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Preliminary communication

Stereoselective effects of gadolinium ions on the relaxation properties of ^{13}C and ^{1}H nuclei of aldohexuronic acids and poly(glycosiduronic acids)

BENITO CASU, GIUSEPPE GATTI, NATSUKO CYR, and ARTHUR S PERLIN

Istituto di Chimica e Biochimica G Ronzoni, Milan (Italy), Italian Research Council Istituto di Chimica delle Macromolecole, Milan (Italy), and Department of Chemistry McGill University Montreal (Canada)

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^{1}H n m r measurements of induced chemical-shifts have shown[1] that europium ions (Eu^{3+}) interact with aldohexuronic acids, exhibiting particularly strong binding with sodium α-D-galacturonate and α-L-guluronate In the course of n m r studies on the interaction of heparin with calcium[2] and other metal ions, we have observed shifts induced by Eu^{3+} and Pr^{3+} for the H-1 and H-5 signals of the L-iduronic acid moieties of the biopolymer, whereas signals due to the hexosamine residues were essentially unaffected Another lanthanide salt, gadolinium nitrate has recently been found[3,4] to act as a ^{1}H relaxation agent in water These findings prompted us to examine the possibility that gadolinium (Gd^{3+}) might interact selectively with some uronic acids, and thereby modify the relaxation pathways of their ^{1}H and, possibly also, ^{13}C nuclei This communication reports that, indeed, such effects have now been observed

Gadolinium nitrate induced striking changes (see Fig 1b) in the ^{13}C spectrum of sodium α,β-D-galactopyranuronate (**1** and **2**) in deuterium oxide (see Fig 1a) Signals due to C-6 and C-1 of the α anomer suffered a marked diminution in apparent intensity, and, although some of the upfield signals were also strongly affected (see later), there was no comparable impact on C-6 and C-1 of the β anomer (The ^{13}C spectrum of α,β-D-galactopyranose was unaltered under these conditions, which clearly implicates the carboxyl group of **1** in the interaction observed) Other important factors are the uronic acid lanthanide ratio and the pH Thus, an increase in the proportion of Gd^{3+} by a factor of 3–4 over that employed for the spectra in Fig 1 (*i.e.*, sugar cation = 1 1 x 10^{-4}) caused a gross distortion in resolution due to general line-broadening By contrast, at these same levels but at pH 2, Gd^{3+} had no appreciable effect whatever on the spectrum of either anomer, and its influence on the α anomer decreased sharply above pH 7 Comparable data were obtained by ^{1}H n m r spectroscopy. for example, the H-1 (and also H-5 signal) of the α anomer was markedly broadened in the presence of Gd^{3+}, whereas that of the β anomer was not noticeably altered In addition, the effects of lanthanide concentration and pH already noted again applied

