HOW THE CHEMICAL FEATURES OF MOLECULES MAY HAVE ADDRESSED THE SETTLEMENT OF METABOLIC STEPS

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ABSTRACT

Introduction While the evolutionary adaptation of enzymes to their own substrates is a well assessed and rationalized field, how molecules have been originally selected in order to initiate and assemble convenient metabolic pathways is a fascinating, but still debated argument.
Objectives Aim of the present study is to give a rationale for the preferential selection of specific molecules to generate metabolic pathways.
Methods he comparison of structural features of molecules, through an inductive methodological approach, offer a reading key to cautiously propose a determining factor for their metabolic recruitment.
Results Starting with some commonplaces occurring in the structural representation of relevant carbohydrates, such as glucose, fructose and ribose, arguments are presented in associating stable structural determinants of these molecules and their peculiar occurrence in metabolic pathways.
*Conclusion*s Among other possible factors, the reliability of the structural asset of a molecule may be relevant or its selection among structurally and, a priori, functionally similar molecules.

Key words: metabolic steps genesis; carbohydrate metabolism; hemiacetal stability; enzyme selectivity.

1. Introduction

Metabolism is the complex, articulated network of chemical reactions that sustain cell life (Hawley et al. 2015; Holmes 2015). While strictly obeying thermodynamic laws (Noor et al. 2014), metabolism is kinetically controlled through the action of a wide and specialized pattern of biocatalysts, enzymes (Fersht 1999; Price and Stevens 1999). As living cells are thermodynamically open systems, they require a continuous supply of molecules to sustain their life-compatible steady states (Sajitz-Hermstein and Nikoloski 2013). The evolutionary adaptation of enzymes to their substrates, aimed at improving their catalytic efficiency, has been well assessed with environmental conditions being an important trigger (Copley 2012; Fields et al. 2015). However, how molecules, whose recruitment may have contributed to the construction of convenient metabolic routes, were originally selected, is still an open question. (Firn and Jones 2009). Starting with some commonplaces occurring in the representation of the structure of various relevant biomolecules, this work aims to find a relationship between the potential of a molecule to offer stable structural determinants and the chances of that molecule becoming a pivotal player in a metabolic pathway.

2. Familiar and less familiar molecular structures.

As we are so used to thinking of formulas of compounds as they appear in metabolic maps, it may be difficult to look at the same compounds outside of the metabolic context. Looking at the formulas shown in Fig. 1 (*a* and *b*), we immediately associate compound *a* with D-glucose; however, it takes a little more to realize that compound *b* is also D-glucose. The only difference between the two structures is simply the different hemiacetal rearrangement, as the glucose molecule is represented either as β-D-glucopyranose (*a*) or as β-D-glucofuranose *(b).*

This identification failure may be because we are used to thinking of glucose in its pyranosidic hemiacetal structure, which we have unequivocally associated with the highest stability in which the cyclic hemiacetal of the aldohexose can be arranged. Now, playing the same game of "What is it?" with two other important and familiar metabolites, such as the ketohexose D-fructose and the aldopentose D-ribose, it is again likely that we may not recognize them right away, if these two compounds are represented as in Fig. 1, *c* and *d,* respectively, instead than in their most common representation (Fig. 1 *e* and *f,* respectively). However also in the case of fructose and ribose, the pyranosidic arrangements of cyclic hemiacetals represent the most stable structures.

In fact, although not as stable as glucose, in which the pyranoside represents approximately 99% of all the possible hemiacetal rearrangements, the pyranosidic form of fructose and ribose also represents as much as 75% and 80%, respectively, of all the possible hemiacetal rearrangements (Angyal and Bethell 1976; Angyal and Pickles 1972). This way of reasoning is not merely limited to defining the most appropriate way to represent these molecules, but may be worth evaluating when considering how the structural features of the above-mentioned molecules could have affected the consolidation of crucial metabolic steps.

3. Recruitment of molecules to assemble metabolic pathways

Biochemists are aware that molecular bio processes are under enzymatic control. Thus, the chemical reactivity and relative stability of reactants and products are overcome in many instances by the specific and powerful enzyme catalytic action. In fact, enzymes, which are also able to integrate endergonic with exergonic reactions, enable molecules to be channeled through processes that are difficult to predict from their chemical reactivity. Nevertheless, the fact that certain molecules have been selected as enzyme substrates from a set of possibly very similar candidates, would seem to indicate some advantage, in terms of chemical reactivity/stability or accessibility,

must have occurred. In this context, returning to considerations on the hemiacetal stability, sugar metabolism is quite instructive.

