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Note

Aldo–enol transition in periodate-oxidized dextrans

Svetlana N. Drobchenko *, Ludmila S. Isaeva-Ivanova, Alexander R. Kleiner, Elena V. Eneyskaya

Petersburg Nuclear Physics Institute, Russian Academy of Sciences, Gatchina, St. Petersburg, 188350, Russian Federation

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The absence of absorption for aldehyde groups in the UV and IR spectra of "dialdehyde polysaccharides" is generally thought as being due to the formation of hemiacetal and hydrated functionalities in water solutions [1]. It was established in our previous reports [2–4] that this is valid only for a narrow pH range (4–5.2), and that formation of the enol and enolate anion is in fact a main feature, at least at higher pH.

The present work deals with ^{1}H, ^{13}C NMR and electrophoretic light scattering (ELS) measurements of oxidized dextrans (ODs) performed at various pH, which supports our early results [4].

1. Experimental

Dextrans with molecular weights of 20,000 (Fluka AG), 40,000, 60,000 (Reachim, Russian Federation), 70,000, 110,000, 500,000 (Loba Chemie) were used in the present study. Dextrans were oxidized as previously described [2]. Aldehyde groups were determined by their iodine number [5], enols by the method of Meyer [6], and the carboxyl groups according to ref. [7]. ODs were reduced with sodium borohydride according to ref. [8].

UV spectra of dextran solutions were recorded with a Specord M-40 spectrophotome-

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* Corresponding author.

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ter. Samples for IR spectroscopic measurements were prepared by pressing with KBr and their spectra were measured with a Specord IR-71 instrument. ^{1}H NMR (200.13 MHz) and ^{13}C NMR (125.7 MHz) spectra were recorded with an AC 200 Bruker spectrometer for samples (150 mg) in D_2O (3 mL).

Electrophoretic mobility by ELS method was measured with a device [9] designed in Petersburg Nuclear Physics Institute (St. Petersburg, Russian Federation).

2. Results and discussion

^{1}H NMR spectra of dextran and dextran products are presented in Figs. 1 and 2, respectively. After periodate oxidation, several new signals at 4.3, 5.15, 5.3–5.5, 5.7, and 6.0 ppm were observed with relative intensities which depended on the pH of the solution. It is worth to note that all signals from ODs detected in these regions disappeared after treatment with sodium borohydride (Fig. 3). It follows that the above-mentioned signals must arise from protons of hydrated aldehyde and aldehyde groups linked by polyacetal bonds. A pH-dependent quantitative change in the ratio between these aldehyde forms results in different relative intensities of these signals at alkaline and acidic pH.

Signals at 8–9 ppm were observable only at alkaline pH (Fig. 2a) and they disappeared at low pH (Fig. 2b). Conversely, when the alkali was added to the product isolated at acidic pH, the signals appeared again (Fig. 2c). In this region, only signals from CH enol protons, carboxyl and α,β-unsaturated aldehyde olefinic protons are expected. Taking into account that signals in this region appear only at alkaline pH, and in view of the observed reversibility, aldo–enol transitions are probably responsible for the observed pH-dependence of OD solutions.

To test this conclusion, ODs were treated with sodium borohydride which is known to reduce aldehyde without affecting carboxyl groups and double carbon–carbon bonds. Absence of signals from the reduced dextran in this spectral region at any pH of the solution (Fig. 3) supports that signals at 8–9 ppm are effectively due to the presence of a tautomeric form of aldehydes.

The fact that a signal appeared at 9.3 ppm after addition of the acid to the alkaline

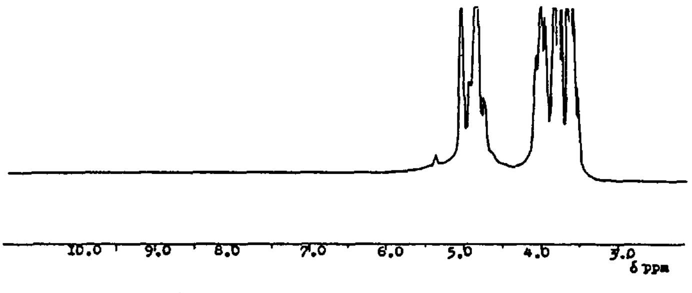


Fig. 1. ^{1}H NMR spectrum of dextran, mol wt 60,000.

