ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 329 (2000) 1–5

CARBOHYDRATE RESEARCH

Rapid Communication

Studies of the complexation of sugars by diffusion-ordered NMR spectroscopy

M. Dolores Díaz, Stefan Berger *

Institut für Analytische Chemie, Universität Leipzig, Linnestraße 3, D-04103 Leipzig, Germany

Received 11 July 2000; accepted 7 August 2000

Abstract

In the presence of lanthanide cations, some sugars with a special relationship between their hydroxyl groups are able to form complexes in water. Diffusion-ordered NMR spectroscopy (DOSY) can be used as a tool to distinguish between the complexed and noncomplexed forms in a mixture due to the differences in their relative diffusion coefficient values. The lowest diffusion was attributed to the complexed species because of the increase in both size and molecular weight when compared with the noncomplexed forms. Mixtures of sugars of the same molecular weight and also the isomers of a single monosaccharide can be 'separated' by DOSY on the basis of their different tendencies to form complexes with different diffusion coefficient values.

Keywords: Lanthanide cations; Sugars, complexation of; Diffusion-ordered spectroscopy; DOSY; Diffusion coefficient

A reducing sugar exists as a mixture of different forms in equilibrium including pyranose, furanose and acyclic forms [1]. If one or more of these forms possess a sequence of hydroxyl groups in axial–equatorial–axial arrangement on a pyranose ring or a sequence of three *cis*-hydroxyl groups on a furanose ring, then, in the presence of inorganic salts, the equilibrium proportions are modified due to the weak but specific complexation of the favored forms with the cations [2].

Based on these structural features, the physical separation of sugar mixtures and the isolation of single tautomeric forms in solution are possible. In fact, since the early 1960s, and as a consequence of such selective complex formation, sugars can be separated on columns of cation-exchange resins using water as eluent [2]. As an example, D-glucose and D-fructose were separated on a kilogram scale by the use of a Ca^{2+} column [3], although for such a kind of separation trivalent cations as La^{3+} or Sm^{3+} are the most effective [4]. A similar method allowed the isolation of the α and β anomers of D-allose (both as pyranose and furanose forms) because the β forms cannot coordinate with cations [5]. On the other hand, the separation of complexed and noncomplexed sugars could be observed by analytical methods such as TLC [4] and electrophoresis [6]. The strength of the complexation between different sugars or forms of the same sugar has been investigated by thermodynamic [7] and spectroscopic methods [8].

* Corresponding author. Fax: +49-341-9711833 or 9736115.

E-mail address: stberger@rz.uni-leipzig.de (S. Berger).

0008-6215/00/$ - see front matter
PII: S0008-6215(00)00239-1

In the present work it will be shown that a new, recently developed NMR technique [9], based on pulsed field gradient (PFG) NMR called diffusion-ordered spectroscopy (DOSY) might be also considered as a useful method to distinguish between complexed and non-complexed sugars.

DOSY enables identification and even structure elucidation of the individual components in a mixture resulting from the differences in their translational diffusion coefficients [10]. A DOSY experiment performed on a mixture displays the conventional NMR spectrum in one dimension, while the diffusion spectrum is displayed in the other dimension. To clarify the NMR assignment of a complicated mixture, the DOSY technique is useful because the NMR signals belonging to one of the components appear aligned with the same diffusion coefficient value.

The resolution of DOSY is related to the molecular size since according to the Stokes–Einstein equation, the diffusion coefficient of a molecule is inversely proportional to the molecular radius [11]. For instance, if some of the tautomeric forms of a reducing sugar were complexed with cations, their global size and molecular weight should be greater than the noncomplexed ones, and consequently they should exhibit different diffusion coefficients values.

Here it will be qualitatively shown that although the complexation is a dynamic process, the DOSY experiment can be successfully used because the observed diffusion is an average of the populations of the diffusion of the complexed and noncomplexed forms in equilibrium. The relative difference between the diffusion coefficient (expressed as $\log D$) of complexing and noncomplexing sugars are comparable to the results obtained by TLC plates in the presence of lanthanide cations [4], but may be obtained in a more simple and shorter NMR experiment. A set of pentoses and hexoses were used to evaluate monosaccharides. The diffusion of the components in aqueous solutions of single monosaccharides as well as binary mixtures of pentoses or hexoses were studied. The differences in diffusion observed after the addition of $LaCl_3$ make the species in the mixture distinguishable. Although the method does not provide a physical separation, it will be shown below that a clear visualization of such 'separation' by DOSY experiments is possible in molecules of high interest such as D-ribose.

