Determination of the Anomeric Configuration of Glycosyl Esters of Nucleoside Pyrophosphates by Fast-Atom Bombardment Tandem Mass Spectrometry

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Very low energy collision-induced dissociation of the deprotonated molecules of glycosyl esters of nucleoside pyrophosphates results in distinct fragmentation patterns that depend on the cis–trans configuration of the phosphodiester and 2″-hydroxyl groups of the glycosyl residue. In tandem mass spectrometry, sugar nucleotides with cis configuration produce only one, very abundant fragment that corresponds to nucleoside monophosphate, whereas nucleotides with trans configuration give weak signals for the nucleoside di- and monophosphates and their dehydration products. This empirical rule holds for sugar nucleotides that have a free 2″-hydroxyl group and no alternative charge location. Owing to its simplicity, sensitivity, and tolerance of impurities, fast-atom bombardment–tandem mass spectrometry represents a suitable method for determination of the anomeric linkage of glycosyl esters of nucleoside pyrophosphates if the absolute configuration of glycosyl residue is known and the compound fulfills the above-mentioned requirements. *(J Am Soc Mass Spectrom 1995, 6, 516–520)*

Nucleoside 5′-glycosyl pyrophosphates, also called sugar nucleotides, are involved in many fundamental biochemical processes because they act as donors of glycosyl residues, as intermediates, and as regulation factors for the biosynthesis of glycoconjugates in all living organisms. Since the discovery by Park and Johnson [1] of uridine diphospho-*N*-acetylmuramic acid-pentapeptide—a key precursor of the cell-wall peptidoglycan in bacteria—an impressive number of sugar nucleotides have been isolated or synthesized [2–4], and new compounds are still being discovered [5–8]. Glycosyltransferases that employ sugar nucleotides as donors are highly specific to only one anomeric form of sugar nucleotide. Knowledge of the anomeric configuration of glycosyl esters of nucleoside pyrophosphates is, therefore, of great importance in understanding the mechanism of glycosyl transfer reactions and also in the preparation of substrates and analogues for specific enzymes [9].

Until now, the anomeric configuration of sugar nucleotides usually was determined either by nondestructive physicochemical methods, such as NMR and optical rotation measurements, or by different hydrolytic treatments, which include alkaline hydrolysis or enzymatic cleavage with specific exoglycosidases and pyrophosphatases, followed by a comparison of the obtained degradation products with authentic sugar and glycosyl phosphates standards [2, 8].

All the above-mentioned methods for determination of the anomeric linkage of sugar nucleotides require relatively large quantities of a highly purified sample, which represents a serious limitation because these compounds are very labile and present in only small amounts in living organisms. For methods based on hydrolytic and enzymatic treatments, the problem can be overcome if radiolabeled samples of high purity are available. However, in the case of sugar nucleotides that contain unusual glycosyl residues, specific exoglycosidases or appropriate sugar phosphate standards are often lacking, which renders this approach of little help.

Recently, fast-atom bombardment was applied to the determination of nucleotides [10–12] and sugar nucleotides [5–7, 13] and proved to be a sensitive method for obtaining information about the molecular mass, the identity of glycosyl and nucleoside moieties, and the presence of covalent modifications.

In the present communication, we demonstrate that further structural information can be obtained by using fast-atom bombardment–tandem mass spectrometry (FAB-MS/MS) for the analysis of glycosyl esters of nucleoside pyrophosphates (also, directly from mixtures) and, in particular, that the tandem mass spec-

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trum can be used to determine the anomeric configuration of glycosyl residues, at least, in certain sugar nucleotides.

Experimental

Chemicals. All the glycosyl esters of nucleoside pyrophosphates were obtained from Sigma Chemical Co. (St. Louis, MO).

Mass Spectrometry. Fast-atom bombardment–mass spectrometry was performed on a Finnigan-MAT (San Jose, CA) TSQ 70 triple quadrupole mass spectrometer with an Ion Tech (Middlesex, UK) FAB gun operating at 7 keV and 1 mA with xenox as the gas. In the tandem mass spectrometry experiments, the collision-offset voltage was 0.5 V and the pressure of the target gas (xenon) was 0.6 mtorr. Sodium salts of sugar nucleotides were dissolved in water to a concentration of 10 mg/mL, and 0.5 μL of the solution was applied to a glycerol matrix.

