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Complexation of vanadium(V) oxyanions with hexopyranose- and mannopyranoseuronic acid-containing polysaccharides: stereochemical considerations

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Abstract—Carbohydrates containing galactopyranosyl and mannopyranosyl units with vicinal *cis*-diols were treated with $NaVO_3$ in D_2O, and complexation was determined by ^{51}V NMR spectroscopy. Me α-Gal*p*, Me β-Gal*p* (3,4-*cis*-diols), and Me α-Man*p* (2,3-*cis*-diol) complexed, but Me β-Man*p* barely did so. This low degree of complexation also occurred with a β-mannan containing alternate (1 → 3)- and (1 → 4)-linkages and an alginate having β-Man*p*A blocks. In contrast, branched α-mannans complexed readily, although the ^{51}V resonances for one with side chains terminated with α-Man*p*-(1 → 3)-α-Man*p*-(1 → differed from another with only α-Man*p*-(1 → 2)-α-Man*p*-(1 → groups. The anomeric configuration of Me α-Gal*p* and Me β-Gal*p*, each with 3,4-*cis*-diols remote from C-1, gave rise to three ^{51}V signals of complexes with similar shifts and proportions. The shifts of a galactomannan with terminal α-Gal*p*-(1 → 2)-α-Man*p*- were the same as those with α-Gal*p*-(1 → 6)-β-Man*p*- groups, but fewer complexes were formed with the former structure, probably due to greater steric crowding of the vanadate esters. Most of the complexes gave rise to a signal in the δ −515 region, consistent with the dimeric trigonal–bipyramidal structure.

Keywords: Hexopyranans; VO_3(V) complexes; Vicinal *cis*-diols; Anomeric configuration; ^{51}V NMR spectroscopy

1. Introduction

^{51}V NMR spectroscopy has been extensively used to show the specificity and degree of complexation in aqueous solution of pentavalent vanadate with polyhydroxylic systems. In 1986, Tracey and Gresser[1] showed that aqueous ethylene glycol and pentavalent Na_3VO_4 formed as a major product, a trigonal–bipyramidal cyclic ester dimer, with a 2:2 stoichiometry, which gave rise to a signal at δ −520. This was followed with a series of other investigations.[2–4] In their review of 1998, Verchére et al.[5] related that for the previous decade, a controversy existed as to the structure of the main complexes in aqueous solution, but that the consensus was that they had a distorted trigonal–bipyramidal geometry (**1**). Clearly, with asymmetric diols, two stereoisomeric complexes can be formed, the simplest system of propylene-1,2-diol giving rise to two signals at δ −523 and −529.[1] Tracey and Gresser extended their investigations to cyclic diols and methyl glycosides.[6] The equilibrium constant for complexation of a great excess of 1,2-cyclohexanediols over vanadate with in HEPES buffer at pH 7.5 favored the *cis*- over the *trans*-isomer to the extent of 10:1, and ^{51}V NMR spectroscopy of both isomers showed signals at $\delta \sim -520$. In the case of Me *β*-Man*p*, with its 2,3-*cis*-diol, a signal at δ −522 was observed close to one at δ −517 from Me α- and β-Gal*p*, which contain 3,4-*cis*-diols, but with additional signals at δ −544 and −502. These would be due to complexes with other structures, perhaps related to the 4,6-OH

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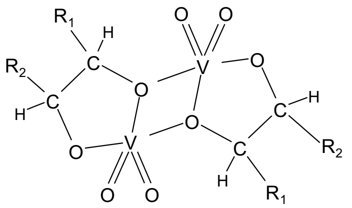
groups, perhaps more favorable to complexation than those in the Man*p* and the α- and β-Glc*p* series. In the latter, with their *trans*-OH groups in the conformationally stable pyranosyl ring, virtually no complexation took place.



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Geraldes and Castro[7] investigated complexation of reducing monosaccharides with ammonium vanadate at pH 7.0. Glucose gave rise to a signal at δ −523, probably due to complexation with the 1,2-*cis*-diol of its α-anomer.

