reducing the temperature of this reaction to 50°C decreased the rate of pyrazinone synthesis by about 50%. The total pyrazinone yield for a similar reaction using dihydroxyacetone was about two-thirds of that observed for glyceraldehyde. Figure 3b shows the pyrazinone yield as a function of the glyceraldehyde and alanine amide concentration. As expected, the absolute yields of the pyrazinone isomers increase as the substrate concentration increases from 25 to 100 mM. However, the total percent yield of pyrazinone (based on glyceraldehyde) decreases from 9.3% to 7.3% as the substrate concentration increases from 25 to 100 mM. The decrease in percent yields of the pyrazinone isomers at the higher reactant concentrations is probably due to intermolecular side reactions that interfere with the synthetic process, or modify the structure of the pyrazinone products.

The prebiotic synthesis of the 2-pyrazinones shown in Fig. 4 was also examined by reacting DL-glyceraldehyde with the glycine amide, L-alanine amide and L-leucine amide; and by reacting D-erythrose with L-alanine amide and L-leucine amide. Preliminary analysis using thin-layer chromatography showed that each reaction yielded products that had the characteristic blue fluorescence and mobility expected for their respective 2-pyrazinone products. In the pyrazinone structures shown in Fig. 4, the amino acid amide reactant contributes the two nitrogen groups and the carbon groups to the right of the nitrogens; the sugar reactant contributes the carbon groups to the left of the nitrogen groups. In the figure the groups derived from glycine amide reactant are enclosed within a dashed box.

Synthesis of 2-pyrazinones from Glyceraldehyde and Ammonia

As shown in Fig. 5a we next examined the time course of the synthesis of the 3,5- and 3,6-dimethylpyrazin-2-one isomers at 65°C from 100 mM DL-glyceraldehyde and 100 mM ammonia in a 500 mM acetate buffer (pH 5.5). As shown, the ammonia reaction, like the alanine amide reaction, yields about twice as much 3,6-isomer as it does 3,5-isomer. However, the ammonia reaction reaches completion in about 3 days instead of the 5 days required by the alanine amide reaction. As shown in Fig. 5b, the absolute yields of the pyrazinone isomers increase as the substrate concentration increases from 50 to 200 mM, but the total percent yield of pyrazinone (based on glyceraldehyde) decreases from 0.049% to 0.032% as the substrate concentration increases from 50 to 200 mM. The table inserted in the figure shows that reacting 100 mM glyceraldehyde with excess 200 mM ammonium chloride increases the total percent yield of pyrazinone from 0.049% to 0.064%. Like the reaction with alanine amide, the observed decrease in pyrazinone yield at the higher reactant concentrations is probably due to intermolecular side reactions that interfere with the synthetic process, or