Results and Discussion

Negative-ion fast-atom bombardment of glycosyl esters of nucleoside pyrophosphates (sugar nucleotides). The high mass region of the negative-ion fast-atom bombardment FAB mass spectra of all the sugar nucleotides tested showed the presence of an intense signal that arises from the deprotonated free acid molecule, $[M - H]^-$ and a weaker signal for the monosodiated species $[M - 2H + Na]^-$ (not shown).

Tandem mass spectrometry of the $[M - H]^-$ ion of sugar nucleotides at low collision energy. At the collision-offset voltage of 0.5 V, deprotonated molecules of sugar nucleotides gave very few, although characteristic, fragments (Figure 1). For example, the product ion spectrum of the $[M - H]^-$ ion of guanosine 5′(α – D-mannopyranosyl pyrophosphate) (GDP-α-D-mannose) of m/z 604 (Figure 1a) displayed the presence of fragments at m/z 442 and m/z 362, which correspond to guanosine diphosphate (GDP) and guanosine monophosphate (GMP), respectively. These ions were accompanied by smaller fragments (−18 u) at m/z 424 and m/z 344, which are attributed to dehydration products of GDP and GMP, respectively.

In the case of guanosine 5′(α-D-glucopyranosyl pyrophosphate) (GDP-α-D-glucose), which differs from GDP-α-D-mannose only by the configuration of hydroxyl group at the C-2″ position, fragmentation of the deprotonated molecule (m/z 604) at low collision energy resulted in only one abundant product ion at m/z 362, which corresponds to guanosine monophosphate (GMP; Figure 2b).

To examine further the role of the glycosyl configuration in the fragmentation of sugar nucleotides, we tested both anomers (α and β) of guanosine 5′(L-

