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Spectroscopic investigation of the VO^{2+}/hyaluronate interaction

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ABSTRACT

An oxovanadium(IV) complex was prepared by interaction of aqueous solutions of $VOSO_4 \cdot 5H_2O$ and sodium hyaluronate, at controlled pH-values, under nitrogen atmosphere, and subsequent precipitation with absolute ethanol. The complex formation and the characteristics of the VO^{2+}/hyaluronate interactions were confirmed by vibrational (IR and Raman) and UV–vis electronic absorption spectroscopy. Only the carboxylate groups of the glucuronic acid moiety of the polymer are involved in coordination, acting as monodentate ligands. The suggested coordination is similar to that found in the analogous Cu(II), Mn(II) and Ni(II) complexes.

1. Introduction

Hyaluronic acid (hyaluronan) is a high molecular polysaccharide present in connective, epithelial and neural tissues of animals, and is a member of a group of polysaccharides that has been termed “connective tissue polysaccharides”, “mucopolysaccharides” or “glycosaminoglycans”. It is a linear, unbranched polymer composed by a repeating disaccharide that consists of N-acetyl-D-glucosamine and D-glucuronic acid, linked by a β 1-4 glycosidic bond. The disaccharides are linked by β 1-3 bonds to form the polymer chain (Fig. 1). Hyaluronic acid plays an important role in many biological processes, including tissue hydration, proteoglycan organization in the extracellular matrix, and tissue repair, and it has found application for several clinical purposes and also for cosmetic use (Lapčik Jr., Lapčik, De Smedt, Demeester, & Chabreček, 1998).

The compound is usually isolated and used in the form of its sodium salt. Two principal preparative routes have been used, a biotechnological process, which involves its extraction from chicken crests and some biosynthetic procedures, employing bacterial enzymes (da Rosa, Hoelzel, Bordin Viera, Barreto, & Beirão, 2008; De Luca et al., 1998; Lapčik et al., 1998; Sugahara, Schwartz, & Dorfman, 1979).

In previous studies, we have performed systematic investigations of the interaction of vanadium species with carbohydrates and related species (for a recent review, cf. Baran, 2009). In this context, the interaction of vanadium species with polysaccharides has been scarcely investigated and the available information is so far restricted to VO^{2+} complexes of polygalacturonic acid (Branca, Micera, Dessi, & Kozlowski, 1989; Deiana, Erre, Micera, Piu, & Gessa, 1980), chondroitin sulfate A (Etcheverry, Williams, & Baran, 1994) and chitosan (Baran, 2008). In order to extend these studies we have now investigated the interaction of the oxovanadium(IV) cation with the hyaluronate anion.

2. Experimental

2.1. Materials

Analytical reagent grade $VOSO_4 \cdot 5H_2O$, NaOH and absolute ethanol were supplied by Merck and used as received.

Chicken crests were used as the source for the isolation of sodium hyaluronate, employing a process developed at INTA (Buenos Aires, Argentina). In the first step an enzymatic digestion of the material is performed, followed by successive fractional precipitations with ethanol to purify the crude product. The molecular weight of the purified material is of about 10^6 Da (protein content <0.3%, water content 3.4%) (Dattilo et al., 2010).

2.2. Spectroscopic measurements

The infrared spectra in the range 4000–400 cm^{-1} were recorded with a FTIR-Bruker-EQUINOX-55 instrument, using the KBr pellet technique. Raman spectra in the same spectral range were measured on powdered samples using the FRA 106 Raman accessory of an IF66 Bruker spectrophotometer. Radiation from a Nd:YAG solid-state laser (1064 nm) was used for excitation. It was rela-

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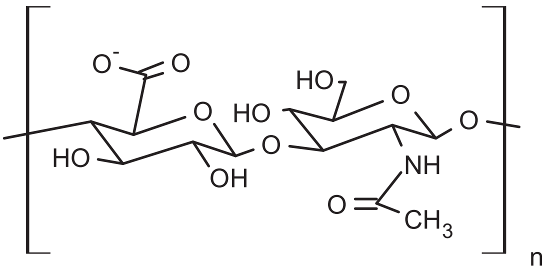

Fig. 1. Schematic structure of the hyaluronate chain.

tively difficult to obtain good quality Raman spectra, especially due to the fibrous characteristics of both, sodium hyaluronate and the complex. We have made a great number of assays, with pure samples and with different KBr/sample mixtures and also varying the number of accumulated scans, without important changes in the spectral quality.