The experimental work is divided in two parts: (i) 'separation' of tautomers of a monosaccharide by selective complexation with lanthanide cations and, (ii) 'separation' of sugars from a mixture of monosaccharides with identical molecular weight by selective complexation with lanthanide cations.

DOSY experiments were performed on systems with different abilities to form complexes with $LaCl_3$ in water [4]. Reducing monosaccharides were divided in favored (e.g. D-ribose or D-allose) and nonfavored complexing species (e.g. D-arabinose, D-glucose or D-galactose), depending on the geometry of their tautomers. The example of ribose is shown in Fig. 1.

(i) DOSY experiments with single reducing monosaccharides: 'separation' of tautomers of a monosaccharide by selective complexation with lanthanide cations. — Four solution samples of D-ribose, D-allose, D-arabinose and D-glucose, respectively, in deuterated water were prepared. After equilibrium was reached, $LaCl_3$ was added.

The simple observation of the new ^1H and ^{13}C NMR spectra reveals, as is known, shifting of signals and changes in the tautomeric ratio of the favored complexing sugars (D-ribose and D-allose), but no changes for the nonfavored ones (D-arabinose and D-glucose). These effects are immediately visualized in the DOSY spectra as differences in the diffusion coefficients of the complexed species compared with their references (Fig. 2).

The small solvent molecule diffuses much faster than the solute molecules (complexed or not), and its DOSY peak always appeared above the others. DOSY spectra of D-ribose or D-allose showed drastic changes in the map of signals after addition of $LaCl_3$, even at the lowest concentration of $LaCl_3$, but no changes at all were detected for the nonfavored sugars. For the complexed isomers of D-ribose or D-allose (both the α-furanose and α-pyranose forms), a lower diffusion coefficient was detected. In contrast, their noncomplexed forms,

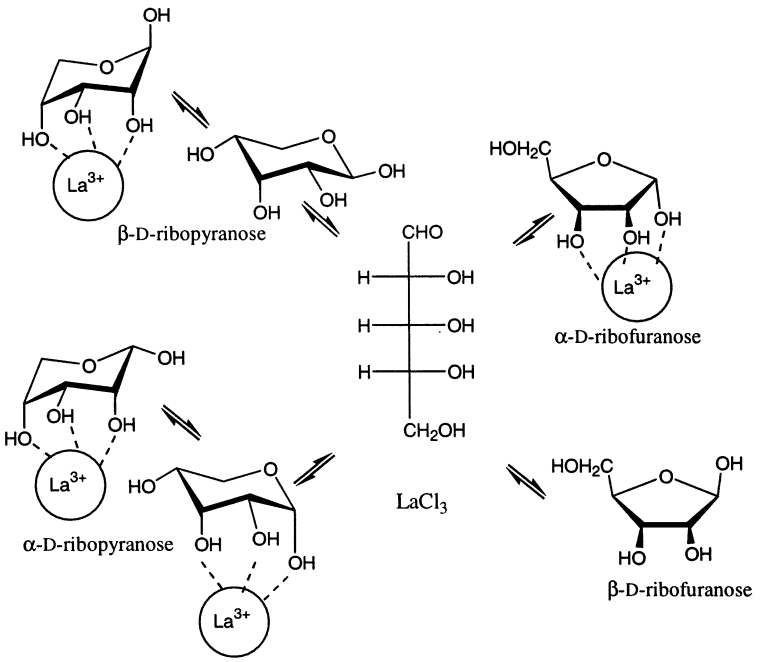

Fig. 1. Tautomeric forms of D-ribose in aqueous solution with $LaCl_3$.

as well as the isomers of the noncomplexing monosaccharides D-arabinose and D-glucose, kept the same diffusion pattern observed in the reference (i.e., without $LaCl_3$) spectrum.

Since the solvent signal was much more intense than that of the solute, and since it lies in the anomeric region under study, a careful phasing and baseline correction was required to avoid artifacts that would give a wrong value for the diffusion coefficient. Such a kind of artifact could not be avoided in the case of the anomeric proton of the complexed α-D-ribopyranose because it was too close to the water signal. As a consequence, its displayed DOSY peak appears higher in the diffusion dimension than expected for the complexed form. This problem was encircled by the observation of the diffusion coefficients of the other signals belonging to α-D-ribopyranose, which were coincident with each other.