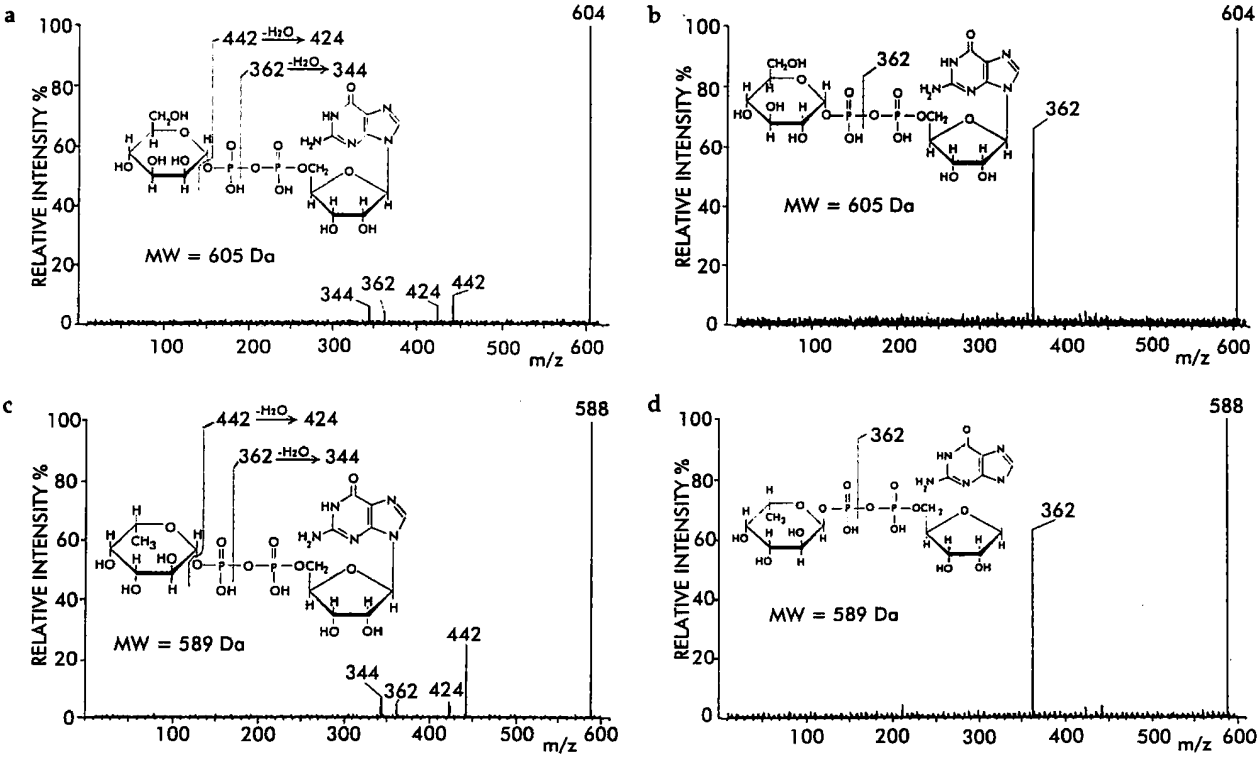

Figure 1. The product ion spectrum of the $[M - H]^-$ deprotonated molecule of (**a**) guanosine 5′(α-D-mannopyranosyl pyrophosphate) (GDP-α-D-Man), (**b**) guanosine 5′(α-D-glucopyranosyl pyrophosphate) (GDP-α-D-Glc), (**c**) guanosine 5′(β-L-fucopyranosyl pyrophosphate) (GDP-β-L-Fuc), and (**d**) guanosine 5′ (α-L-fucopyranosyl pyrophosphate) (GDP-α-L-Fuc).

fucopyranosyl pyrophosphate) (GDP-L-fucose) (Figure 1 c and d). GDP-β-L-fucose has the same trans configuration of the phosphodiester group and the 2″-hydroxyl group as GDP-α-D-mannose. The fragmentation pattern of the GDP-β-L-fucose $[M - H]^-$ ion (m/z 588) at low collision energy (Figure 1c) is similar to that of GDP-α-D-mannose (Figure 1a) and shows the presence of fragments that correspond to GDP, GMP, and their dehydration products. On the contrary, in GDP-α-L-fucose, the phosphodiester and the 2″-hydroxyl groups are in cis configuration, as in GDP-α-D-glucose. The product ion spectrum of the deprotonated molecule of GDP-α-L-fucose (Figure 1d) is different from that of GDP-β-L-fucose (Figure 1c) and very similar to that of GDP-α-D-glucose (Figure 1b); it shows only one intense signal at m/z 362, which corresponds to GMP.

Two other compounds, namely, uridine 5′(α-D-mannopyranosyl pyrophosphate) (UDP-α-D-mannose) and uridine 5′(α-D-glucopyranosyl pyrophosphate) (UDP-α-D-glucose), which differ in their cis–trans geometry of the phosphodiester and 2″-hydroxyl groups (Figure 2), also fragment in two different ways. The product ion spectrum of the deprotonated molecule of UDP-α-D-mannose (m/z 565; Figure 2a) reveals abundant ions at m/z 403 and m/z 323, which corresponds to uridine diphosphate (UDP) and uridine monophosphate (UMP), respectively. The tandem mass spectrum of the $[M - H]^-$ ion of UDP-α-D-mannose is, therefore, comparable to those of GDP-α-D-mannose and GDP-β-L-fucose, because in each of these spectra, the most prominent fragments are the nucleoside di- and monophosphates (NDP and NMP) and their dehydrated forms.