The electronic absorption spectra were measured with a Hewlett-Packard 8452 diode-array spectrophotometer, using 10-mm quartz cells.

2.3. Preparation of the complexes

To 0.050 g of sodium hyaluronate distilled water (6 mL) was added and left to swell for 24 h. The pH of the solution was adjusted to pH 9.40 with 0.1 M NaOH and 2 mL of a 0.0285 M solution of $VOSO_4{\cdot}5H_2O$ was slowly added. The resulting mixture was stirred for one hour under a N_2 atmosphere. The final pH of the reacting mixture was 4.48. The pH-adjustment was crucial because at pH-values above 6.00, gel formation was observed and the product could not be precipitated.

The mixture was added to cold, absolute ethanol (50 mL), under vigorous stirring. The blue solid threaded product was filtered with a fritted glass funnel, washed twice with cold absolute ethanol and, finally, air dried.

3. Results and discussion

3.1. Complex formation

The interaction of a VO^{2+} salt with a sodium hyaluronate solution, at a controlled pH-value, and subsequent complex precipitation by the addition of absolute ethanol is a procedure which has also been employed for the synthesis of other metallic complexes of hyaluronate, for example those of Cu(II) (Tratar Pirc, Arčon, Bukovec, & Kodre, 2000), Mn(II), Co(II) and Ni(II) (Tratar Pirc, Arčon, Kodre, & Bukovec, 2004) or Zn(II) (Nagy et al., 1998). In the present case, the complex is obtained in the form of a blue threaded solid.

3.2. Infrared and Raman spectra

The FTIR spectra of sodium hyaluronate and of one of the obtained VO^{2+}/hyaluronate complex samples, in the medium IR range between 1800 and 800 cm^{-1}, are shown in Fig. 2. The proposed assignments are presented in Table 1. They are based mainly in a previous analysis of the FTIR spectra of sodium hyaluronate and its oligomers (Gilli, Kakuráková, Mathlouthi, Navarini, & Paoletti, 1994) and on the information provided by a recent Raman study of the polymer (Synytsya et al., 2011), as well as by some standard references (Parker, 1971; Smith, 1999). The results are briefly discussed, as follows:

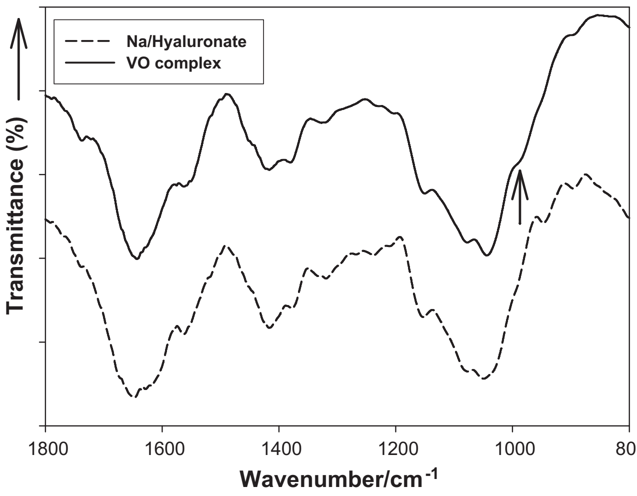

Fig. 2. FTIR spectra of sodium hyaluronate and of the VO^{2+}/hyaluronate complex in the spectral range between 1800 and 800 cm^{-1} (the arrow identifies the position of the characteristic (V=O) stretching mode).