More recently, Noleto et al.[8] studied the complexation of NaVO$_3$ in D$_2$O at its natural pD, with Me α-Man*p*, Me α-Gal*p*, and its β-isomer, and selected *O*-methyl derivatives with defined vicinal *cis*- and OH-4,6-diols. Me α-Man*p* and its derivatives having free *cis*-OH-2,3 groups complexed (the 2,3-complexation site was shown by ^{13}C NMR spectroscopy), giving principal ^{51}V signals in the δ −515 to −517 region. As previously found,[6] a variety of complexes were formed with Me α- and β-Gal*p* giving signals at $\delta \sim -494$, −508, and −535.[8] Complexation occurred with Me 6Me-α-Gal*p* and Me 2,6-Me$_2$-α-Gal*p*, which have 3,4-*cis*-diols, but not with Me 3Me-β-Gal*p*, which contains free 4,6-OH groups.[8]

As an aqueous solution of a complex of oxovanadium(IV) with a lichen galactomannan gave rise to activities related to peritoneal macrophages and leishmanicidal activity,[9] we now examine the formation of esters of selected polysaccharides with NaVO$_3$ without accompanying buffer, which could be biologically prejudicial. Tested were galactopyranose, mannopyranose-, and mannopyranoseuronic acid-containing polysaccharides, taking into account a surprising preliminary experiment, in which ^{51}V NMR spectroscopy showed an almost complete lack of complexation with methyl β-mannopyranoside (Table 1).

2. Results and discussion

2.1. Experiments with low-molecular-weight standards

NaVO$_3$ dissolved in D$_2$O solution in the presence of carbohydrates, gave rise to ^{51}V signals of ester complexes at lower field than those of vanadate oligomers, whose signals (areas in parentheses) were at δ −549 (1%), −565 (2%), −570 (78%), and −578 (19%): The latter have been attributed to monomers, dimers, tetramers, and pentamers, respectively.[6,7] Experiments were now carried out using a 3:1 molar ratio of carbohydrate to NaVO$_3$, with an equivalent of 194 being used for Me α- and Me β-Man*p*. The value for polysaccharides corresponded to the mass of each glycosidic unit.

As previously shown, Me α-Man*p* gave rise to signals of complexes at δ −499 (1%), −506 (2%), and −514 (16%) (Table 1; Fig. 1A),[8] whereas now the β-anomer was

Table 1. Chemical shifts and percentage areas of ^{51}V NMR signals of vanadate(V) esters

Substrate	^{51}V NMR signals of complexes (δ in ppm)
Ethylene glycol, pD 7.5	−515 (3%)
Me α-Man*p*, pD 7.0[a]	−492 (5%), −507 (12%), −515 (20%)
Me α-Man*p*, pD 7.2[b]	−515 (3%)
Me α-Man*p*, pD 7.5[a,8]	−499 (1%), −506 (2%), −514 (16%)
Me α-Man*p*, pD 8.0[a]	−499 (1%), −506 (2%), −515 (17%)
Me α-Man*p*, pD 8.5[a]	−499 (1%), −507 (2%), −515 (16%), −541 (2%)
Me α-Man*p*, pD 9.0[a]	−499 (1%), −506 (1%), −515 (14%), −533 (6%)
Me α-Man*p*, pD 10.0[a]	−515 (7%), −530 (31%)
S. rouxii α-mannan (**2**)[a]	−516 (6%)
S. cerevisiae α-mannan (**2**,**3**)[a]	−494 (4%), −508 (6%)
S. cerevisiae α-mannan (**2**,**3**)[c]	−493 (24%), −508 (35%)
Partly hydrolyzed *S. cerevisiae* α-mannan[a]	−491 (5%), −517 (5%)
Me β-Man*p*[a]	−496 (1.1%), −506 (0.7%)
R. glutinis (1→3),(1→4)-β-mannan (**4**)[a]	−518 (0.3%)
L. brasiliensis β-Man*p* A block (**5**)(Na$^+$)[a]	−492 (0.3%), −507 (0.5%), −529 (0.1%), −542 (0.2%)
Me α-Gal*p*[a,8]	−495 (9%), −508 (10%), −535 (4%)
T. fermentans α-galacto-α-mannan (**6**)[a]	−493 (1%), −508 (1%)
M. scabrella α-galacto-β-mannan (**7**)[a]	−493 (2%), −508 (3%)
Me β-Gal*p*[a,8]	−493 (10%), −509 (9%), −534 (3%)