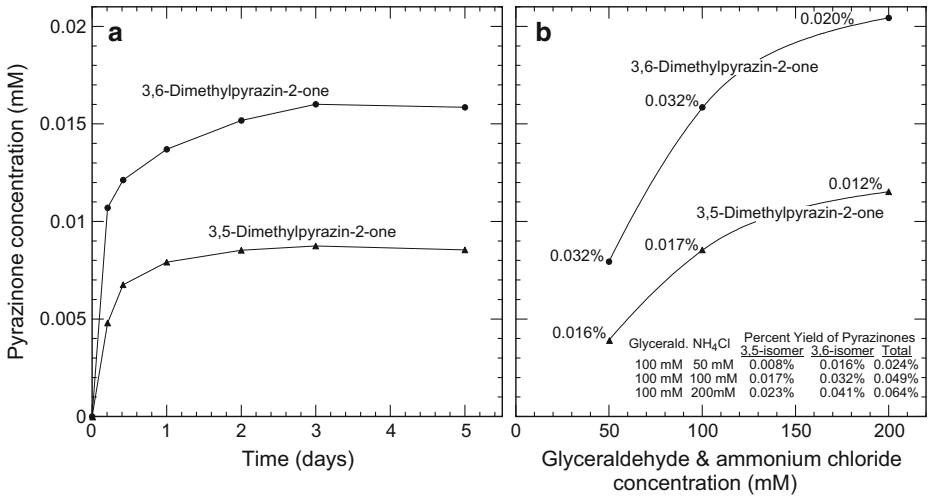

Fig. 5 Synthesis of 3,5-dimethylpyrazin-2-one and 3,6-dimethylpyrazin-2-one from DL-glyceraldehyde and ammonium chloride in 500 mM sodium acetate at pH 5.5 and 65°C as a function of **a** reaction time using 100 mM glyceraldehyde and 100 mM ammonium chloride, and **b** reactant concentration (yields measured at 5 days). The percentages shown are percent yields based on glyceraldehyde

modify the structure of the pyrazinone products. For comparison, the percent yield of the pyrazinone isomers from 50 mM pyruvaldehyde and 100 mM ammonium chloride under the same conditions was 0.88%, or about 10-times greater than the glyceraldehyde reaction.

Figure 6 depicting the pH and buffer dependence of pyrazinone synthesis from glyceraldehyde and ammonium chloride shows that the pH 7 reaction is slower than the other reactions carried out in the pH 4–5.5 region which are near completion at 5 days. The highest pyrazinone yields also occur in the pH 4.5–5.5 region. The yield at pH 5.5 in the succinate buffer is appreciably higher than in the acetate buffer.

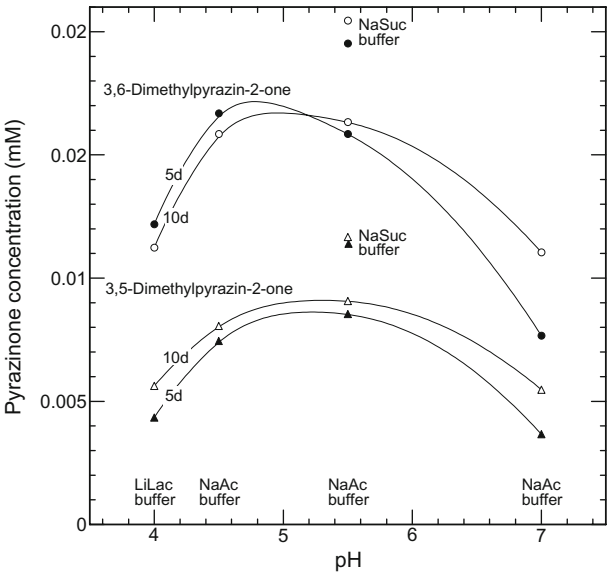

Fig. 6 Buffer and pH dependence of the synthesis of 3,5- and 3,6-dimethylpyrazin-2-one from 100 mM DL-glyceraldehyde and 100 mM ammonium chloride in 500 mM buffers at 65°C. *NaAc* Sodium acetate, *NaSuc* sodium succinate, *LiLac* lithium lactate

