

# Aldolase as a Chirality Intersection of L-Amino Acids and D-Sugars

Toratane Munegumi

Received: 15 November 2014 / Accepted: 7 January 2015 /

Published online: 26 February 2015

© Springer Science+Business Media Dordrecht 2015

**Abstract** Aldolase plays an important role in glycolysis and gluconeogenesis to produce D-fructose-1,6-bisphosphate (D-FBP) from dihydroxyacetone phosphate (DHP) and D-glyceraldehyde-3-phosphate (D-GAP). This reaction is stereoselective and retains the D-GAP 2*R* configuration and yields D-FBP (with the configuration: 3*S*, 4*S*, 5*R*). The 3- and 4-position carbons are the newly formed chiral carbons because the 5-position carbon of D-FBP comes from the 2-position of D-GAP. Although four diastereomeric products, (3*S*, 4*R*, 5*R*), (3*R*, 4*R*, 5*R*), (3*R*, 4*S*, 5*R*), (3*S*, 4*S*, 5*R*), are expected in the nonenzymatic reaction, only the (3*S*, 4*S*, 5*R*) diastereomer (D-FBP) is obtained. Therefore, the chirality in the 3- and 4-positions is induced by the chirality of the enzyme composed of L-amino acid residues. D-Glucose-6-phosphate (D-G6P), which is generated from D-FBP in the gluconeogenesis pathway, produces D-ribose-5-phosphate (D-R5P) in the pentose phosphate pathway. D-R5P is converted to PRPP (5-phosphoribosyl- $\alpha$ -pyrophosphate), which is used for the *de novo* synthesis of nucleotides. Ribonucleic acid (RNA) uses the nucleotides as building blocks. The configurations of the 4*R*-carbon and of the 3*S*-carbon are retained. The stereochemical structure of RNA is based on 3*S* as well as 4*R* (D). The consideration above suggests that aldolase is a key enzyme that determines the 3*S* configuration in D-R5P. It is thus a chirality intersection between amino acids and sugars, because the sugar chirality is determined by the chiral environment of an L-amino acid protein, aldolase, to produce D-FBP.

**Keywords** Aldolase · Metabolism · L-amino acid · D-sugar · Chirality

## Introduction

All organisms on Earth use L- amino acids in proteins and D-form nucleotides in nucleic acids. Although each monomeric compound in proteins and nucleotides is homochiral, overall the monomeric compounds are heterochiral to each other, as shown in Fig. 1.

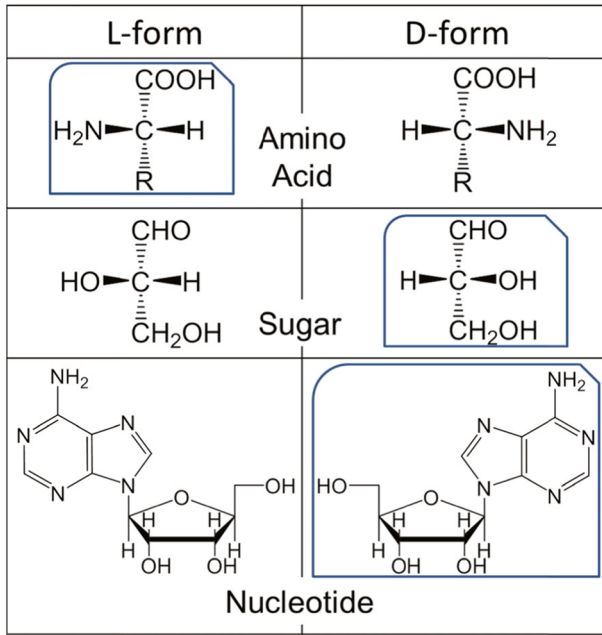
L-Amino acids, D-sugars, and D-nucleotides form the polymers proteins, polysaccharides, and polynucleotides. The origin and development of homochirality in biology have been

---

Paper presented at ORIGINS 2014, Nara Japan, July 6-11 2014.