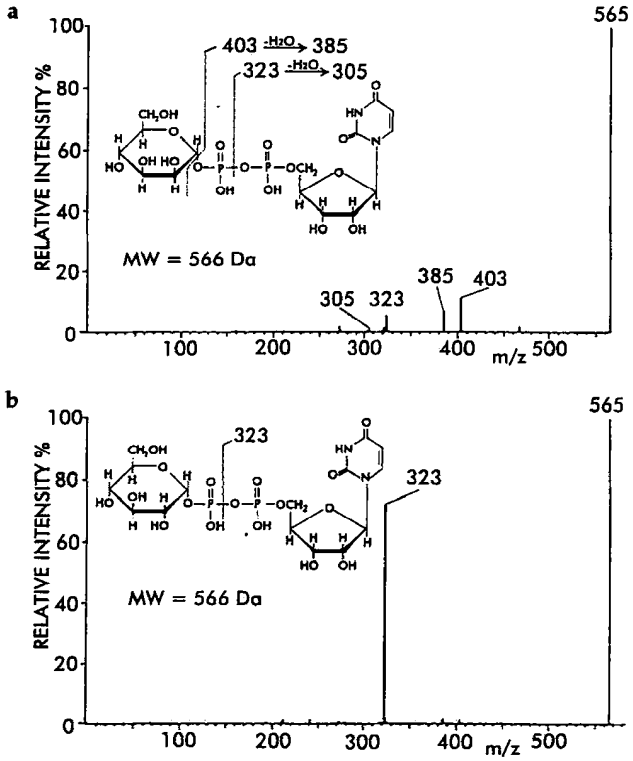

Figure 2. The product ion spectrum of the $[M - H]^-$ deprotonated molecule of (**a**) uridine 5′(α-D-mannopyranosyl pyrophosphate) (UDP-α-D-Man) and (**b**) uridine 5′(α-D-glucopyranosyl pyrophosphate) (UDP-α-D-Glc).

Examination of the fragmentation pattern of the deprotonated molecule of UDP-α-D-glucose (Figure 2b) reveals a striking similarity with patterns obtained for GDP-α-D-glucose (Figure 1b) and GDP-α-L-fucose (Figure 1d). Accordingly, the only abundant fragment at m/z 323 is the nucleoside monophosphate (i.e., uridine monophosphate). Moreover, other sugar nucleotides with preserved cis orientation of their phosphodiester and 2″-hydroxyl groups behaved in the same manner in the tandem mass spectrometry experiments (Table 1). Thus, for example, UDP-α-D-galactose (a 4″-epimer of UDP-α-D-glucose) and a pentose-containing derivative, UDP-α-D-xylopyranose, give identical fragmentation spectra, as does UDP-α-D-glucose (Table 1). Similarly, the product ion spectrum of the deprotonated molecule of thymidine 5′(α-D-glucopyranosyl pyrophosphate) (TDP-α-D-glucose) shows the presence of only one fragment at m/z 321: thymidine monophosphate (Table 1).

However, in the case of uridine 5′-(α-*N*-acetyl-2-amino, 2-deoxy-D-glucopyranosyl pyrophosphate) (UDP-GlcNAc), the 2″-hydroxyl group of which is replaced by the acetylated amino group, the molecular ion gave a distinct tandem mass spectrum (Table 1). Unlike UDP-α-D-glucose, it does not produce any uridine monophosphate (at m/z 323), but a significant ion at m/z 385, which corresponds to $[UDP - H_2O - H]^-$. This observation again confirms the role of the 2″-hydroxyl group of the glycosyl residue in the fragmentation pathways of sugar nucleotides.

The product ion spectrum of the $[M - H]^-$ ion of UDP-α-D-glucuronic acid (Table 1) also reveals important differences in comparison with those of other sugar nucleotides that have cis orientation of the phosphodiester and 2″-hydroxyl groups. Abundant fragments that correspond to UDP, UMP, and glucuronic acid 1-PO_3 are observed at m/z 403, 323, and 255, respectively.

Obviously, the above-presented fragmentation patterns of sugar nucleotides that contain a neutral monosaccharide (Figures 1 and 2) do not hold for species that bear an alternative charge location or a substitution at the C-2″ position of the glycosyl residue.

Differential behavior of isomers of sugars and sugar sulfates in FAB was described in previous literature [14–16], and the use of collision-induced dissociation (CID) to distinguish anomeric or positional isomers of some sugar derivatives, such as C-glycosides [17], sulfates [18], and unusual nucleosides [19, 20], also was reported. However, as far as we know there is no such report on phosphorylated forms of sugars. Additionally, because the above-mentioned tandem mass spectrometry studies of sugar derivatives dealt with very few examples of quite particular compounds [17–20],

Table 1. Negative-ion fast-atom bombardment–tandem mass spectrometry of glycosyl esters of nucleoside pyrophosphates[a]