Discussion

Pathways of 2-pyrazinone Synthesis from Glyceraldehyde and Alanine Amide

Figure 7a shows possible reaction pathways for the synthesis of 3,5- and 3,6-dimethylpyrazin-2-ones from glyceraldehyde and alanine amide. Since at 50°C the rate of synthesis of the pyrazinone isomers is much faster than the observed uncatalyzed rate at 50°C of glyceraldehyde dehydration to pyruvaldehyde (an intermediate needed for pyrazinone synthesis) the dehydration of glyceraldehyde to pyruvaldehyde undoubtedly involves covalent catalysis by alanine amide as previously described for structurally similar alanylalanine (Weber 2001). As shown in the figure, this catalytic process most likely begins by addition of glyceraldehyde to alanine amide to give either glyceraldehyde–carbinolamide (I) by pathway A1, or glyceraldehyde–carbinolamine (II) by pathway A2 (Challis and Challis 1970; Ogata and Kawasaki 1970). Glyceraldehyde–carbinolamine (II) then undergoes a sequence of transformations involving: (a) dehydration of the carbinolamine bond that yields glyceraldehyde–imine (IV), (b) irreversible β-dehydration of the glyceraldehyde residue of imine (IV) to give pyruvaldehyde–imine (VI) (Weber 2001), (c) cyclization of imine (VI) yielding dihydropyrazin-2-one (VIII), and finally (d) dehydration of pyrazinone (VIII) to give 3,6-dimethylpyrazin-2-one. Alternatively, glyceraldehyde–imine (IV) could be converted to pyruvaldehyde–imine (V) and/or (VI) by pathway B that involves cyclization of glyceraldehyde–imine (IV) to imidazolidinone (IX) that dehydrates to imidazolidinone (X) which can open, yielding pyruvaldehyde–imines (V) and/or (VI) that undergo the transformations described earlier to give their respective 3,5- and 3,6-

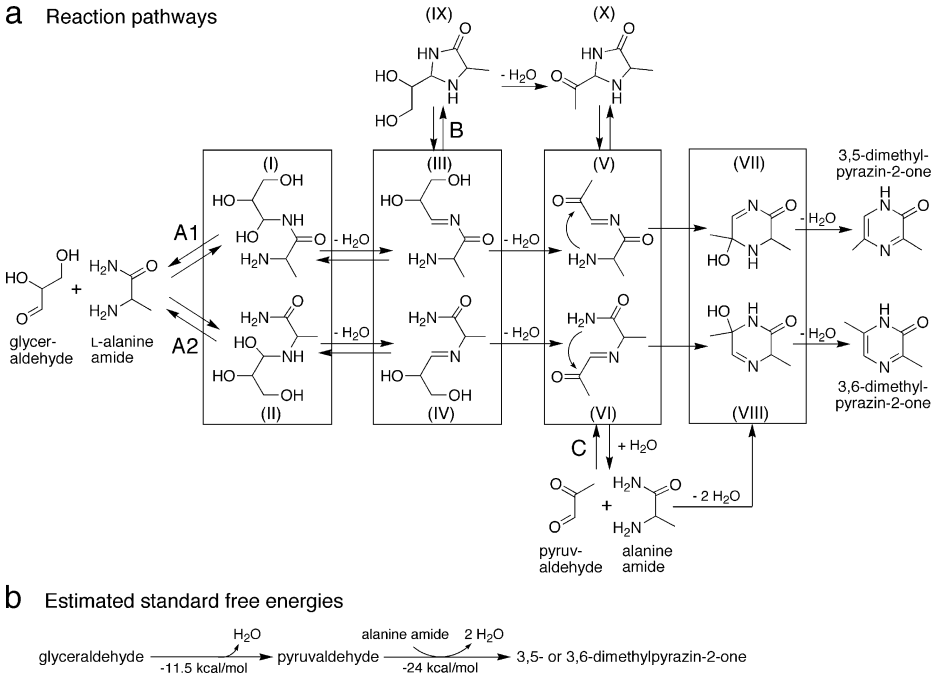

Fig. 7 Synthesis of 3,5- and 3,6-dimethylpyrazin-2-ones from DL-glyceraldehyde and L-alanine amide at pH 5.5 and 65°C: **a** plausible reaction pathways showing intermediates, **b** estimated standard free energies of reaction at pH 7 (Mavrovouniotis 1991)

dimethylpyrazin-2-one isomers. In addition, the conversion of intermediates (V) and (VI) to cyclic intermediates (VII) and (VIII) could occur by pathway C that involves hydrolysis of intermediates (V) and (VI) to free pyruvaldehyde and alanine amide that recombine to give, respectively, cyclic dihydropyrazin-2-ones (VII) and (VIII) that are converted as described earlier to their respective 3,5- and 3,6-dimethylpyrazin-2-one isomers.