T. Munegumi (✉)

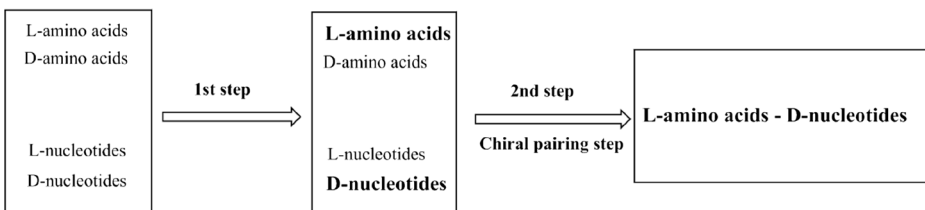
Naruto University of Education, Naruto, Tokushima 772-8502, Japan  
e-mail: tmunegumi@naruto-u.ac.jp



**Fig. 1** Heterochiral and homochiral monomeric compounds of biopolymers

discussed for many years (Avalos et al. 1998; Palyi et al. 2004; Breslow et al. 2010; Pavlov and Klabunovskii 2014). However, most hypotheses are restricted to the chirality of monomeric compounds and do not explain the heterochiral origins of products formed between amino acids and nucleotides as well as the homochirality in biopolymers (Munegumi and Shimoyama 2003), although several research results have been published (Pizzarello and Weber 2004; Tamura and Schimmel 2004). The heterochiral relationship between amino acids and nucleotides has been discussed by Weber (1987); he used the expression ‘chiral pairing’ (Weber 1987) between L and D, as shown in Fig. 2.

The first step is characterized as ‘the origin of optical activity, either the L-amino acid or D-nucleotide was initially a small excess of enantiomer’ (Weber 1987). The first step includes the enantiomeric resolution of monomeric compounds: L-amino acids or D-amino acids. The second step is characterized as the step in which ‘either the chiral L-amino acids caused the selection of D-nucleotides or, conversely, D-nucleotides brought about the selection of L-amino acids’ (Weber 1987). He explains the selection by the chemical difference between the L-amino acid–D-nucleotide pair and its diastereomers. The second step can lead to stereospecific (Smith and March 2007) condensation to give L-homochiral polypeptides plus D-



**Fig. 2** Chiral pairing (heterochiral pairing) between L-amino acids and D-nucleotides

homochiral polynucleotides or D-homochiral polypeptides plus L-homochiral polynucleotides. Modern terrestrial organisms use homochiral condensation to give polypeptides from heterochiral pairs. Several experimental results of stereospecific condensation reactions have been shown by Profy and Usher (1984) and Weber (1987).

'Chiral pairing' seems to be a key expression in solving the puzzle of the heterochiral relationship between amino acids and nucleotides. The chiral pairing that Weber (1987) showed suggests that the heterochiral chiral pairing between L-amino acids and D-nucleotides is a stronger interaction than the homochiral interaction between L and L or D and D. However, we should call the chiral pairing between L and D 'heterochiral pairing' because there may be 'homochiral chiral pairing' between L and L or D and D (Fig. 2). Because heterochiral pairing is stronger than homochiral pairing, modern organisms use the heterochiral pairing between L-amino acids and D-nucleotides.

As well as the chemical evolution of L-amino acids and D-sugars, the early metabolic pathways for the biosynthesis of those compounds may serve to reinforce heterochiral pairing. I concentrate here on several enzymes, namely transaminase, enolase, and aldolase in the amino acid biosynthesis, the glycolytic, and/or gluconeogenesis, respectively (Say and Fuchs 2010; Voet and Voet 2011). In particular, this research stresses the role of aldolase, which might have played an important role in the origin and development of the heterochiral pairing between amino acids and sugars. I discuss the theme of this paper using the results in the literature and some calculations using molecular modeling software to explore some viewpoints on heterochiral pairing.