[a] Molar equivalent ratio of carbohydrate to NaVO$_3$, 3:1.

[b] A molar equivalent ratio of carbohydrate was used, but the solution was diluted 10-fold.

[c] Molar equivalent ratio of carbohydrate to NaVO$_3$, 12:1.

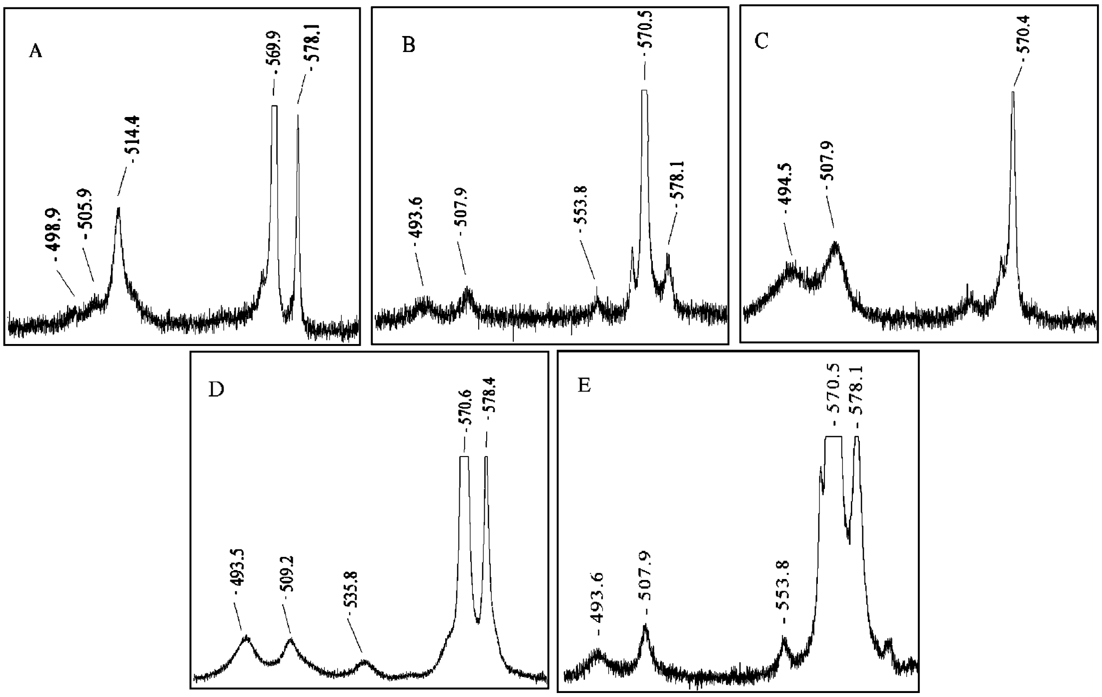


Figure 1. ^{51}V NMR spectra of D_2O solutions (30 °C) of $NaVO_3$+Me α-Man*p* (A), *S. cerevisiae* α-mannan (mannan to $NaVO_3$ molar ratio, 3:1) (B), *S. cerevisiae* α-mannan (mannan to $NaVO_3$ molar ratio, 12:1) (C), Me β-Gal*p* (D), and galactomannan from *M. scabrella* (E).

evidenced by small signals at δ −496 (1.1%) and −506 (0.7%) (Table 1). This low level of complexation, referring to structure **1**, could be due to steric interaction of the 1-OMe with the vanadate group(s), both being on the β-face of the pyranosyl ring.