Glycolysis, which is one of the most studied metabolic pathways, offers a unique opportunity to challenge this line of thought. The efficiency and even the elegance of the strategic design of glycolysis is very apparent (Bar-Even et al. 2012). Briefly, an aldohexose (D-glucose) is converted into two threoses (glyceraldehyde-3-phosphate, G3P and dihydroxy aceton phosphate, DHAP) linked by an equilibrium reaction that enables only one molecular species to be channeled until the metabolic goal is fulfilled. In principle, the above pathway could act as a molecular funnel, for all the eight different aldohexose diastereoisomers of the D-configuration series (Fig. 2). Once all the eight D-aldoses have been converted to their four corresponding 2-ketoexoses, they could generate the same end-product, the pyruvate, through the oxidation of the same intermediates G3P and DHAP.

Thus, even though this metabolic pathway appears to be conceived to include different, apparently equivalent substrates, glucose, through its phosphorylation at the C6 hydroxyl group, is by far the most likely aldose to enter glycolysis. This happens despite other aldoses being able to enter this metabolic pathway as phosphorylated sugars. Indeed, mannose, which is susceptible to phosphorylation at the C6 position by a mannokinase, may enter the glycolitic pathway. The mannose-6-phosphate (M6P) thereby generated is isomerized to fructose-6-phosphate (F6P) by an M6P isomerase. The latter step, which may be used in the reverse direction to replenish M6P from F6P, is the link between mannose and glucose metabolism. However, despite the possible convergence of the two sugars into the same catabolic pathway, mannose is mainly used for N-glycan synthesis and thus involved in N-glycation pathways **(**Sharma et al. 2014; Ichikawa et al., 2014)

Also galactose can be found phosphorylated at the C6 position but only through the isomerization of galactose-1-phosphate (Gal-1-P). The latter is the main product of the galactokinase action, which is linked to glucose metabolism through the Leloir pathway (Frey 1996). Thus, even though tagatose-6-phosphate, generated by Gal-1-P epimerase, may reach, at least in some bacteria, the bottom of the glycolytic funnel forming GA3P and DHAP by specific aldolases (Zgiby et al. 2000; Hall et al., 2002), galactose as well mannose metabolism essentially diverge from the glycolytic flux, leaving glucose as the main player in glycolysis.

What could have led to, or at least, what could have contributed to this preferential choice for a metabolic pathway so common in biodiversity? It is difficult to answer such a Darwinian-like approach to molecule selection. However, on the basis that the true substrate of kinases is the hemiacetal of the aldose (Mulichak et al. 1998), glucose must certainly have appeared right from the

beginning as the most stable and structurally reliable substrate, since its pyranosidic hemiacetal structure is by far the most stable of all the aldoses.

On the same bases as the structural stability of the glucose hemiacetal, the evolutionary selection of glucose has been proposed to be linked to its poor tendency to glycate proteins, thus avoiding the risk of protein damage **(**Bunn and Higgins 1981). For an idea of this particular primacy of glucose, it is worth looking at the relative content of free aldehyde for different aldoses (Fig. 3) (Dworkin and Miller 2000). As can be seen, given its possible access to the glycolitic flux and given its hemiacetal stability, mannose possesses a free aldehyde content only three fold higher than glucose. The special features of glucose certainly concern, among other structural thermodynamic factors (Goldberg and Tewari 1989), its special stabilization associated with a chair structure of the β-anomer, in which the groups with the highest steric hindrance in the molecule (i.e. the hydroxyl groups and the C6 methoxy group) may all be allocated to the less bulky equatorial positions (Fig.2). The relevance of this feature is well represented by the comparison between D-glucose and D-idose (Fig. 3). The latter, sharing with D-glucose only the steric configuration at C5, is not able to allocate all groups in the equatorial position and displays a percentage of free aldehyde which is approximately 100-fold higher than D-glucose.(Dworkin and Miller 2000).