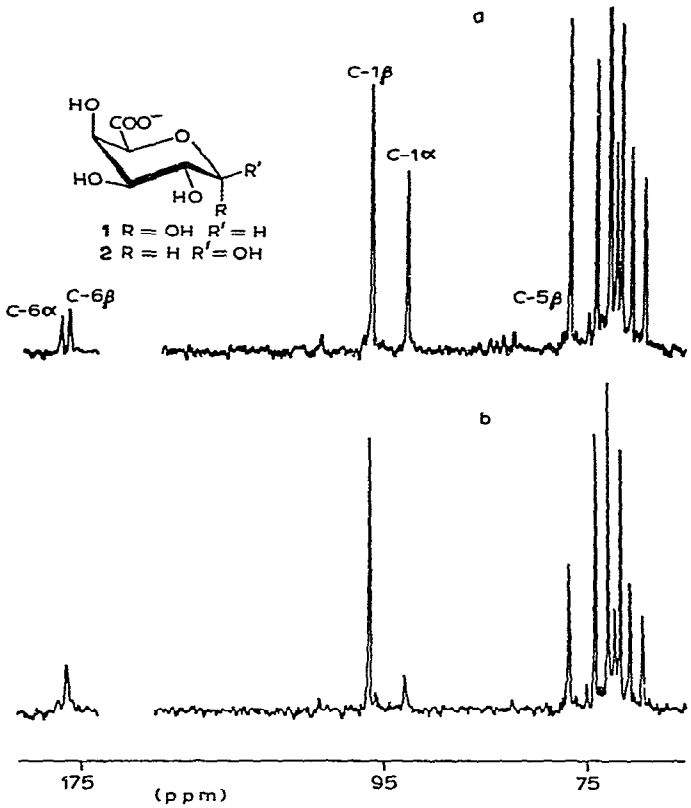

Fig 1 ^{13}C n m r spectrum (22 6 MHz) of (a) sodium α,β-D-galacturonate (mM) in deuterium oxide, and (b) the same solution containing $Gd(NO_3)_3$ 100 nM

Sodium α,β-D-glucopyranuronate and -L-idopyranuronate gave results very similar to those illustrated in Fig 1, showing that there is a general stereoselectivity on the part of Gd^{3+} for the anomer (α) having the anomeric hydroxyl group axially attached

A close analogy was also found between these observations and the relaxation behavior of ^{13}C and ^{1}H nuclei of uronic acid residues in glycosaminoglycans Addition of gadolinium nitrate to a solution of heparin caused a marked decrease in the apparent intensity of the C-6 and C-1 signals[5] of the L-iduronic acid residues Similarly, the H-1 and H-5 signals[6] of these residues experienced marked line-broadening In neither spectrum were any signals attributable to the aminodeoxyhexose residues affected Therefore, this interaction of the polymer with Gd^{3+} furnishes support for earlier evidence[6,7] favoring the α-L configuration for the L-iduronic residues of heparin.

Selective line-broadening was also observed for C-1 and C-6 of the L-iduronic residues in dermatan sulfate due to Gd^{3+}, whereas these ions had no impact on the ^{13}C spectrum of chondroitin 6-sulfate Hence, both results were in accord with expectation, in

that the D-glucuronic acid residues of the latter polymer undoubtedly have the β-D configuration[8] whereas the acid residues of the former are regarded as of the α-L type[6]

It has been supposed[1,2] that the binding site of Eu^{3+} in α-D-galacturonic acid consists of a carboxylic oxygen atom, O-4, and O-5, with the ion so positioned that the magnetic axis points towards H-1*e*, this accounts for the fact that the H-1 signal of the α anomer experiences by far the largest of the induced shifts observed Undoubtedly, O-4 is not so important for binding with Gd^{3+}, because of the strong affinity of this cation for the *gluco* (O-4*e*) and *galacto* (O-4*a*) configurations, and a more attractive model is one incorporating the axial O-1 of α anomers Nevertheless, the matter of defining spatial requirements in these systems is not straightforward This is emphasized by the remarkable fact that, although the signals for C-6 and C-1 of the β anomer (2) remained essentially unchanged in Fig 1b (relative to those in Fig 1a), the C-5 signal of this compound became much smaller; indeed, it appears that C-5 was the only ^{13}C nucleus of 2 affected (Similar behavior was exhibited also by the β-D-*gluco* isomer) Yet the carboxylate group of 2 must be implicated, because, as already noted, β-D-galactopyranose is totally unreactive under comparable conditions Presumably, however, effective bonding takes place between this group and Gd^{3+} at a distance (the broadening induced shows[3,4] an internuclear dependence of r^{-6}) large enough to cause only a minor effect on the relaxation of C-6, but in a manner such that the cation is relatively closer to C-5 With the α anomer, many of the nuclei are affected, an indication that the binding of Gd^{3+} by the carboxylate group of 1 brings the cation into close proximity with a large portion of the molecule Irrespective of the source, however, these different effects that are produced – on C-1, C-6, and H-1 signals in particular – make Gd^{3+} a sensitive, diagnostic reagent for distinguishing between anomeric forms of uronic acids

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