Tracey and Gresser[1] showed that ethylene glycol complexed in Tris–Cl buffer at pH 7.5 to give a 2:2 ester dimer with a signal at δ −520. Ray et al.[10] in an aqueous system containing predominantly ethylene glycol showed that a signal at δ −523 was obtained. In our D_2O solution, an isotope effect was observed, since the ester resonance appeared at δ −515 (Table 1).

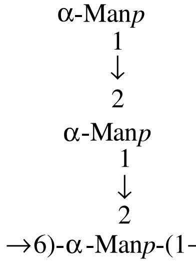
The number and proportions of vanadate esters of Me α-Man*p* varied according to the pD (Table 1). At all pDs, a signal of a stereoisomeric 2:2 dimer appeared at δ −515. Other minor signals appeared at −499 and −506, which were also not pD dependent. At pD 8.5–10.0, a signal at higher fields became increasingly prominent, finally appearing at δ −533 (31%).

An aqueous solution of $NaVO_3$, at the same concentration as used in ^{51}V experiments, had pH 7.49. It changed very little to pH 7.55 on addition of a 3:1 amount of Me α-Man*p*.

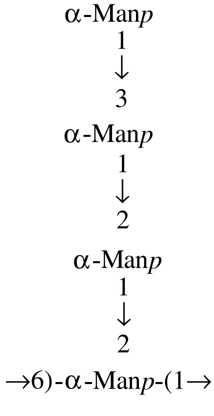
2.2. Complexation of vanadate with branched α-mannans

An α-mannan from *Saccharomyces rouxii* contains structure **2**, and another with one less nonreducing end-unit, both containing 2,3-*cis*-diols.[11,12] Each, with its terminal α-Man*p*-(1 → 2)-unit, gave rise to a single ^{51}V NMR signal at δ −516 (Table 1).

The α-mannan from *Saccharomyces cerevisiae* (baker's yeast), with predominant repeating structure **3**[13] having terminal α-Man*p*-(1 → 3)- and some α-Man*p*-(1 → 2)-units, as in **2**, gave rise to two signals at δ −494 (4%) and −508 (6%) (Table 1, Fig. 1B). On increasing the α-mannan to vanadate ratio by a factor of 4 to 12:1, the resulting ^{51}V spectrum, as expected, showed greatly enlarged ester signals at δ −494.5 (24%) and −508 (35%) (Table 1; Fig. 1C).


2

$$\begin{array}{c}\alpha\text{-Man}p\\1\\\downarrow\\2\\\alpha\text{-Man}p\\1\\\downarrow\\2\\\rightarrow6)\text{-}\alpha\text{-Man}p\text{-}(1\rightarrow\end{array}$$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$$\begin{array}{c}\alpha\text{-Man}p\\1\\\downarrow\\3\\\alpha\text{-Man}p\\1\\\downarrow\\2\\\alpha\text{-Man}p\\1\\\downarrow\\2\\\rightarrow6)\text{-}\alpha\text{-Man}p\text{-}(1\rightarrow\end{array}$$

Partial hydrolysis of *S. cerevisiae* α-mannan preferentially removed its side chains[14] to form a product with 41% of (1 → 6)-linkages, as determined by the relative area of its H-1 signal at δ 5.16, which resonates at highest field of all its H-1 signals. However, even with more nonreducing end units than those of the parent α-mannan, no appreciable increase in complexation took place with signals at δ −491 (5%) and −507 (5%) (Table 1).