## Results and Discussion

### Chiral Intersections of L-Amino Acids and D-Sugars in the Metabolic Pathways

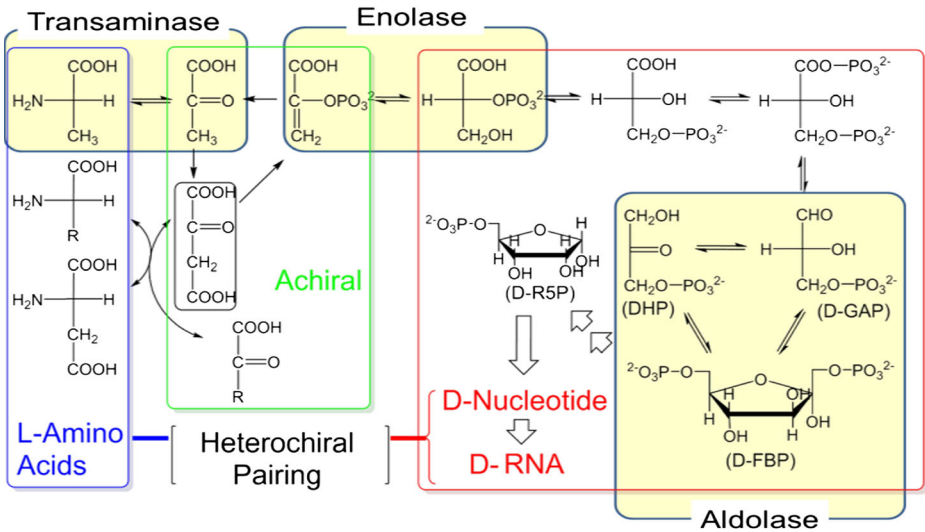
Figure 3 summarizes the main metabolic pathways involving chirality inversion and retention of amino acids and sugars. Transaminase catalyzes the amino acid formation from pyruvate and its reversible reaction. The enzyme transaminase is composed of L-amino acids, which forms an achiral compound, pyruvate, and L-amino acids.

Enolase catalyzes the interconversion between phosphoenol pyruvate and D-2-phosphateglycerate (D-2PG). One of the reversible pathways is glycolysis and another is gluconeogenesis. Therefore, the transformation of L-amino acids to D-2PG and its reversible reactions are concentrated in these processes in metabolism.

D-2PG is converted to 3-phosphoglycerate (D-3PG) and then 1,3-bisphosphoglycerate (D-1,3PG), which affords two sugars: glyceraldehyde 3-phosphate (D-GAP) and dihydroxyacetone 3-phosphate (DHP). These two compounds are coupled to give D-fructose 1,6-bisphosphate (D-FBP) by aldolase in gluconeogenesis.

In the pentose phosphate pathway, D-FBP yields ribose-5-phosphate (D-R5P) which is a building block for nucleotide synthesis. Thus, D-polynucleotides play a role of heterochiral pairing with L-amino acids.

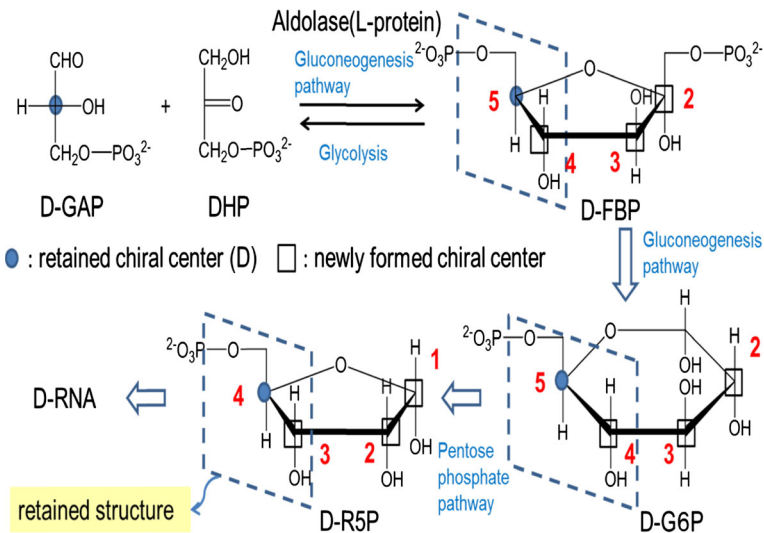
In the formation of D-FBP by aldolase, the configuration of D-GAP is retained at the 5-position of D-FBP, which has four chiral centers at the 2-, 3-, 4- and 5-positions, as shown in Fig. 4. The chiral centers at the 2-, 3- and 4-positions are newly formed by aldolase, and the configurations of the three chiral centers are retained during the synthesis of D-G6P in gluconeogenesis. However, in the pentose phosphate pathway, the chiral center at the 3-position of D-G6P is inverted to give D-R5P by ribose-5-phosphate isomerase. The chiral centers at the 4- and 5-positions in D-FBP are retained in the metabolic pathways to D-R5P, as shown in Fig. 4.