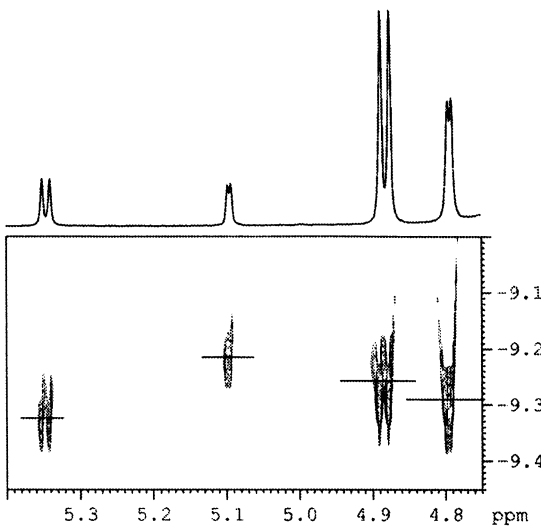

Fig. 2. The H-DOSY spectrum shows the anomeric region of D-ribose in aqueous solution with $LaCl_3$ (molar ratios of ribose: $LaCl_3$ of 1:0.5). The gradient strength of 1 ms duration was incremented in 32 steps, with diffusion times of 50 ms. *D* (the diffusion coefficient) is given in m^2/s.

Fortunately, in the case of D-allose, only the anomeric signal of β-D-allopyranose, which is one of its noncomplexing forms, was obscured by the water signal. The other anomeric signals of allose could be properly analyzed.

The predicted lowest diffusion could be always easily observed for the favored complexing isomers α-furanose and α-pyranose. However, the DOSY spectrum cannot separate the complexed furanose from pyranose forms.

(ii) DOSY experiments with mixtures of reducing monosaccharides[1]*: 'Separation' of*

[1] The DOSY experiment could separate pentoses and hexoses: a mixture of D-arabinose and D-galactose in deuterated water was successfully separated because of the difference in their diffusion coefficients. As was expected, the lower molecular weight arabinose diffuses faster than galactose. The experiment was performed in the absence of inorganic salts.

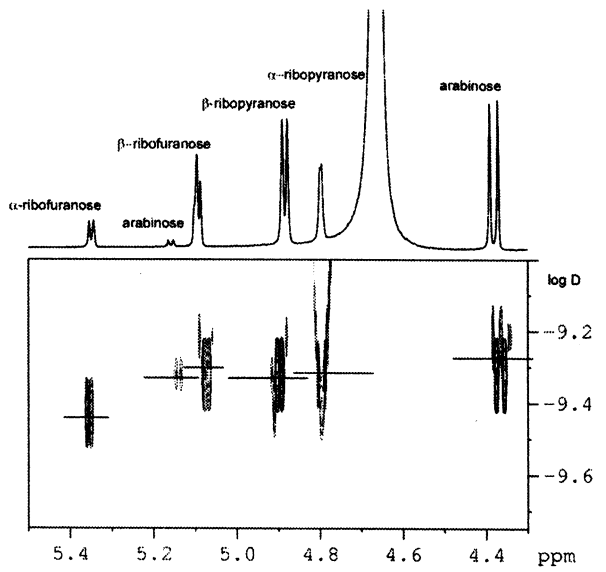

Fig. 3. The H-DOSY spectrum shows the anomeric region of the mixture of D-arabinose and D-ribose in aqueous solution with $LaCl_3$ (molar ratios of arabinose:ribose: $LaCl_3$ of 1:1:2). At $\delta = 5$ there is overlapping of the signals corresponding to the anomeric proton of arabinose and β-ribofuranose. The gradient strength of 1 ms duration was incremented in 16 steps, with diffusion times of 100 ms. *D* (the diffusion coefficient) is given in m^2/s.

sugars from a mixture of monosaccharides with identical molecular weight by selective complexation with lanthanide cations. — Water solutions containing binary mixtures of favored and nonfavored reducing monosaccharides with identical molecular weight were studied under the same conditions described above for single monosaccharides. Their corresponding DOSY spectra, recorded as reference, showed no differences in diffusion for the individual components. However, the addition of $LaCl_3$ changed the diffusion coefficients because only some of the isomers are complexed (D-ribose and D-allose), while their homologues in their respective binary mixture are not (D-arabinose and D-glucose) (Fig. 3).