An attempt was made to determine the complexation sites in the α-mannan using ^{13}C NMR spectroscopy, despite the broadness of its signals when compared with those of Me α-Man*p*. However, two possible signals were detected at δ 87.1 and 89.7, which when compared with those of esters of Me α-Man*p*,[8] at δ 87.4 (C-2) and 80.0 (C-3), suggested that the former could arise via esterification at C-2. However, any accompanying C-3 signal would overlap with those of 2-O- and 3-O-substituted units of the α-mannan, which would appear in the δ 80.0 region.[15]

2.3. Complexation with linear β-mannan and β-mannopyrananuronic acid

The low degree of complexation found with Me β-Man*p* also extended to β-linked polysaccharides. Two with free *cis*-2,3-diols were examined. One was from *Rhodotorula glutinis* (**4**),[16] which formed only a very small signal of complex at δ −518 (0.3%) (Table 1).

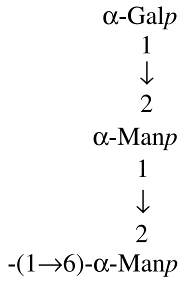
$[\text{-}\beta\text{-Man}p\text{-}(1\rightarrow3)\text{-}\beta\text{-Man}p\text{-}(1\rightarrow4)\text{-}]_n$ **4**

$[\text{-}\beta\text{-Man}p\text{A-}(1\rightarrow4)\text{-}]_n$ **5**

The other was the sodium salt of a (1→4)-linked β-mannopyranosyluronic acid block (**5**), isolated from alginic acid of the marine alga *Laminaria brasiliensis*.[17] This also barely complexed, as shown by small signals at δ −492 (0.3%), −507 (0.5%), −529 (0.1%), and −542 (0.2%) (Table 1).

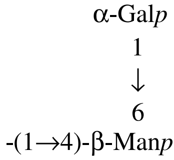
2.4. Complexation of vanadate with α-galactopyrano-α-mannan and α-galactopyrano-β-mannan

The degrees of complexation of vanadate with an α-galacto-α-mannan and an α-galacto-β-mannan, each having terminal α-galactopyranosyl units, were determined. It has been shown that there was no anomeric effect influencing its complexation with Me α-Gal*p* and Me β-Gal*p*, each giving rise to three signals with similar chemical shifts and proportions (Table 1),[8] with only those of Me β-Gal*p* being presented in Table 1 and Figure 1D. A galactomannan of the yeast *Trichosporon fermentans*[18] should complex with vanadate by virtue of its Me α-Gal*p* terminal units present in repeating structure **6**. However, although two similar resonances were present at −493 (1%) and −508 (1%), these were much smaller that those of the Me α-Gal*p* standard, which are at δ −495 (9%), −508 (10%), and −535 (4%) (Table 1).[8]



$$\begin{array}{c}\alpha\text{-Gal}p\\1\\\downarrow\\2\\\alpha\text{-Man}p\\1\\\downarrow\\2\\\text{-(1}\rightarrow\text{6)-}\alpha\text{-Man}p\text{-}\end{array}$$

6



$$\begin{array}{c}\alpha\text{-Gal}p\\1\\\downarrow\\6\\\text{-(1}\rightarrow\text{4)-}\beta\text{-Man}p\text{-}\end{array}$$

7

8

$\text{-}(1\rightarrow4)\text{-}\beta\text{-Man}p\text{-}$

A somewhat greater degree of complexation, although smaller than that observed with Me α-Gal*p*, was found with the galactomannan from seeds of *Mimosa scabrella*, which contains structures **7** and **8**[19,20] (it is assumed that the 4-O- and 4,6-di-O-substituted β-Man*p* units of the main chain would not contribute to the vanadate ester signals). When compared with signals of structure **6**, similar ones of complexes were formed, but in a greater proportion at δ −494 (3%) and −508 (3%) (Table 1; Fig. 1E). It is interesting that the high field of the methyl galactosides does not appear in the spectra of the two galactomannans. This point will be further investigated.

In both galactomannans, the low proportion of signals of the complexes could be due to nonbonded hindrance of the adjacent α-Man*p* unit, which would crowd the vanadate ester of the 3,4-*cis*-diol. It would be expected that structure **6**, which has →2)-α-Man*p*-, rather than the less sterically hindering →6)-α-Man*p*-units in **7**, would give rise to a greater free energy and less complexation.