Glycosyl–NDP (MW)	Configuration of the phospho-diester and 2″-hydroxyl groups of the glycosyl residue	Relative abundances[b] of fragments (m/z) obtained from $[M - H]^-$ parent ion (100%) at 0.5-V collision-offset voltage (%)			
		$[NDP - H]^-$	$[NDP - H_2O - H]^-$	$[NMP - H]^-$	$[NMP - H_2O - H]^-$
GDP-α-D-Man	trans	10	6	5	6
(605)		(442)	(424)	(362)	(344)
GDP-β-L-Fuc	trans	24	5	4	7
(589)		(422)	(424)	(362)	(344)
GDP-α-D-Glc	cis	—	4	66	—
(605)		(442)	(424)	(362)	(344)
GDP-α-L-Fuc	cis	—	—	63	—
(589)		(442)	(424)	(362)	(344)
UDP-α-D-Man	trans	12	7	6	—
(566)		(403)	(385)	(323)	(305)
UDP-α-D-Glc	cis	—	—	63	—
(566)		(403)	(385)	(323)	(305)
UDP-α-D-Gal	cis	—	—	36	—
(566)		(403)	(385)	(323)	(305)
UDP-α-D-Xyl_p	cis	—	—	80	—
(536)		(403)	(385)	(323)	(305)
TDP-α-D-Glc	cis	—	—	38	—
(564)		(401)	(383)	(321)	(303)
UDP-α-D-GlcA[c]	cis	25	—	15	—
(580)		(403)	(385)	(323)	(305)
UDP-α-D-GlcNAc		—	9	—	—
(607)		(403)	(385)	(323)	(305)

[a] Abbreviations: Fuc, fucose; Gal, galactose; Glc, glucose; GlcA, glucuronic acid; GlcNAc, *N*-acetyl-2-amino, 2-deoxy-D-glucose; glycosyl-NDP, glycosyl ester of nucleoside pyrophosphate; Man, mannose; MW, molecular weight; NDP, nucleoside diphosphate; NMP, nucleoside monophosphate; Xyl_p, xylopyranose.

[b] Only signals of relative abundance equal to or greater than 4% are given.

[c] A signal of m/z 255 (relative abundance 4%) that corresponds to [glucuronosyl 1-PO_3]$^-$ fragment also was observed.

they do not provide sufficient information for prediction of tandem mass spectrometry patterns of the anomeric forms of related substances.

In the present study, we used very low collision energies to obtain simple diagnostic CID spectra of anomeric forms of sugar nucleotides. Increasing the collision energy results in generation of additional new fragments (Wolucka, B. A. et al., manuscript in preparation) and, consequently, in more complex (nevertheless still specific for a given anomeric form) tandem mass spectra of glycosyl esters of nucleoside pyrophosphates.

Conclusions

The results described here demonstrate that the anomeric configuration of a glycosyl ester of nucleoside pyrophosphate of known absolute configuration can be determined by fast-atom bombardment–tandem mass spectrometry at very low collision energy provided the glycosyl residue does not contain an alternative charge location and has a free hydroxyl group at the C-2″ position. Then the product ion spectrum of a deprotonated molecule, $[M - H]^-$ shows a characteristic pattern that depends on cis–trans configuration of the phosphodiester and 2″-hydroxyl groups of the sugar moiety. For sugar nucleotides with cis orientation of the above-mentioned groups (as in NDP-α-D-Glc), the diagnostic fragment is a nucleoside monophosphate $[NMP - H]^-$ and this is the only abundant ion observed. In the case of sugar nucleotides with trans configuration of the phosphodiester and 2″-hydroxyl groups (as in NDP-α-D-Man), four major fragments that are obtained from the $[M - H]^-$ ion are the nucleoside diphosphate $[NDP - H]^-$ and nucleoside monophosphate $[NMP - H]^-$ and their dehydration products $[NDP - H_2O - H]^-$ and $[NMP - H_2O - H]^-$. Further studies on low collision-energy-induced dissociation of chemically synthesized α/β isomers of glycosyl esters of nucleoside pyrophosphates that contain other kinds of monosaccharides will be necessary to define fragmentation pathways and the underlying mechanisms, and to verify the general applicability of the FAB-MS/MS technique and other, more sensitive, mass spectrometry approaches, such as electrospray tandem mass spectrometry, for the determination of the anomeric configuration of sugar nucleotides.

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