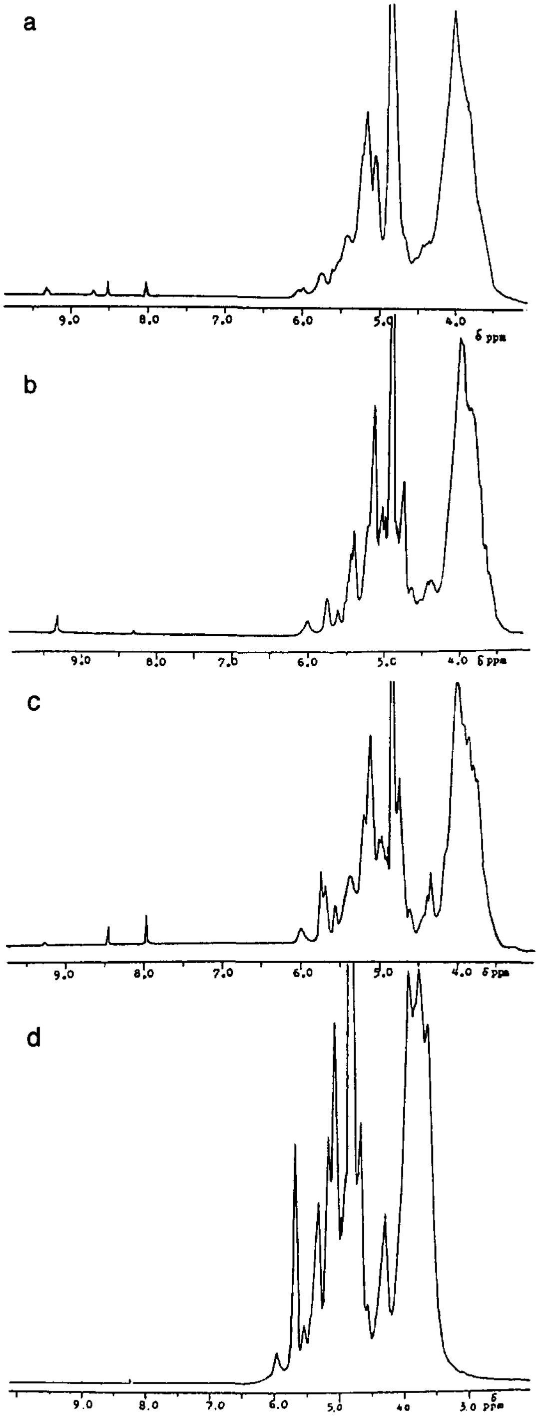


Fig. 2. ^{1}H NMR spectra of the oxidized dextran (mol. wt. 60,000, do 60). (a) OD isolated at pH 7.5, in 0.01 N NaOD; (b) OD isolated at pH 7.5, in 0.01 N DCl; (c) OD isolated at pH 3.0, in 0.01 N NaOD; (d) OD isolated at pH 3.0, in 0.01 N DCl.