Although glyceraldehyde–carbinolamide (I) is known to dehydrate to glyceraldehyde–(*N*-acyl)-imine (III) (Ogata and Kawasaki 1970), the irreversible β-dehydration of the glyceraldehyde residue of (*N*-acyl)-imine (III) to give the pyruvaldehyde–(*N*-acyl)-imine (V) is uncertain, since we have not found a description of this reaction in the chemical literature. However, if the conversion of imine (III) to imine (V) is blocked, the synthesis of 3,5-dimethylpyrazin-2-one could use pyruvaldehyde–(*N*-acyl)-imine (V) synthesized by pathway B and/or dihydropyrazin-2-one (VII) formed via Pathway C.

Figure 7b shows that the synthesis of the pyrazinones from glyceraldehyde and alanine amide has a very favorable free energy of about −35 kcal/mol. In this synthesis the dehydration of glyceraldehyde to pyruvaldehyde contributes −11 kcal/mol, and the pyruvaldehyde–alanine amide condensation–dehydration supplies the remaining −24 kcal/mol of pyrazinone synthesis. These very favorable energy values show that the synthesis of 2-pyrazinone from glyceraldehyde and alanine amide is thermodynamically feasible at submicromolar substrate concentrations.

Pathways of 2-pyrazinone Synthesis from Glyceraldehyde and Ammonia

Figure 8a shows reaction pathways likely to be involved in the synthesis of 3,5- and 3,6-dimethylpyrazin-2-ones from glyceraldehyde and ammonia. Since at 65°C the rate of synthesis of the pyrazinones is much faster than the expected uncatalyzed rate of glyceraldehyde dehydration to pyruvaldehyde needed for pyrazinone formation, the dehydration of glyceraldehyde to pyruvaldehyde undoubtedly involves covalent catalysis by ammonia as described

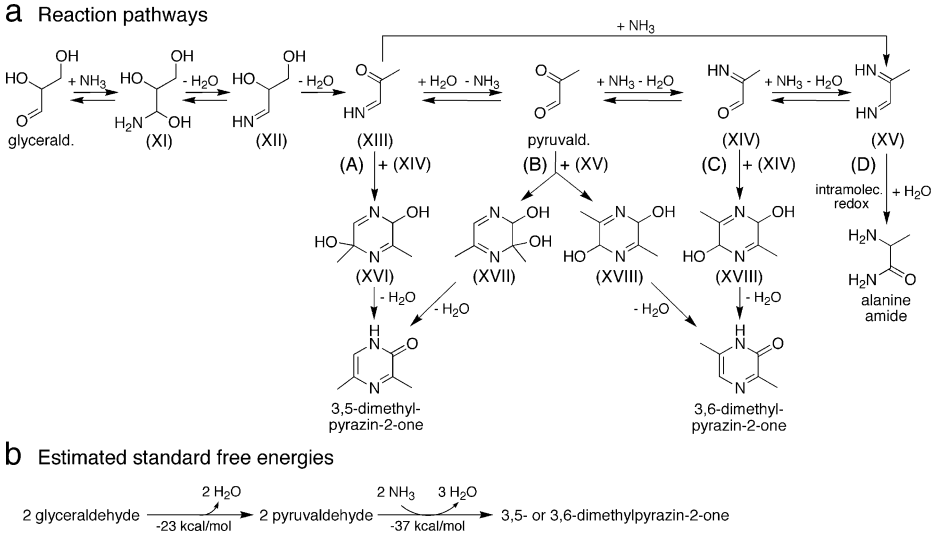

Fig. 8 Synthesis of 3,5- and 3,6-dimethylpyrazin-2-ones from DL-glyceraldehyde and ammonia at pH 5.5 and 65°C: **a** plausible reaction pathways showing intermediates, **b** estimated standard free energies of reaction at pH 7 (Mavrovouniotis 1991)