3. Conclusions

A number of mannans, galactomannans, and a β-mannopyrananuronic acid, containing vicinal *cis*-diol groups, were treated with in D_2O solution with $NaVO_3$ (pD ~7.5) to form complexes of vanadate ester, detected and quantified by ^{51}V NMR spectroscopy. Mannose-containing polysaccharides with 2,3-*cis*-diols in the α-Man*p* series and galactomannans with 3,4-*cis*-diols in the α- and β-Gal*p* series complexed, as evidenced by low-field signals of their esters. Much less complexation occurred with a β-mannopyranan and a β-mannopyrananuronic acid, each containing 2,3-*cis*-diols, unlike polysaccharides containing α-Man*p* units, due to steric effects. The greatest degree of complexation occurred with the branched α-mannan from *S. cerevisiae*, followed by the branched galactomannan of *M. scabrella*. The complexes of these polysaccharides will be tested as targeting devices to see if they have leishmanicidal activity.

4. Experimental

4.1. Materials

Sodium metavanadate ($NaVO_3$) was obtained from Sigma-Aldrich, as was methyl β-D-mannopyranoside, which was labeled in the catalogue as its *iso*-propylate. Its ^{13}C NMR spectrum showed that it was the glycoside with 2-propanol of crystallization. To remove this, it was dissolved in H_2O and freeze dried, and ^{13}C NMR examination of the residue showed it to be identical with authentic material.[21] Other carbohydrates were available in our laboratory.

4.2. pH effect on addition of Me α-Man*p* to vanadate solutions

As an indication of pD values used in ^{51}V examination of polysaccharides, a solution of vanadate (110 mg) in water (4 mL) had pH 7.49. On addition of Me α-Man*p* (450 mg), the pH was 7.55. At a 10-fold dilution of the above, the pH was 7.20, before and after addition of Me α-Man*p*.

4.3. Preparation of solutions of carbohydrate with sodium vanadate

Aqueous solutions of polysaccharide (500 mg in 10 mL) were adjusted, if necessary, to pH 7.5, freeze-dried, and 45 mg of the residues were dissolved in D_2O (0.5 mL). To this solution, sodium vanadate (11 mg) in D_2O (0.4 mL) was added dropwise slowly with shaking, in order to avoid formation of clots of complex.

In the calculation of millimolar concentrations, a molar equivalent of 194 was used for Me α-Man*p* and Me β-Man*p*, 162 for hexosans, 198 for the alginate (Na^+ salt), and 62 for ethylene glycol. In general, 3:1 carbohydrate to $NaVO_3$ molar ratios were used, although one of 12:1 ratio was also examined with the α-mannan of *S. cerevisiae*. The galactomannan of *M. scabrella*, which formed viscous solutions, was dissolved in 3 mL of D_2O. In general, the solution of vanadate was mixed with that of polysaccharide solution with vigorous shaking, in order to avoid possible clots of vanadate complex.

4.4. NMR spectroscopy

NMR measurements were carried out in 5-mm tubes, on D_2O solutions at 30 °C, using a Bruker DRX-400 Avance spectrometer. ^{13}C spectra were obtained at 100.6 MHz with 1 s delay and 0.6 s acquisition times, and 80,000 transients in attempts to detect minor signals of polysaccharide complexes (this was only successful with the *S. cerevisiae* α-mannan). Chemical shifts are expressed as δ (ppm) relative to that of external Me_4Si ($\delta = 0$).

The ^1H spectrum which was of acid-degraded *S. cerevisiae* mannan was obtained at 30 °C at 400.16 MHz with presaturation with 2 s delay and acquisition time and 16 transients.

^{51}V spectra were obtained at 105.2 Hz, with 0.5 s delay and 0.10 s acquisition times, and 1000 transients. Chemical shifts are in δ (ppm) relative to that of external $VOCl_3$ ($\delta = 0$).

Acknowledgements

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