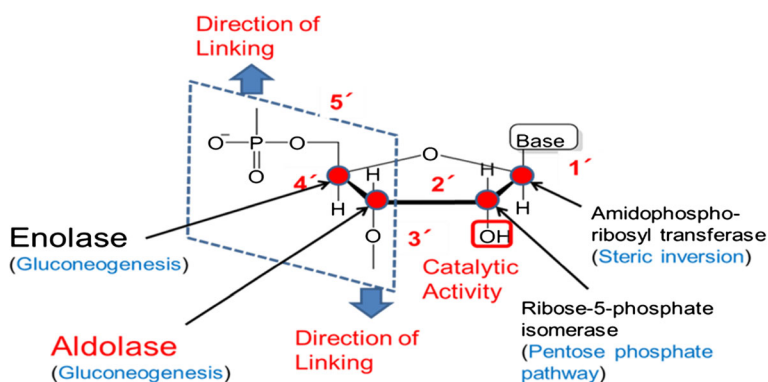
**Fig. 3** Metabolic pathways involving chiral inversion and retention in amino acids and sugars

Moreover, the chiral center at the 1-position of D-R5P is inverted by aminodophosphoribosyltransferase during the construction of D-ribonucleotides, in which the chiral center at the 1'-position (Fig. 5) corresponds to the 1-position of D-R5P.

Figure 5 summarizes the chiral retention and inversion reactions during the metabolic pathways from glyceraldehyde 2-phosphate to nucleotides. In the gluconeogenesis and pentose phosphate pathways to the direction of ribose, initially the 4-position has the *R* configuration, which enolase directly links to the achiral precursor. The chiral centers at the 3'- and 4'-positions are introduced by aldolase and enolase, respectively. The chiral carbon at the 2'-position is introduced by ribose-5-phosphate isomerase in the pentose phosphate pathway. The



**Fig. 4** Retention of configuration at the 4- and 5-positions of D-FBP



**Fig. 5** The enzymes that originally introduce chiral centers to the ribose moiety in RNA

chiral center at the 1'-position is introduced by amidophosphoribosyltransferase. The reaction is a steric inversion from the original ribose. These chiral centers are retained in RNA. The combination of  $4R$  and  $3R$  determines the direction of the linkage to RNAs.

Table 1 summarizes the configuration change in the metabolic pathways from phosphoenolpyruvate to nucleotides.

As shown in Table 1, carbon (6) in all of the compounds is achiral and the chirality ( $2R$ ) of carbon (5) in D-2PG is first elaborated by enolase. The chirality ( $5R$ ) of carbon (5) in D-FBP is retained and the chirality ( $4S$ ) of carbon (4) is created by aldolase, which forms two more

**Table 1** Configuration of chiral centers of sugars in the metabolic pathways

Metabolic pathway	Gluconeogenesis					Pentose phosphate pathway	<i>De novo</i> biosynthesis of nucleotides	
Enzyme <sup>a</sup>	Enolase	Three enzymes	Aldolase	Two enzymes		Four enzymes	More than ten enzymes	
Identical <sup>b</sup> carbon	PEP <sup>d</sup>	D-2PG	DHP <sup>e</sup> D-GAP	D-FBP	D-G6P	D-R5P	PRPP	D-Nuc
(1)	–	–	1-non	1-non	$1S$	–	–	–
(2)	–	–	2-non	$2S$	$2R$	$1S$	$1R$	$1'R$
(3)	–	–	3-non	$3S$	$3S$	$2R$	$2R$	$2'R$
(4)	1-non	1-non	1-non	$4S$	$4S$	$3S$	$3S$	$3'S$
(5)	2-non	$2R$	$2R$	$5R$	$5R$	$4R$	$4R$	$4'R$
(6)	3-non <sup>e</sup>	3-non	3-non	6-non	6-non	5-non	5-non	$5'$ -non

<sup>a</sup> Enzymes: The enzymes shown as names catalyze the reactions of the compounds just below the columns from left to right. Numbers plus enzymes indicate how many enzymes catalyze the reactions of the compounds just below the columns from left to right. For instance, D-2PG passes through the sequential reactions catalyzed by three enzymes to lead D-GAP in the metabolic pathways

<sup>b</sup> Identical carbon means the identity of carbon atoms in the metabolic pathways as well as the same carbon in the same line in the table, e.g., carbon (6) exists at the position 6 of D-G6P, etc