earlier by Weber (2001). As shown in the figure, this catalytic process begins by covalent addition of ammonia to glyceraldehyde's aldehyde group to give glyceraldehyde–carbinolamine (XI) that undergoes dehydration to give glyceraldehyde–imine (XII) (Ogata and Kawasaki 1970). This reaction is followed by irreversible β-dehydration of the glyceraldehyde residue of imine (XII) that yields pyruvaldehyde–imine (XIII) which undergoes a sequence of transformations involving the reversible addition and removal of water and ammonia to generate free pyruvaldehyde (Weber 2001), and pyruvaldehyde–ammonia adducts (XIV) and (XV). As shown, pyruvaldehyde and its imines (XIII), (XIV), and (XV) undergo condensation reactions with each other by pathways A, B, and C to give cyclic intermediates (XVI, XVII) and (XVIII) which dehydrate to the 3,5- and 3,6-dimethylpyrazin-2-one isomers, respectively. Alternatively, as shown in pathway D, intermediate (XV) could undergo intramolecular reduction–oxidation to give alanine amide, since similar α-ketoaldehyde–amine adducts have been shown to rearrange to amino acid amides (Maurer and Woltersdorf 1938). If this is the case, then some fraction of the pyrazinone product could be synthesized by reaction of glyceraldehyde with alanine amide synthesized in situ.

Figure 8b shows that the synthesis of the 3,5 and 3,6-dimethylpyrazinones from glyceraldehyde and ammonia has a very favorable free energy of about −60 kcal/mol that makes it thermodynamically feasible at very low substrate concentrations. In this synthesis the dehydration of two glyceraldehyde molecules to give two pyruvaldehyde molecules contributes −23 kcal/mol. The subsequent condensation of the two pyruvaldehyde molecules with two ammonia molecules to give cyclic intermediates that dehydrate forming the pyrazinone isomers supplies the remaining −37 kcal/mol of pyrazinone synthesis.

Synthesis and Oligomerization of Reactive Pyrazine Monomers

Complementary pyrazine residues could have been the templating residues of oligomers with or without the phosphodiester backbone of modern RNA or its simpler analogs (Benner 2004; Orgel 2004; Schoning et al. 2002; Zhang et al. 2005; Bean et al. 2006). The results presented here indicate that phosphorylated pyrazine monomers capable of forming pyrazine–glycol RNA "PZNA" could be synthesized by reaction of pentose phosphates with amino acid amides (or ammonia) as shown in Fig. 9. This primitive polymer, like glycol–RNA (Zhang et al. 2005), has one asymmetric carbon per backbone residue that could complicate its replication chemistry. However, the primitive polymer is stereochemically much simpler than RNA that has 4-asymmetric carbons for each β-D-ribose residue. The elimination of the asymmetry at carbons 1 thru 3 of the pentose-phosphate substrate during pyrazine ring synthesis allows the homochiral primitive polymer to be made from

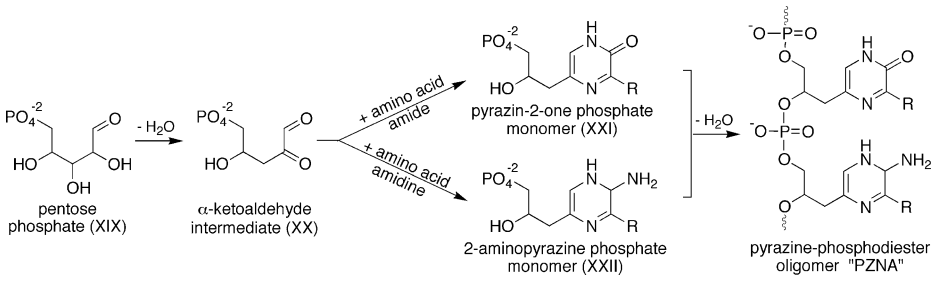

Fig. 9 Proposed pathway for the synthesis of pyrazine phosphodiester oligomers from pentose-5-phosphate reacted with an amino acid amide and amino acid amidine

any D-pentose (i.e. the α- and β-forms of D-ribose, D-arabinose, D-xylose, D-lyxose, D-ribulose and D-xylulose) or alternatively any L-pentose. However, since the prebiotic plausibility of this process depends on the uncertain availability of the labile phosphate anhydrides needed for sugar phosphate synthesis and polymerization, we have begun to consider, within the constraints of sugar chemistry, other pyrazine polymer backbone structures that do not contain phosphate.