The case of fructose is in line with the reliable accessibility of functional groups as a factor influencing the settlement of metabolic steps toward one molecule with respect to others. Here, the game of "What is it?" may explain why fructose cannot directly be used as a substrate for glycolysis, despite being well represented in the cell and despite being an important player in its phosphorylated form in this metabolic pathway. In fact, as the C6 hydroxyl group is mostly unavailable because of the prevailing pyranosidic structure, fructose appears to be recruited in the metabolic transformations essentially through the phosphorylation at the C1 carbon atom. This is an unavoidable event in glycolysis, where the isomerization of glucose 6 phosphate leaves the C1 hydroxyl group as the only extra ring group accessible to phosphofructokinase. This is also true for the phosphorylation of the free sugar by fructokinase, which in the liver and muscle specifically transfers the γ-phosphate of ATP to the C1 position of the ketose **(**Slein 1950). The possibility of phosphorylation at the C6 hydroxyl group, as is reported to occur in the brain (Slein 1950) cannot be disregarded, since the existence of the furanosidic form. Indeed, fructose phosphorylation may occur at C6 even in muscle or adipose tissues but only when the level of this sugar either for abnormal dietary intake or for metabolic defects, become high (Steinmann and Santer 2012**)**. However, it remain the fact that the main route of dietary fructose utilization is the phosphorylation at the C1 position **(**Campos and Tappy 2016; Havel 2015).

This explains why through the evolutionary assessment of a metabolic pathway, one molecule, amongst other very similar candidate molecules, was the prominent starting point of the way. This selection deals only with the starting point of the growth of a reaction network. Thus, these considerations should not be confused either with the structural evolution of the enzyme proteins aimed at improving substrate recognition, or with the recruitment of metabolites from equilibrium steps to be enrolled in metabolic pathways. This applies, for instance, to the isomerization of DHAP into GA3P, an equilibrium thermodynamically favoring DHAP, which, although being able to occur spontaneously, is metabolically supported by an efficient triosephosphate isomerase and then is driven towards GA3P by an oxidative step, which is crucial in the catabolic breakdown of glucose. Another example of an extreme case of a molecule, used in its truly least stable and least available form, is the rather improbable, but real, use of glucose through its free aldehyde form by aldose reductase (Balestri et al. 2015) in the polyol pathway. In this case, however, we cannot disregard the complex inhibitory action on the reaction exerted by glucose hemiacetal (Del Corso et al. 2008) and the fact that the glucose flux through the polyol pathway, which is significant and damaging in the hyperglycemic conditions associated with diabetes, is almost negligible in physiological normoglycemic conditions (Brownlee 2005).

A further example of how the structural features of a molecule may affect its metabolic use is the case of another aldose, the D-ribose. This is quite a diffuse molecule, for example as hydrophilic moiety of purinic and pyrimidine nucleosides, and as the natural pylon of the phosphate bridge between bases in nucleic acids, is recruited for its use as ribose 5-phosphate (Rib-5-P). Returning to the “What is it ?” game, Rib-5-P production by the direct phosphorylation of free ribose is improbable, the hydroxyl group at the C5 atom of the sugar being mainly involved in the formation of the rather stable pyranosidic hemiacetal ring. Indeed, although a phosphorylation at position 5 of the ribose has been reported to occur by a kinase acting on the open chain of ribose (Agranoff and Brady 1956), it is beyond doubt that the massive production of Rib-5-for vital cell processes is not the direct phosphorylation of ribose, but is due to the hexose monophosphate shunt pathway (Stincone et al. 2015). It is thus conceivable, though hard to demonstrate, that the availability of such a crucial reagent for cell life as Rib-5-P, as highlighted for glucose concerning glycolysis, must rely on stable and structurally well-defined molecular species.

4. Conclusions

As a general consideration, it may be possible to state that before becoming metabolites, bio-molecules are simply molecules, whose reactivity and also structural features, before their

enzymatic recruitment, may right from the beginning have played a subtle role in the development of the most convenient metabolic pathways.

COMPLIANCE WITH ETHICAL STANDARDS

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REFERENCES

Agranoff, B.W. & Brady, R.O. (1956). Purification and properties of calf liver ribokinase. *Journal of Biological Chemistry, 219,* 221-229.

Angyal, S.J. & Bethell, G.S. (1976). Conformational analysis in carbohydrate chemistry III. The 13C NMR spectra of the hexuloses. *Australian Journal of Chemistry, 29,* 1249-1265.

Angyal, S.J. & Pickles, V.A. (1972). Equilibria between pyranoses and furanoses. *Australian Journal of Chemistry, 25,* 1695-1710.

