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Influence of the photosynthetic pathway on the hydrogen isotopic profile of glucose

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Abstract The SNIF-NMR method (site-specific natural isotope fractionation studied by Nuclear Magnetic Resonance) was used to examine the isotopic profile of glucoses derived from plants with different photosynthetic pathways. It is shown that the type of photosynthetic metabolism, either C3 (beet-root, orange, grape), C4 (maize, sugar-cane) or CAM (pineapple), exerts a strong influence on the deuterium distribution in the sugar molecules. The isotopic profile also depends, secondarily, on the physiological status of the precursor plant. Consequently, the isotopic fingerprint of glucose may be a rich source of information in mechanistic comparisons of metabolic pathways. Moreover, from an analytical point of view, it may provide complementary criteria with respect to the ethanol probe for origin inference of sugars.

Key words deuterium • glucose • isotope ratio • plant metabolism • SNIF-NMR

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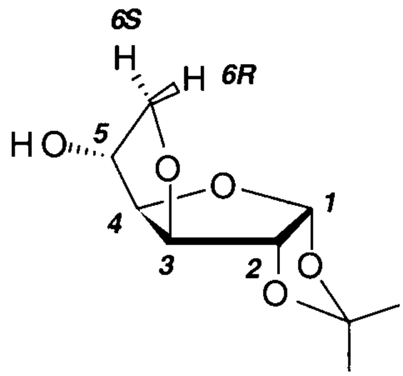
Introduction

Whereas the overall carbon isotope ratios of sugars, provide unambiguous criteria for distinguishing C3 and C4 photosynthetic pathways, the overall hydrogen isotope ratio, $(D/H)_{tot}$, is not discriminating in terms of metabolic type. We have shown previously that ethanol, obtained by fermenting different kinds of sugars in standardized conditions, offers a general and very efficient isotopic probe for identifying the botanical species, and even the geographical origin, of the plant precursor [3]. However, since the partly independent information contained in the seven carbon-bound hydrogens of the glucose skeleton is strongly reduced by the transformation into a molecule with only two carbon-bound hydrogen sites, it was interesting to investigate directly the glucose moiety of sugars. To this aim the glucose molecule has been converted into 3,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose in a two-step reaction with high yield [5]. The derivatives of glucose samples extracted from plants with different photosynthetic pathways were then analyzed using the SNIF-NMR method [2, 3, 7].

Results and discussion

A SNIF-NMR methodology involving a suitable isotopic reference (pyridine) was developed [8] in order to determine, with good repeatability and reproducibility, the site specific hydrogen isotope ratios, $(D/H)_i$, of derivatives (Scheme 1) of glucose samples derived from plants with different photosynthetic metabolisms: beet, orange, grape (C3), cane, maize (C4), pineapple (CAM).



Scheme 1. Structure of 3,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose.

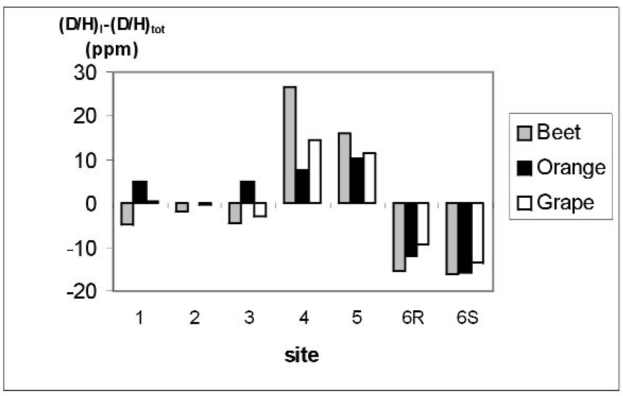


Fig. 1. Isotopic profiles of C3 glucoses.

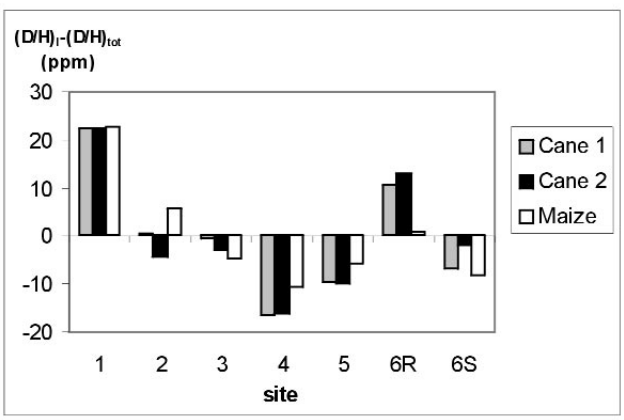


Fig. 2. Isotopic profiles of C4 glucoses.

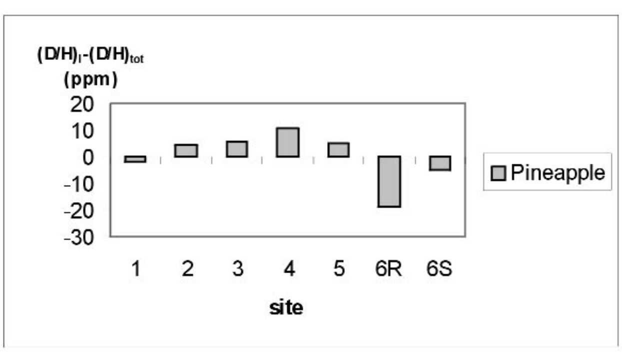


Fig. 3. Isotopic profile of a CAM glucose.