Figure 10 shows two processes that could have driven the irreversible oligomerization of non-phosphorylated 2-pyrazinone and 2-aminopyrazine complementary monomers. In Fig. 10a the first type of oligomerization process begins by formation of a reversible hemiacetal bond between 2-pyrazinone (XXIII) and 2-aminopyrazine (XXVI) to give hemiacetal oligomer (XXIV) that dehydrates irreversibly to enol ether oligomer (XXV) (March 1992; Ogata and Kawasaki 1970). The second type of oligomerization process proceeds by aldol condensation yielding aldol oligomer (XXVII) that dehydrates irreversibly to dehydrated aldol oligomer (XXVIII) (Reeves 1966). In both cases, the initial condensation reaction that reversibly joins the monomer to the growing polymer is followed by irreversible dehydration driven by the conjugation of its unsaturated product with the aromatic pyrazine ring. For the dehydration reactions to yield a ring-conjugated double bond, the monomer's carbonyl group must be located at the β-position of the side chain. In the hemiacetal condensation process, the alcohol group can be located at any position in the monomer's side chain, thereby allowing the formation of backbones having four, six, or more atoms. However, the aldol condensation process is limited to side chains having three or five carbons, since the nucleophilic hydroxymethyl group must be located adjacent to the β-positioned carbonyl group.

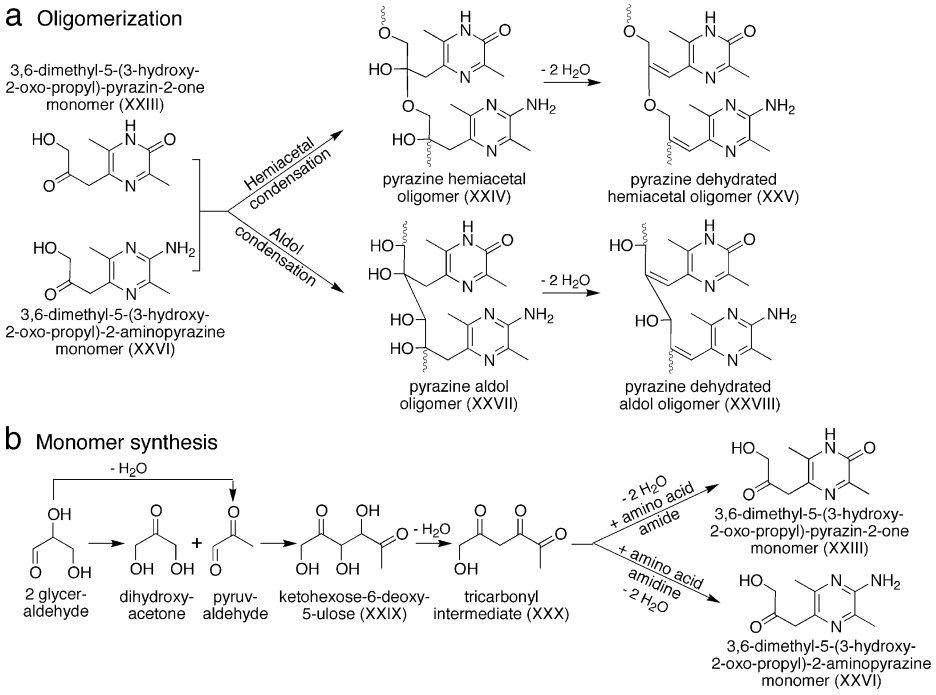

Fig. 10 Proposed pathways for the sugar-driven **a** oligomerization and **b** synthesis of polymerizable 2-pyrazinone and 2-aminopyrazine monomers