1. Conclusions

It has been shown that the DOSY technique possesses enough sensitivity to distinguish between compounds with an almost identical structure and the same molecular weight. In spite of the weakness and the dynamic character of the complexes of sugars and cations, the distinction between complexed and noncomplexed forms was possible. In order to use DOSY experiments for quantitative evaluation of the strength of sugar complexation, new experiments under different conditions are currently being carried out.

2. Experimental

General reagents.—The sugars were commercially available from Fluka Chemical Co. (D-arabinose, D-ribose, D-galactose and D-glucose) and Aldrich Chemical Co. (D-allose), and they were used without other previous treatment.

The heptahydrated lanthanide chloride was purchased from Aldrich. To avoid a strong water signal from $LaCl_3 \cdot 7H_2O$, a solution of $LaCl_3 \cdot nD_2O$ in deuterated water was prepared as follows: after drying the commercial $LaCl_3 \cdot 7H_2O$ under vacuum, D_2O was added. The solution was stirred to facilitate the proton–deuterium exchange, and then the solvent was evaporated under vacuum. This procedure was repeated three times, and finally a solution of $LaCl_3$ in deuterated water was obtained.

Methods.—All NMR experiments were conducted on a Bruker DRX 400 MHz spectrometer. Samples of the sugars with a concentration of 0.5 M in deuterated water were used for NMR experiments. After the conformational equilibrium was established, a solution of $LaCl_3$ was added. The study was performed using molar ratios of sugar: $LaCl_3$ of 1:0, 1:0.5, 1:1, 1:1.5 and 1:2.

The NMR spectra in the absence of $LaCl_3$ were considered as references and then compared with experiments performed after addition of $LaCl_3$. The ^1H NMR as well as ^{13}C NMR and DOSY spectra were recorded and compared with the references. To illustrate the changes in the diffusion coefficient most clearly, the spectra shown in the figures contain only an expansion of the anomeric region.

A stimulated echo sequence incorporating bipolar gradients with a longitudinal eddy delay of 5 ms (BPPLED) was used for acquiring DOSY spectra. Gradient strengths of 1 ms duration were incremented in 16 or 32 steps, and the diffusion times were optimized for

every experiment. Typical values found for diffusion delay range from 50 to 100 ms for complete decay of the signals. The projections are the corresponding ^{1}H NMR spectra recorded immediately before the DOSY.

Acknowledgements

M. Dolores Díaz gratefully acknowledges the grant of a fellowship (EX 99 25391200) by Ministerio de Educación y Cultura of Spain.

References

[1] (a) S. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15–68. (b) S. Angyal, *Adv. Carbohydr. Chem. Biochem.* 49 (1991) 19–35.

[2] (a) S. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 1–43. (b) B. Gyurcsik, L. Nagy, *Coord. Chem.*, 203 (2000) 81–149.

[3] P.E. Parker, S. Thawait, *Chem. Ind. (London)*, (1983) 817–821.

[4] (a) Y. Israeli, J.-P. Morel, N. Morel-Desrosiers, *Carbohydr. Res.*, 263 (1994) 25–33. (b) Y. Israeli, C. Lhermet, J.-P. Morel, N. Morel-Desrosiers, *Carbohydr. Res.*, 289 (1996) 1–10.

[5] S. Angyal, G.S. Bethell, R.J. Beveridge, *Carbohydr. Res.*, 73 (1979) 9–18.

[6] (a) S. Angyal, J.A. Mills, *Aust. J. Chem.*, 32 (1979) 1993-2001. (b) B. Bettler, R. Amado, H. Neukom, *J. Chromatogr.*, 498 (1990) 223–230.

[7] (a) N. Morel-Desrosiers, Lhermet, C., J.-P. Morel. *J. Chem. Soc., Faraday Trans.*, 87 (1991) 2173–2177. (b) N. Morel Desrosiers, C. Lhermet, J.-P. Morel, *J. Chem. Soc., Faraday Trans.*, 89 (1993) 1223–1228.

[8] R.E. Lenkinski, J. Reuben, *J. Am. Chem. Soc.*, 98 (1976) 3089–3094.

[9] C.S. Johnson, Jr., *Progr. NMR Spectrosc.*, 34 (1999) 203–256.

[10] A. Chen, C.S. Johnson, Jr., M. Lin, M.J. Shapiro, *J. Am. Chem. Soc.*, 120 (1998) 9094–9095.

[11] K.F. Morris, P. Stilbs, C.S. Johnson, Jr., *Anal. Chem.*, 66 (1994) 211–215.