The results expressed as percent deviations, $\delta(D/H)_i(\%)$, with respect to the mean hydrogen isotope ratio of the glucose skeleton, $(D/H)_{tot}$, are given in Table 1.

As illustrated in Figures 1–3, these results show that the hydrogen isopic profile is typical of the photosynthetic pathway. Thus, it is observed that sites 4 and 5 of the glucose skeleton are relatively enriched in the C3 plants, whereas the highest deuterium contents are found in sites 1 and 6 of the C4 sugars. Although it strongly differs from the others, the isotopic profile of the CAM fruit, pineapple, is closer to that of the C3 species than to that of the investigated C4 sugars.

In contrast with the carbon-13 distribution, which is similar in glucose molecules from C3 or C4 origin [4], the hydrogen isotopic profile exhibits a discriminating behavior in terms of metabolic type.

However, in spite of the similarities observed in the isotopomeric contents of sugars pertaining to the same metabolic family, the site specific ratios, $(D/H)_i$, may not be considered as the simple product of a constant metabolic profile by an overall deuterium content, which would depend on environmental factors. The fractionation phenomena which govern the relative distribution of deuterium among the molecular sites are also influenced secondarily by physiological features of the plant. Thus, within the C3 family, the relative deuterium enrichment at site 5 and at the C-4 position which originates from NADPH, is more accentuated in beet tuber than in fruits such as orange. Taking into account the existence of three different sources of triose phosphate precursors in C3 plants, variations in the relative importance of photorespiration phenomena [1, 6] are expected to exert some influence on the hydrogen isotopic profile. In this respect, the present strategy provides an attractive approach for investigating physiological responses of plants to environmental variations.

From a general point of view, the deuterium contents of sugars depend on those of the source water which is enriched in the warmer and drier countries where orange trees are grown. Moreover active evapo-transpiration phenomena in leaves further enhance the D/H ratio of water entering the photosynthetic process, and photorespiration plays an increasing role when C3 plants are grown in warmer regions. The present results show that the differences in physiological characteristics of plants may be responsible for variations in the fractionation factors. Such fractionation effects mainly result from kinetic isotope effects which may significantly influence the isotopic distribution when they intervene at a metabolic branch point. Consequently, since every molecular site may be specifically affected, a simple correlation of overall or specific deuterium contents with climatic parameters may be expected only at constant isotopic profile of the molecular probe. This behavior must be kept in mind when isotopic data are used for climate inference. Further investigation of samples grown in controlled conditions should now be conducted in order to estimate more precisely the role of the different environmental factors.

It should also be emphasized that the described analytical strategy can be efficiently applied not only to glucose, but to

Table 1. Site-specific hydrogen isotopic parameters of glucoses extracted from plants with different photosynthetic pathways.

Plant	$(D/H)_{tot}$[a]	Site-specific isotopic deviation, $\delta(D/H)_i(\%)$[a,b]						
	(ppm)	**1**	**2**	**3**	**4**	**5**	**6R**	**6S**
Beet	128.1	-3.75 (1.62)	-1.56 (1.35)	-3.43 (1.54)	20.84 (1.68)	12.41 (1.25)	-12.10 (1.51)	-12.72 (1.25)
Orange	146.2	3.42 (1.52)	0.07 (1.09)	3.42 (1.59)	5.13 (0.91)	7.05 (0.89)	-8.34 (1.34)	-10.74 (1.30)
Grape	144.8	0.35 (1.79)	-0.28 (1.11)	-2.14 (1.41)	10.01 (1.00)	8.01 (1.02)	-6.56 (1.26)	-9.25 (1.67)
Cane 1	137.4	16.30 (1.75)	0.29 (1.67)	-0.51 (1.32)	-12.08 (1.74)	-6.91 (1.72)	7.71 (1.22)	-5.02 (1.92)
Cane 2	143.1	15.58 (1.63)	-3.07 (1.15)	-2.17 (0.93)	-11.25 (1.81)	-6.99 (2.93)	9.15 (0.77)	-1.26 (2.90)
Maize	151.0	15.10 (1.04)	3.77 (1.72)	-3.05 (0.89)	-7.09 (2.92)	-3.71 (1.44)	0.46 (1.12)	-5.50 (0.98)
Pineapple	153.5	-1.30 (1.19)	2.67 (1.14)	3.52 (1.57)	7.10 (1.34)	3.39 (1.13)	-12.25 (1.11)	-3.26 (1.14)

[a] $(D/H)_{tot}$ and all site-specific isotopic ratios were determined by the SNIF-NMR method.
[b] The site-specific isotopic parameters, measured on the derivative represented in Scheme 1, are expressed as percent deviations, $\delta(D/H)_i(\%)$, of the isotopic ratios, $(D/H)_i$, with respect to the mean value, $(D/H)_{tot}$, of the glucose skeleton: $\delta(D/H)_i(\%) = 100[(D/H)_i-(D/H)_{tot})]/(D/H)_{tot}$.
The error, given within parentheses, is defined as: $[\text{standard deviation}/(D/H)_i]\cdot 100$

mixtures of glucose and fructose; to sucrose (beet, cane), and to mixtures of glucose, fructose and sucrose (fruit juice). The isotopic profile of the glucose moiety can therefore be used as fingerprint for identifying the origin of many commodities consisting of carbohydrates.

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