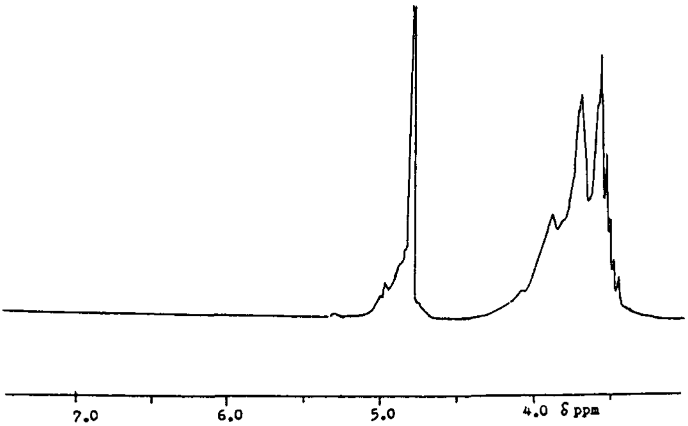


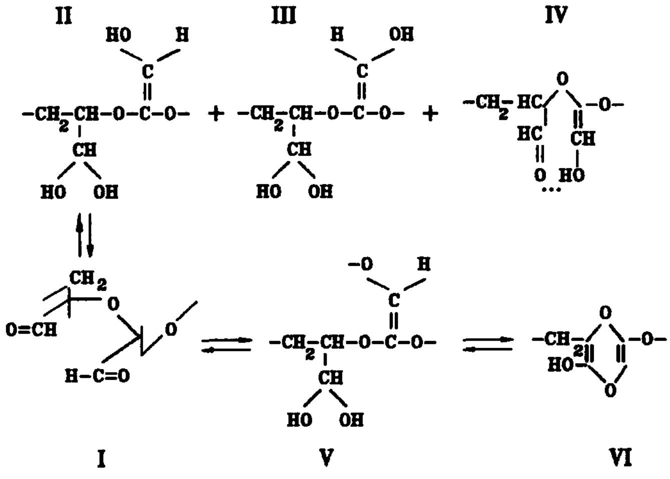
Fig. 3. ^{1}H NMR spectrum of OD product after reduction with sodium borohydride in 0.01 N NaOD.

solution of the OD (Fig. 2b) may be explained by the conversion of an enolate ion into the corresponding aldehyde.

Furthermore, the close coincidence in number of enol groups, as calculated from the total of relative intensities of signals at 8–9 ppm (7 and 11% are given by the spectra in Figs. 2a and 2c, respectively) and also determined by the method of Meyer [6] (9%), confirmed our assignment of these signals to CH enol protons.

The presence of several signals in the region 8–9 ppm might be due to formation of different isomeric enol forms, as well as enolate anions and cyclic derivatives (Scheme 1). The final tautomeric distribution would then be a function of pH-dependent, reversible aldo–enol transitions and hydration, rehydration processes.

^{13}C NMR spectra of alkaline solutions of ODs were in further support of this hypothesis (Fig. 4). The spectrum measured at pH 7.5 displayed signals at 113.7, 156.9,



Scheme 1.