- The so called amide I band, which is essentially the C=O stretching vibration of the amide group (Parker, 1971; Smith, 1999) is seen as a well defined and relatively broad band in both, the hyaluronate anion and the complex.
- The amide II band, which is mainly a mixed vibration involving the N–H in plane bending and the C–N stretching vibration (Parker, 1971) is present as a medium intensity band on both spectra.
- The so called amide III band originated in a similar vibrational mixing appeared also as a well-defined peak in both spectra. It may be, eventually, superimposed to δ(COH) and δ(CH) deformational modes (Synytsya et al., 2011).
- The ν_{as}(C–O–C) bridge stretching vibration is observed at a similar energy as in chitosan and its VO^{2+} complex (Baran, 2008). The corresponding symmetric mode could not be identified with certainty, but it is located, probably, at about 900 cm^{-1} (Synytsya et al., 2011).
- The characteristic $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ stretching vibrations of the carboxylate groups could be clearly identified in both spectra: the first one as a weak shoulder on the lower energy side of the broad amide I band and the second one, as a relatively strong and well-defined band. As it can be seen from Table 1, the $\nu_s(COO^-)$ mode remains unchanged after complex formation whereas the

Table 1
Assignment of the infrared spectra of sodium hyaluronate and of the corresponding VO^{2+} complex (band positions in cm^{-1}).

Na–hyaluronate	VO^{2+}–hyaluronate complex	Assignment
3560 sh, 3465 vs, 3337 vs	3426 vs, br	ν(O–H) + ν(N–H)
2925 m	2926 m	$\nu(CH_3) + \nu(CH_2) + \nu(CH)$
1647 vs, br	1643 vs, br	Amide I
1616 sh	1626 sh	$\nu_{as}(COO^-)$
1563 m	1562 m	Amide II
1416 s	1416 s	$\nu_s(COO^-)$
1376 m	1376 m	δ(O–H)
1319 m	1320 m	Amide III
1154 m	1150 s	$\nu_{as}(C–O–C)_{bridge}$
1077 m	1077 m	ν(CO) + δ(C–OH)
1048 vs	1049 vs	ν(C–OH)
	992 sh	ν(V=O)
947 w	945 sh	ν-Ring (out-of-phase)
894 w	897 w	δ(C1–H) [β-anomer]
613 m	617 m	$\rho(H_2O)$
	473 w	ν(V–O)

vs, very strong; s, strong; m, medium; w, weak; sh, shoulder; br, broad.

corresponding antisymmetric mode is slightly displaced to higher energies. This behavior implies participation of the carboxylato groups in bonding. The energy separation of the two stretching motions is slightly higher in the complex (210 cm^{-1}) than in the free hyaluronate ion (200 cm^{-1}), suggesting unidentate binding of this group (Nakamoto, 2009; Tratar Pirc et al., 2004).

- The typical V═O stretching vibration of the oxocation is seen as a shoulder at 992 cm^{-1} in the FTIR spectrum presented in Fig. 2 and as medium intensity line at 996 cm^{-1} in the Raman spectrum, lying in the region which is characteristic for complexes in which VO^{2+} interacts with carboxylate groups (Baran, 2001). Additionally, in the lower part of the FTIR spectrum (not shown in Fig. 2) we have identified a weak band at 473 cm^{-1} (466 cm^{-1}, Raman), which can be assigned with certainty to one of the V–O (ligand) motions (Baran, 2001).

Finally, and as shown in Table 1, the medium intensity band found at 2925 cm^{-1} in hyaluronate (2926 cm^{-1} in the complex) is surely of complex origin, because several symmetric and antisymmteric C–H stretching modes appear in this region: ν(CH), $\nu(CH_2)$ from the CH_2OH groups and $\nu(CH_3)$ from the N-acetyl groups (Baran, 2008). Besides, the strongest band in this region, which is clearly splitted in sodium hyaluronate, can be assigned to the O–H and N–H– stretching vibrations. The deformational mode of water, $\delta(H_2O)$, surely contributes, in certain extent, to the broadening of the strong IR band centered at about 1650 cm^{-1}.

Concerning the Raman spectra, and although, as mentioned above, it is relatively difficult to obtain good quality spectra we could approximately reproduce the recently published spectrum of sodium hyaluronate (Synytsya et al., 2011). As in the case of the IR spectra, also the Raman spectra of the VO^{2+}-complexes were rather similar to those of the free hyaluronate ion. Again in this case, changes in band positions were only found for the two carboxylate stretching bands. Besides, and as stated above, the ν(V═O) vibration could also be clearly identified in the Raman spectrum of the complex.