<sup>c</sup> non: no chiral carbon

<sup>d</sup> Abbreviations of names are as follows: PEP: phosphoenol pyruvate; PRPP: 5-phosphoribosyl- $\alpha$ -pyrophosphate; D-Nuc: D-nucleotide

<sup>e</sup> Dotted line squares relate to DHP and its chirality

chiral carbons ( $2S$ ) and ( $3S$ ) in the reaction of D-GAP and DHP. The configuration of carbons ( $4S$ ) and ( $5R$ ) is consistently retained as  $3S$  and  $4R$  in ribose and ribonucleotides. However, other carbon atoms, ( $2S$ ) and ( $3S$ ) in D-FBP, are changed in their chirality several times by the enzymes, including ribose-5-phosphate isomerase and amidophosphoribosyltransferase in the pentose phosphate pathway and the *de novo* biosynthesis of nucleotides.

Enolase produces the D-configuration ( $2R$ ) of D-2PG from achiral PEP. This fact means that an L-protein generates a D-configuration chirality in a sugar. Furthermore, aldolase produces some more chiral carbon centers using its L-chiral environment and the D-chirality of D-2PG.

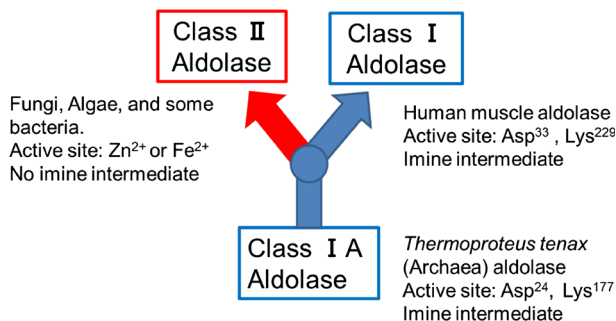
The D-chirality ( $2R$ ) of D-2PG must interact with the active site of enolase as L-protein to accelerate the differentiation reaction for making D-FBP. This is a heterochiral interaction.

### Classification of Aldolases

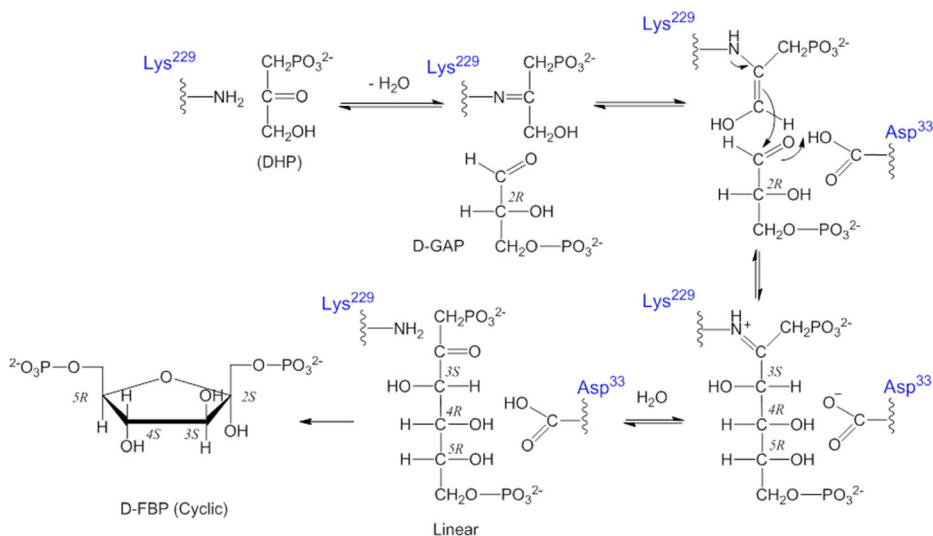
Aldolases have been classified into class I and class II (Voet and Voet 2011). Class I aldolases, which are found in animals and plants, form an imine intermediate in the active site which Asp and Lys catalyze (Dalby et al. 1999; Voet and Voet 2011). Class II aldolases, which are found in fungi, algae, and some bacteria, need zinc and ferrous ions for catalysis and bypass imine formation. However, a different type of aldolase, class IA, has been found (Siebers et al. 2001) in an archaeobacterium, and one of the class IA aldolases, found in *Thermococcus kadakaraensis* KOD1, was characterized using gene cloning (Imanaka et al. 2002). The class I and class IA aldolases are homologous (Lorentzen et al. 2004) in having similar catalytic groups, which are Asp and Lys, to form an imine intermediate. The enzyme class IA aldolase appears to have a common evolutionary origin with class I and class II aldolases (Andreeva et al. 2008; Say and Fuchs 2010) (Fig. 6). The similarity between class I and class IA suggests that the search for the stereo-differentiation reaction mechanism in the well-known class I aldolase may lead to the primordial reactions in class IA aldolase. Aldolase is considered to play a role in gluconeogenesis as well as glycolysis (Ronimus and Morgan 2003). Moreover, an aldolase IA possessing phosphatase activity has recently been found (Say and Fuchs 2010), which may be considered as an ancestral gluconeogenic enzyme.