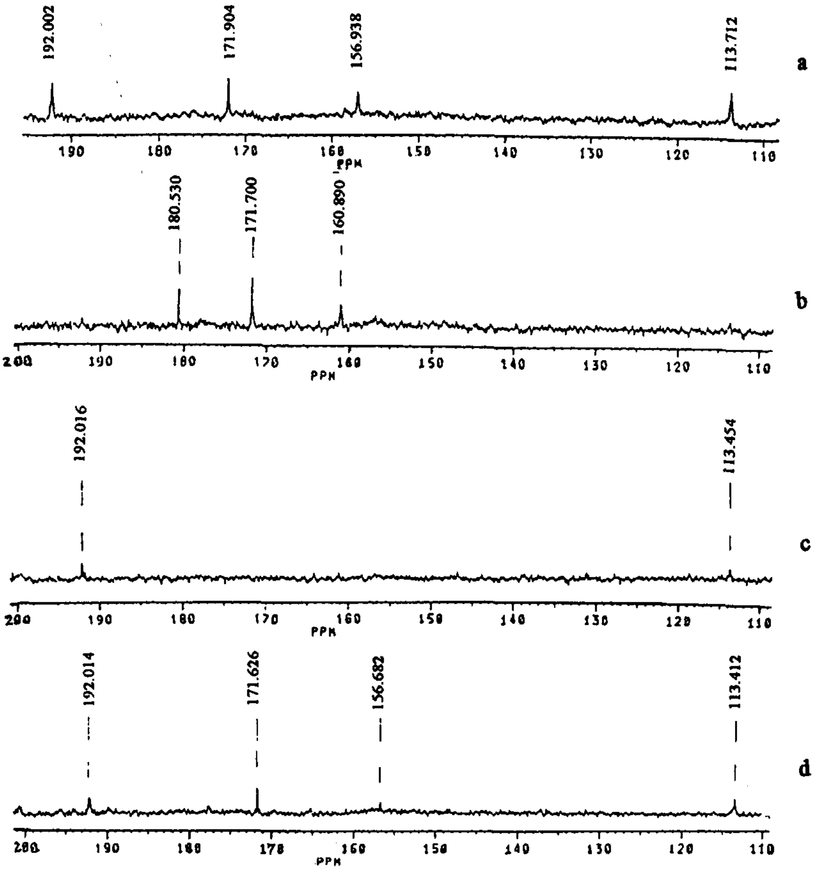


Fig. 4. ^{13}C NMR spectra of dextran products. (a) OD (mol. wt. 60,000, do 40), pD 7.5; (b) OD (mol. wt. 60,000, do 40), pD 9.3; (c) OD (mol. wt. 60,000, do 60) in D_2O, isolated at pH = 3.5, (d) OD (mol. wt. 60,000, do 60) in 0.01 N NaOD, isolated at pH 3.5.

158, 171.9 and 192 ppm (Fig. 4a). ODs derived at acidic pH and solubilized in alkali gave a similar spectrum (Fig. 4d). Increasing the pH of the solution to values higher than 9 resulted in the appearance of new signals in this region, at 171.7 and 180.5 ppm (Fig. 4b). ODs recovered at pH 3.5 and dissolved again in D_2O gave rise to only a few signals in this spectral region (Fig. 4c). We could not obtain the NMR spectrum at acidic pH since the solubility of ODs was insufficient for NMR measurements. Most likely, the signal at 192 ppm can be assigned to hydrogen-bonded aldehyde groups (IV). In this case, as pH increases, enolization, formation of an enolate anion, and eventually ring closure may occur, resulting in the disappearance signal. Consequently, the signals at 150–160 ppm should belong to the olefinic carbon atoms (structures II–VI) and signals at 171.9 and 180 ppm to the carbon atoms of the enol group and enolate ions II–V.

ELS experiments on reduced dextrans confirmed that a charge on the ODs resulted from the enolate ion but not from the carboxyls, because dextrans revealed no electrophoretic mobility (EPM) at any pH after selective reduction of their aldehyde group by sodium borohydride.

The absence of UV and IR absorption in the region typical of aldehydes and enols after sodium borohydride treatment provides additional support for our conclusion. The absorption at 267 nm and 1740 cm^{-1} in the spectra of acidic solutions of ODs must be caused by the aldehyde groups, while absorptions at 240 and 290 nm and 1620 cm^{-1} in the spectra of alkaline dextran solutions may arise from aldo–enol transitions.

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References

[1] J.W. Sloan, B.H. Alexander, R.L. Lohmar, I.A. Wolff, and C.E. Rist, *J. Am. Chem. Soc.*, 76 (1954) 4429–4434.

[2] S.N. Drobchenko, L.S. Isaeva-Ivanova, S.A. Grachev, and G.N. Bondarev, *Vysokomol. Soedin., Ser. B*, 32 (1990) 254–258; *Chem. Abstr.*, 113 (1990) 132631 t.

[3] S.N. Drobchenko, L.S. Isaeva-Ivanova, A.R. Kleiner, I.V. Kulintsova, A.V. Lomakin, V.A. Noskin, and S.D. Forofontova, *Vysokomol. Soedin., Ser. B*, 33 (1991) 220–224; *Chem. Abstr.*, 115 (1991) 29773h.

[4] S.N. Drobchenko, L.S. Isaeva-Ivanova, A.R. Kleiner, A.V. Lomakin, A.R. Kolker, and V.A. Noskin, *Carbohydr. Res.*, 241 (1993) 189–199.

[5] H.A. Rutherford, F.W. Minor, A.R. Martin, and M. Harris, *J. Res. Natl. Bur. Stand.*, 29 (1942) 131–141.

[6] K.H. Meyer, *Justus Liebigs Ann. Chem.*, 380 (1911) 212–242.

[7] G.F. Davidson and T.P. Nevell, *J. Text. Inst.*, 39 (1948) T59–T64; T65–T86.

[8] A. Hajos, *Komplexe Hydride*, Veb Deutscher Verlag der Wissenschaften, Berlin, 1966.

[9] A.R. Kleiner, N.V. Klopov, V.M. Osokin, G.N. Stabnikova, and N.N. Tylez, Prepr. LPNI-1613, Leningrad, 1990.