Balestri, F., Cappiello, M., Meschini, R., Rotondo, R., Abate, M., Del Corso, A., Mura, U. (2015). Modulation of aldose reductase activity by aldose hemiacetals, *Biochimica et Biophysica Acta, 1850,* 2329-2339.

Bar-Even, A., Flamholz, A., Noor, E. & Milo, R. (2012). Rethinking glycolysis: on the biochemical logic of metabolic pathways. *Nature Chemical Biology 8,* 509-517.

Brownlee, M**.** (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes, 54,* 1615-1625.

Bunn, H.F.& Higgins, P.J. (1981). Reaction of monosaccharides with proteins: possible evolutionary significance. *Science, 213,* 222-224.

Campos, V.C. & Tappy, L. (2016). Physiological handling of dietary fructose-containing sugars: implications for health. *Internationl Journal of Obesity 40,* S6-S11.

Copley, S.D. (2012). Toward a systems biology perspective on enzyme evolution. *Journal of Biological Chemistry, 287,* 3-10.

Del Corso, A., Cappiello, M. & Mura, U. (2008). From a dull enzyme to something else: facts and perspectives regarding aldose reductase. *Current Medicinal Chemistry, 15,* 1452–1461.

Dworkin, J.P. & Miller, S.L. (2000). A kinetic estimate of the free aldehyde content of aldoses, *Carbohydrate Research, 329,* 359-363.

Fersht, A. (1999). Structure and Mechanism in Protein Science: Guide to Enzyme Catalysis and Protein Folding. New York: Freeman WH and Company.

Fields, P.A., Dong, Y., Meng, X.& Somero, G.N. (2015). Adaptations of protein structure and function to temperature: there is more than one way to 'skin a cat'. *Journal of Experimental Biology, 218,* 1801-1811.

Firn, R.D. & Jones, C.G. A (2009). Darwinian view of metabolism: molecular properties determine fitness. *Journal of Experimental Botany, 60,* 719–726.

Frey, P.A. (1996). The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose. *FASEB Journal, 10,* 461-470.

Goldberg, R.N. & Tewari, Y. (1989). Thermodynamic and transport properties of carbohydrates and their monophosphates: the pentoses and hexoses *Journal of Physical and Chemical Reference Data, 18,* 809–880.

Hall, D.R., Bond, C.S., Leonard, G.A., Watt, C.I., Berry, A., Hunter, W.N. (2002). Structure of tagatose-1,6-bisphosphate aldolase insight into chiral discrimination, mechanism, and specificity of class II aldolases. *Journal of Biological Chemistry, 277,* 22018–22024.

Havel, P.J. (2005). Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutrition Reviews, 63,* 133-157.

Hawley, J.A., Maughan, R.J. & Hargreaves, M. (2015). Exercise metabolism: historical perspective. *Cell Metabolism, 22,* 12-17.

Holmes, E. (2015). The Metabolism of Living Tissues. Cambridge: Cambridge University Press.

Ichikawa, M., Scott, D.A., Losfeld, M.E. & Freeze, H.H. (2014). The metabolic origins of mannose in glycoproteins. *Journal of Biological Chemistry, 289,* 6751-6761.

Mulichak, A.M., Wilson, J.E., Padmanabhan, K. & Garavito, R.M. (1998). The structure of mammalian hexokinase-1. *Nature Structural Biology, 5,* 555-560.

Noor, E., Bar-Even, A., Flamholz, A., Reznik, E., Liebermeister, W., Milo, R. (2014). Pathway thermodynamics highlights kinetic obstacles in central metabolism. *PLOS Computational Biology, 10,* doi:10.1371/journal.pcbi.1003483.

Price, N.C. & Stevens, L. (1999). Fundamentals of Enzymology: The cell and Molecular Biology of Catalytic proteins. New York: Oxford University Press.

Sajitz-Hermstein, M. & Nikoloski, Z. (2013). Structural Control of Metabolic Flux. *PLOS Computational Biology, 9,* doi:10.1371/journal.pcbi.1003368.

Sharma, V., Ichikawa, M., Hudson, H. & Freeze, H.H. (2014). Mannose metabolism: More than meets the eye. *Biochemical and Biophysical Research Communications, 453,* 220-228.

Slein, M.W., Cori, G.T.& Cori, C.F. (1950). A comparative study of hexokinase from yeast and animal tissues. *Journal of Biological Chemistry, 186,* 763-80.