### Reaction Mechanism of Class I Aldolases

Figure 7 shows a flowchart for the reaction mechanism in the human muscle aldolase (Dalby et al. 1999; Voet and Voet 2011) belonging to class I aldolases. The first stage is imine formation between DHP and the catalytic side chain, Lys<sup>229</sup>. The imine intermediate reacts



**Fig. 6** Classification of aldolases



**Fig. 7** Plausible reaction mechanism (Voet and Voet 2011) of the human muscle aldolase (Dalby et al. 1999) belonging to class I aldolases. Chemical structure of sugars is displayed using Fischer projections; the configuration of chiral centers are shown as *RS*-nomenclature

with D-GAP, *via* general base catalysis by Asp<sup>33</sup> to afford a hexose imine intermediate, which is hydrolyzed to D-FBP (Voet and Voet 2011). In the formation of the imine of D-FBP, a stereo-differentiation reaction proceeds using an active site composed of L-amino acid residues (including Lys<sup>229</sup>, Asp<sup>33</sup>, and the substrate binding site), D-GAP, and DHP. This is thus due to a heterochiral interaction between L-amino acids and D-sugars. The reaction results in two new chiral centers in the linear product. However, these newly formed chiral centers have only one configuration (*3S* and *4R*, respectively). On the other hand, the configuration (*2R*) of D-GAP is retained (*5R*) in the product. Furthermore, the final linear product (*2S*, *3S*, *4R*, *5R*) is cyclized to D-FBP (*2S*, *3S*, *4S*, *5R*). Although the expression of *RS*-nomenclature at 4-position between linear and cyclic D-FBP are different, the configuration at this position is retained.

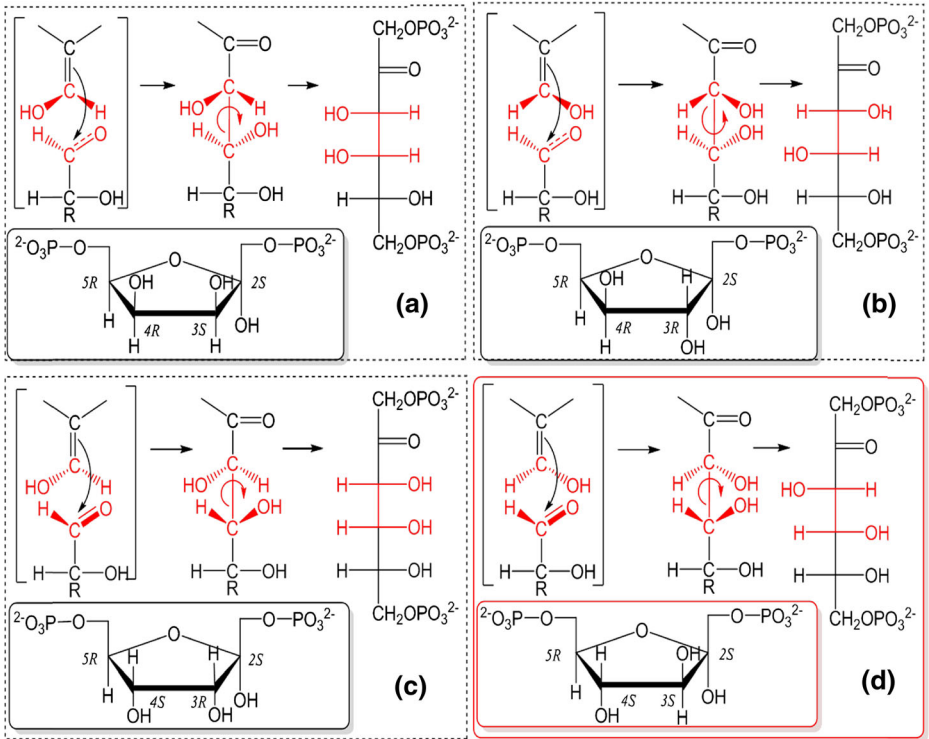
### Predicted Products by Aldol Condensation