3.3. Electronic absorption spectrum

The typical electronic absorption spectrum of $[VO(H_2O)_5]^{2+}$ (C_{4v} symmetry) consists of three bands: $b_2 \rightarrow e$, $b_2 \rightarrow b_1$ and $b_2 \rightarrow a_1$ (generally obscured by overlapping with a charge transfer band). The first two transitions are found at 763 and 625 nm, respectively (Lever, 1984).

The prepared VO/hyaluronan complex shows these bands at 784 and 616 nm. This behavior is characteristic for carboxylato coordinated VO^{2+} complexes, in which the lower energy band suffers a red-shift, whereas the other one presents a blue-shift after complex formation (Allegretti, Ferrer, González-Baró, & Williams, 2000; Berto, Daniele, Foti, Prenesti, & Sammartano, 2008; Williams, Kögerler, & Baran, 1998).

3.4. Structural model

A comparison of the FTIR spectra of our VO^{2+}/hyaluronate complex with those of the corresponding Cu(II) (Tratar Pirc et al., 2000), Mn(II), Co(II) and Ni(II) complexes (Tratar Pirc et al., 2004) shows the presence of totally similar spectral patterns.

In the case of the Cu(II) complex, the coordination polyhedron of the metal center has been elucidated by EXAFS and XANES spectroscopy, demonstrating that the Cu(II) cations are octahedrally coordinated, with four Cu–O bonds with lengths of 1.95 Å, and two longer axial bonds of 2.46 Å. Additionally, also in this case FTIR spectroscopy reveals only the participation of the carboxylate groups of hyaluronate in bonding. These results allow to suggest a coordination sphere conformed by four water molecules (occupying the equatorial positions) and two O-atoms from carboxylate groups belonging to two different and parallel polymer chains (Tratar Pirc et al., 2000). The same behavior was found in the case of the Mn(II) and Ni(II) complexes, whereas those of Co(II) is slightly different (Tratar Pirc et al., 2004).

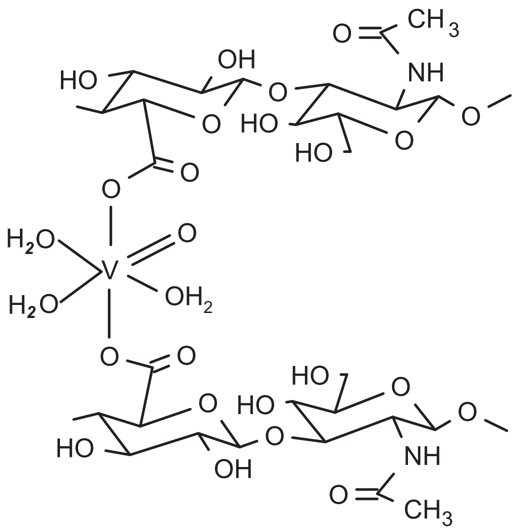

Fig. 3. Proposed structural model for the VO^{2+}/hyaluronate complex.

On the basis of these comparisons it is possible to assume that the coordination of the VO^{2+} cation by the hyaluronate anion occurs in a similar way, involving two monodentate bindings with carboxylate oxygen-atoms of two different polymer chains, occupying axial positions. The coordination sphere is completed by three H_2O molecules; one bonded *trans* to the oxo-group and the other two completing the equatorial bonding plane. The proposed model is shown schematically in Fig. 3.

4. Conclusion

This study confirms the interaction of the oxovanadium(IV) cation, VO^{2+}, with hyaluronate, at a controlled pH-value. The vibrational-spectroscopic study of the generated complex points to the exclusive participation of the carboxylate groups present in the glucuronic acid moiety of the polymer, acting as monodentate ligands. The VO^{2+}/carboxylate interaction is additionally supported by the electronic absorption spectrum of the complex. It is suggested that the VO^{2+} complex presents a similar coordination behavior than those found in the respective complexes of Cu(II), Mn(II) and Ni(II).

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