Steinmann, B., Santer, R. (2012). Disorders of Fructose Metabolism. In Saudubray, J.M., van der Berghe, G., Walter, J. H. (Eds.), *Inborn Metabolic Diseases: Diagnosis and Treatment* (5th ed.) (pp. 157–165). New York: Springer.

Stincone, A., Prigione, A., Cramer, T., Wamelink, M.M.C, Campbell, K., Cheung, E., Olin-Sandoval, V., Grüning, N.M, Krüger, A., Alam, M.T., Keller, M.A., Breitenbach, M., Brindle, K.M., Rabinowitz, J.D., Ralser, M. (2015). The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biological Reviews* 90, 927-963.

Zgiby, S.M., Thomson, G.J., Qamar, S. & Berry, A. (2000). Exploring substrate binding and discrimination in fructose1, 6-bisphosphate and tagatose 1,6-bisphosphate aldolases. *European Journal of Biochemistry, 267,* 1858-1868.

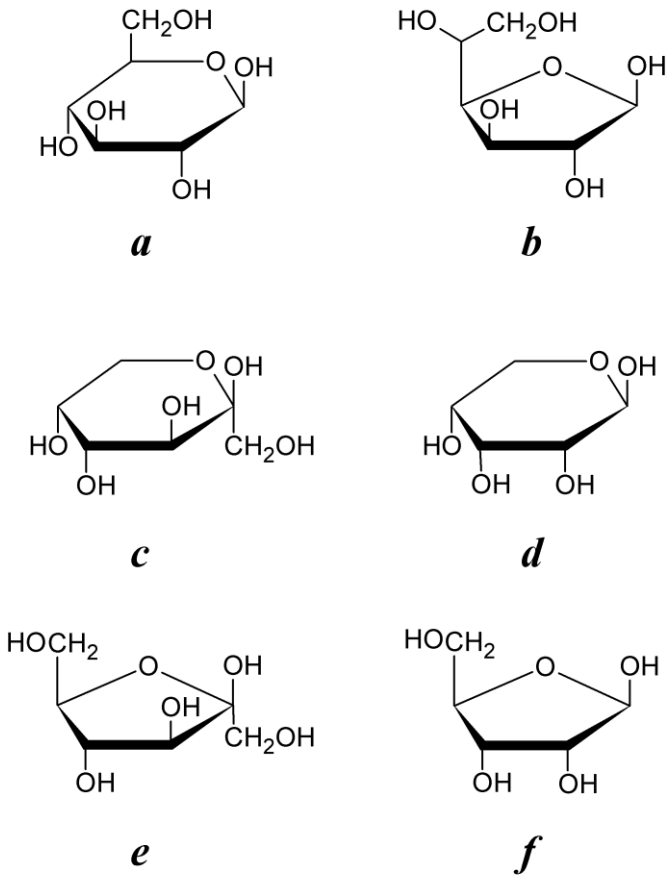

Fig. 1 Structure representation of β-D-glucose, β-D-fructose and β-D-ribose. β-D-glucose is represented in its pyranosidic (*a*) and furanosidic (*b*) hemiacetal form; β-D-fructose is represented in its pyranosidic (*c*) and furanosidic (*e*) hemiacetal form; β-D-ribose is represented in its pyranosidic (*d*) and furanosidic (*f*) hemiacetal form