The catalysis by aldolase is classified into C–C bond formation, which may yield two chiral centers in a cyclic product, as described above. Figure 8 shows the chiral structures of the predicted four diastereomers without aldolase catalysis. However, aldolase produces only one diastereomer, as shown in Fig. 8d. The reaction is a stereo-differentiation reaction.

Table 2 shows the heat of formation ( $\Delta H_f^\circ$ ) calculated using the semiempirical method MOPAC based on PM6 (Stewart 2007) for the stereoisomers which would be generated without aldolase. Cyclic stereoisomer (d) is the most stable in both vacuum ( $\Delta H_f^\circ = -505.8$  kcal/mol) and water ( $\Delta H_f^\circ = -1104.6$  kcal/mol). The difference after cyclization of the linear form also shows the highest value, which suggests that stereoisomer (d) is most stabilized by cyclization compared with the isomers (a) to (c). The calculation error of the heat of formation is shown as the averaged value, 4.4 kcal/mol for the subset of 1373 compounds involving only the elements H, C, N, O, F, P, S, Cl, and Br (Stewart 2007).

The calculated results show that aldolase catalyzes the stereo-differentiating condensation between D-GAP and DHP to give the most stable stereoisomer, D-FBP. The configuration of





**Fig. 8** The four stereoisomers ((a)–(d)) produced by aldol-type condensation without aldolase and their  $\alpha$ -D-furanose form. Stereoisomer (d) is D-FBP

the two chiral carbons ( $4S$ ,  $5R$ ) in D-FBP is retained through PRPP ( $3S$ ,  $4R$ ) to D-ribonucleotides ( $3'S$ ,  $4'R$ ), which also have heterochiral pairing with L-amino acids in Fig. 3.

The stereochemical relationship between  $3'S$ -carbon and  $4'R$ -carbon in D-ribonucleotides may be most important in the heterochiral pairing between D-RNA and L-amino acids.  $4'R$ -carbon in D-ribonucleotides dictate the steric direction of the  $5'$ -carbon of D-AMP, in which a

**Table 2** Heat of formation calculated for D-FBP and its stereoisomers

Stereoisomer: (Configuration)	Heat of formation <sup>a</sup> : $\Delta H_f^\circ$ (kcal/mol) in vacuum			Heat of formation: $\Delta H_f^\circ$ (kcal/mol) in water		
	Linear <sup>b</sup>	Cyclic <sup>c</sup>	Difference <sup>d</sup>	Linear	Cyclic	Difference
(a): ( $2S$ , $3S$ , $4R$ , $5R$ )	−488.0	−495.7	−7.7	−1081.1	−1098.6	−17.5
(b): ( $2S$ , $3R$ , $4R$ , $5R$ )	−487.7	−499.6	−11.9	−1080.7	−1097.2	−16.5
(c): ( $2S$ , $3R$ , $4S$ , $5R$ )	−494.3	−497.5	−3.2	−1083.2	−1099.2	−16.0
D-FBP(d): ( $2S$ , $3S$ , $4S$ , $5R$ )	−491.0	−505.8	−14.8	−1083.0	−1104.6	−21.6

<sup>a</sup> Heat of formation was calculated by a semiempirical method (MOPAC based on PM6)

<sup>b</sup> 'Linear' means the ring-opened structure of stereoisomers of D-FBP

<sup>c</sup> 'Cyclic' means the cyclic structure of stereoisomers of D-FBP

<sup>d</sup> 'Difference' means the difference in the computed  $\Delta H_f^\circ$  between the linear and cyclic forms



phosphoric group at the 5'-carbon of a terminal residue links to a carboxyl group of an L-amino acid in aminoacyl-adenylates. A hydroxyl group at the 3'S-carbon of a t-RNA links to an L-amino acid specially selected for the t-RNA to form an aminoacyl-t-RNA. This is the heterochiral pairing for protein synthesis, as shown in Fig. 3.