Figure 10b shows feasible pathways for the syntheses of the polymerizable 2-pyrazinone (XXIII) and 2-aminopyrazine (XXVI) monomers shown in Fig. 10a. These syntheses use the same sugar transformation reactions as the 2-pyrazinone syntheses reported here (Weber 2001). As depicted, the synthesis of the polymerizable pyrazinone monomer (XXIII) begins by glyceraldehyde isomerization to dihydroxyacetone, and dehydration to pyruvaldehyde. The dihydroxyacetone and pyruvaldehyde products then condense to give ketohexose (XXIX) that dehydrates to tricarbonyl intermediate (XXX). Next, the α-ketoaldehyde group of intermediate (XXX) condenses either with an amino acid amide or amino acid amidine to give cyclic intermediates (not shown) that dehydrate to polymerizable 2-pyrazinone (XXIII) and 2-aminopyrazine (XXVI) monomers, respectively. The reaction of glyceraldehyde with ammonia is also expected to yield polymerizable 2-pyrazinone monomer (XXIII), because, as reported here, the reaction of glyceraldehyde with ammonia produced a small amount of 5(6)-dimethylpyrazin-2-one by a similar process.

Although 2-pyrazinone and 2-aminopyrazine are an attractive complementary pair, it may be that the earliest replicating system used 2-pyrazinone paired with another heterocyclic ring that did not have an exocyclic amino group, since (a) structurally similar 2-pyridinone forms self-dimers even though it lacks an exocyclic amino group and contains only the –CO–NH– hydrogen-bonding group (Beak et al. 1980), and (b) the versatility of sugar-based synthesis suggests that it has the potential to produce, in addition to 2-pyrazinones, other heterocyclic molecules with complementary hydrogen-bond interactions, like 2-pyridinones, 1,5-dihydro-4-imidazolones, and 3,4-dihydro-2-pyrrolones containing sides chains that could modify their complementary interactions. In this regard, sugar reactions with amines and ammonia are known to yield imidazoles, pyrroles, and pyridines (Kort 1970; Ledl and Schleicher 1990). Template-directed oligomerization of the non-phosphorylated polymerizable monomers is envisioned as occurring within organic domains composed of coalesced template-synthesized catalytic oligomers that control monomer synthesis and oligomerization.

Attractive Characteristics of Sugar-driven 2-pyrazinone Synthesis

The synthesis of uracil-like 2-pyrazinone from glyceraldehyde and alanine amide (or ammonia) demonstrates that sugars have the ability to drive the synthesis of nitrogen-heterocycles that could have acted as coding residues in a primitive replicating molecule before the advent of RNA replication. This sugar-based synthesis of 2-pyrazinones is an attractive model prebiotic process for several reasons. First, this synthesis is a spontaneous "one-pot" aqueous process under mild aqueous conditions that does not require an external source of energy, since it is driven by energy stored in the sugar molecule (Weber 2000, 2002). Second, the synthetic process has the ability to generate a variety of 2-pyrazinones with different 3,5- and 3,6-positioned substituents that could have functioned as catalytic groups or polymer backbone residues. The incorporation of the side chain of the amino acid amide substrate into the 3-position of the 2-pyrazinone ring is especially attractive, because it adds the catalytic potential of amino acid side chains to the 2-pyrazinone ring, thereby giving the pyrazinone ring both hereditary (nucleic acid-like) and catalytic (protein-like) properties. Furthermore, the –CO–NH– group of 2-pyrazinone could have catalytic properties, since the same group in 2-pyridinones has been shown to coordinate metal ions, and catalyze both sugar mutarotation and ester aminolysis (Fischer et al. 2005; Kuzuya et al. 1984; Rawson and Winpenny 1995).