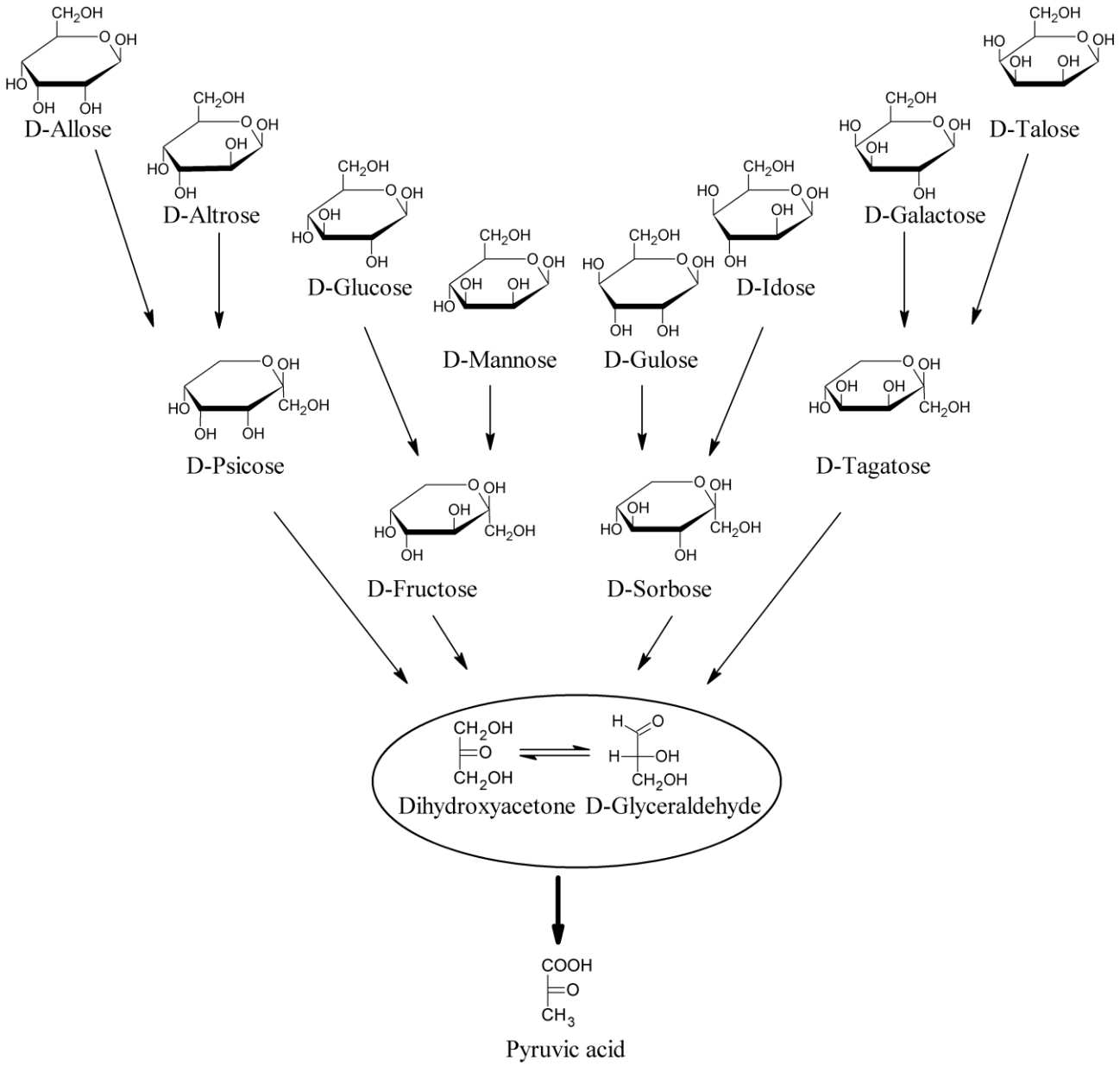

Fig. 2 The glycolytic funnel. Following the metabolic implant of glycolysis, all the eight different D-aldohexoses could potentially generate pyruvate. GA and DHA stand for D-glyceraldehyde and dihydroxy aceton, respectively

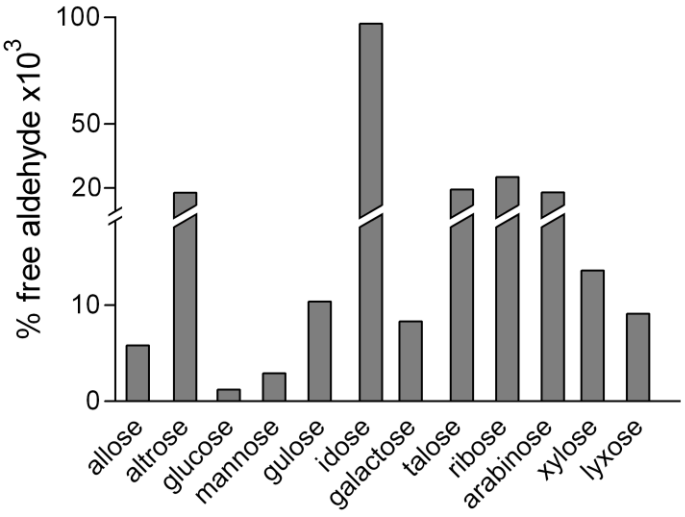

Fig. 3 Relative content of free aldehyde in solution of different D-aldoses. The data, adapted from Dworkin and Miller (2000)**,** refer to D-idose, the sugar displaying the highest content of free aldehyde form at equilibrium