## Conclusions

Chiral pairing may be classified as heterochiral pairing or homochiral pairing. The chiral pairing between L-amino acids and D-nucleotides can be called a heterochiral pairing. The D-configuration of glyceraldehyde formed by enolase is retained through gluconeogenesis, the pentose phosphate pathway, and *de novo* synthesis of nucleotides. An important heterochiral pairing between L-proteins and D-sugars can be seen in the reaction by aldolase, which conserves the D-configuration resulting from 5R in D-FBP as the product to make the 4S-carbon. The configurations (4S and 5R) are consistently retained by RNAs, which have heterochiral pairing with L-amino acids for protein synthesis. Therefore, aldolase can be considered a chiral intersection between L-amino acids and D-nucleotides. The role of aldolase IA found in archaea also has to be discussed more from the viewpoint of origin and development of homochirality or heterochiral pairing.

## References

- Andreeva A, Howorth D, Chandonia J-M, Brenner SE, Hubbard TJP, Chothia C, Murzin AG (2008) Data growth and its impact on the SCOP database: new developments. *Nucleic Acid Res* 36:D419–D425
- Avalos M, Babiano R, Cintas P, Jimenez JL, Palacios JC, Barron LD (1998) Absolute asymmetric synthesis under physical fields: facts and fictions. *Chem Rev* 98:2381–2404
- Breslow R, Levine M, Cheng Z-L (2010) Imitating prebiotic homochirality on Earth. *Orig Life Evol Biosph* 40: 11–26
- Dalby A, Dauter Z, Littlechild JA (1999) Crystal structure of human muscle aldolase complexed with fructose 1, 6-bisphosphate: mechanistic implications. *Protein Sci* 8:291–297
- Imanaka H, Fukui T, Atomi H, Imanaka T (2002) Gene cloning and characterization of fructose-1,6-bisphosphate aldolase from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. *J Biosci Bioeng* 93: 237–243
- Lorentzen E, Siebers B, Hensel R, Pohl E (2004) Structure, function and evolution of the Archaeal class I fructose-1,6-bisphosphate aldolase. *Biochem Soc Trans Part 2*(32):259–263
- Munegumi T, Shimoyama A (2003) Development of homochiral peptides in the chemical evolutionary process: Separation of homochiral and heterochiral oligopeptides. *Chirality* 15:S108–S115
- Palyi G, Zucchi C, Caglioti L (2004) Progress in biological chirality. Elsevier, Amsterdam, p 429
- Pavlov VA, Klabinovskii EI (2014) Homochirality origin in nature: possible versions. *Curr Org Chem* 18:93–114
- Pizzarello S, Weber AL (2004) Prebiotic amino acids as asymmetric catalysts. *Science* 303:1151
- Profy AT, Usher DA (1984) Stereoselective aminoacylation of a dinucleotide monophosphate by the imidazolides of dl-alanine and *N*-(*tert*-butoxycarbonyl)-dl-alanine. *J Mol Evol* 20:147–156
- Ronimus RS, Morgan HG (2003) Distribution and phylogenies of enzymes of the Embden–Meyerhof–Parnas pathway from archaea and hyperthermophilic bacteria support a gluconeogenic origin of metabolism. *Archaea* 1:199–221
- Say RF, Fuchs G (2010) Fructose 1,6-bisphosphate aldolase/phosphatase may be an ancestral gluconeogenic enzyme. *Nature* 464:1077–1081
- Siebers B, Brinkmann H, Dörr C, Tjaden B, Lilie H, van der Oost J, Verhees CH (2001) Archaeal fructose-1,6-bisphosphate aldolases constitute a new family of archaeal type class I aldolase. *J Biol Chem* 276: 28710–28718

- Smith MB, March J (2007) March's advanced organic chemistry: reactions, mechanisms, and structure. Wiley, New York, pp 194–195
- Stewart JJP (2007) Optimization of parameters for semiempirical methods V: modification of NDDO approximations and application to 70 elements. *J Mol Model* 13:1173–1213
- Tamura K, Schimmel P (2004) Chiral-selective aminoacylation of an RNA minihelix. *Science* 305:1253
- Voet D, Voet JG (2011) *Biochemistry*, 4th edn. Wiley, Hoboken
- Weber AL (1987) Stereoselective formation of 2'(3')-aminoacyl ester of a nucleotide. *J Mol Evol* 25:7–11