Third, 2-pyrazinones and 2-aminopyrazines appear to be considerably more stable than the nucleic acid bases as assessed by Shapiro (2006 and ref. therein) and Levy and Miller (1998).

For example, 2-pyrazinone and 2-aminopyrazine can be heated 3–5 min at 280°C in vacuo without any measurable decomposition (Konakahara and Takagi 1977). Also, 2-pyrazinones are stable under the extreme conditions used in their syntheses, like refluxing in either 25% potassium hydroxide for 50 h or 5 N hydrochloric acid for 18 h (Barlin 1982 and ref. therein). Likewise, 2-aminopyrazines are stable during their preparation from lumazines in 10% sodium hydroxide at 170°C for 2 h. Only under the more extreme condition of 20% sodium hydroxide at 170°C for 20 h, are 2-aminopyrazines hydrolyzed to 2-pyrazinones (Weijlard et al. 1945).

Finally, 2-pyrazinone synthesis is prebiotically plausible, since it uses simple substrates, like sugars, ammonia, and amino acid amides that have been synthesized under reasonable prebiotic conditions. Sugars have been synthesized abiotically from formaldehyde and glycolaldehyde (Schwartz and de Graaf 1993; Weber 2001). Formaldehyde has been formed under a variety of prebiotic conditions (Chittenden and Schwartz 1981; Hubbard et al. 1971; Stribling and Miller 1987), including the photochemical synthesis of formaldehyde in the prebiotic atmosphere (Canuto et al. 1983; Ferris and Chen 1975; Kasting and Pollack 1984) and hydrosphere (Aurian-Blajeni et al. 1980; Halmann et al. 1981). Glycolaldehyde could have been made from formaldehyde by the formose reaction (Schwartz and de Graaf 1993), or alternatively, synthesized photochemically in the atmosphere (Bacher et al. 2001). Ammonia has been prebiotically synthesized by the reduction of nitrite (or nitrate) by soluble ferrous ion or ferrous sulfide (Summers 2005). Amino acid amides could have been synthesized by the Strecker synthesis (Miller and Van Trump 1981); by rearrangement of sugar-derived α-ketoaldehyde intermediates (Maurer and Woltersdorf 1938); or by ammonolysis of amino acid thioesters formed by rearrangement of sugar-derived α-ketoaldehyde intermediates in the presence of ammonia and a thiol (Weber 1998).

The unique ability of sugars to drive the synthesis of 2-pyrazinones and other heterocyclic molecules (i.e. pyrroles, imidazoles, pyrazines, pyridines) comes from (a) the favorable energy of sugar alcohol group dehydration that is responsible for the synthesis of the double bonds of the heteroaromatic rings (Weber 2000, 2002), (b) the enhanced reactivity imparted by the sugar carbonyl group (Weber 2004), and (c) the ability of the carbonyl group to form catalytically active, reversible, covalent adducts with ammonia, amines and amides (Challis and Challis 1970; Ogata and Kawasaki 1970; Weber and Pizzarello 2006). These reversible C–N bonds that lead to incorporation of nitrogen into the pyrazinone ring also mediate amine-catalyzed dehydration of sugars that generate the essential α-ketoaldehyde intermediates (Weber 2001). Since amino acid amides and ammonia catalyze not only sugar dehydration, but also sugar synthesis from formaldehyde and glycolaldehyde (Weber 2001; Weber and Pizzarello 2006), it seems likely that 2-pyrazinones can be synthesized from formaldehyde and glycolaldehyde reacted with amino acid amides (or ammonia) which acts as both a reactant and catalyst. The synthesis of pyrazinones starting with formaldehyde, glycolaldehyde, and ammonia has an estimated favorable free energy of about −60 or −10 kcal/mol of carbon.

Acknowledgments I thank Esther Varon for technical assistance in these studies. This investigation was supported by a grant (NNA05CP68A) from the Exobiology Program of the National Aeronautics and Space Administration.

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