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Susceptibility to hydrolysis of phenylboronic pinacol esters at physiological pH

Short Communication

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Abstract: Boronic acids and their esters are highly considered compounds for the design of new drugs and drug delivery devices, particularly as boron-carriers suitable for neutron capture therapy. However, these compounds are only marginally stable in water. Hydrolysis of some phenylboronic pinacol esters is described here. The kinetics is dependent on the substituents in the aromatic ring. Also the pH strongly influences the rate of the reaction, which is considerably accelerated at physiological pH. Therefore, care must be taken when considering these boronic pinacol esters for pharmacological purposes.

Keywords: *Phenylboronic acid esters • Hydrolysis • Boron-carriers • Boron neutron capture therapy*

1. Introduction

Boronic acid compounds have received increasing attention in the field of medicinal chemistry, as potential pharmaceutical agents: development of enzyme inhibitors, controlled drug delivery polymers, saccharide sensors, and boron neutron capture therapy (BNCT) [1]. BNCT is a non-invasive approach for the destruction of cancer cells, based on the selective accumulation of ^{10}B-containing compounds into malignant cells. Subsequent irradiation with low-energy neutrons promotes ^{10}B decay to ^{4}He and ^{7}Li atoms, which can both exert a localized cytotoxicity for the tumour cell [2].

Boronophenylalanine, a boronic acid derivative of phenylalanine, is the best boron-carrier presently used in BNCT, but its selectivity and biocompatibility are not completely satisfactory [3,4]. Many other phenylboronic acids could be potentially efficacious as boron-carriers but, unfortunately, they are very reactive with carbohydrates, with which they produce a series of 5- or 6-membered cyclic esters [5]. Carbohydrates are present at high concentrations in biological systems, as free molecules or bound to proteins and lipids, especially on the cell surface. The covalent interaction with cell-surface carbohydrates may be the cause of the toxicity and inflammatory properties of several phenylboronic acid compounds [6].

The esterification of boronic acids with diols may restrict their reactivity with carbohydrates *in vivo*. Pinanediol, propanediol and pinacol are common diols used for the protection of boronic acids. However, the corresponding boronic esters are not completely stable. In fact, they may be subject to transesterification with other diols, with the possible binding to carbohydrates, and to hydrolysis. The kinetics of hydrolysis is strongly dependent both on the substituents of boron and on the type of diol: there are compounds that are more susceptible to hydrolysis than others, and some are relatively stable in water [5,7]. Concerning the stability of phenylboronic pinacol esters, early works described full stability in aqueous solutions [8,9] while, in more recent publications, evidence of pronounced susceptibility to hydrolysis of some phenylboronic pinacol esters has been presented [10,11].

In the attempt to rationalize the discrepant reports of the literature, we have studied here the kinetics of

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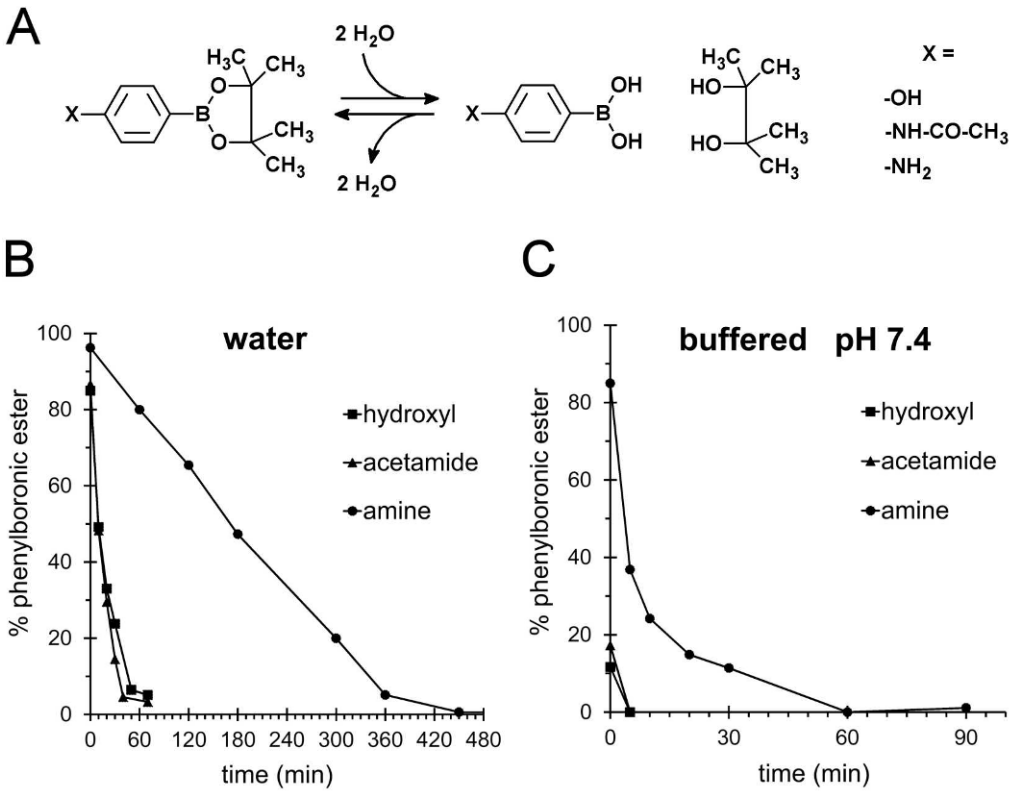

Figure 1. The picture summarizes the interconversion between phenylboronic acid plus pinacol and the corresponding pinacol ester (A). The stability of different phenylboronic pinacol esters in water (B) and in 50 mM sodium phosphate pH 7.4 buffer (C) was assayed by detection of the formed phenylboronic acid using reverse-phase HPLC (see the "Experimental" section). The diagrams show the percentage of residual phenylboronic ester as a function of time (min).

hydrolysis in water and in physiological buffered solutions of phenylboronic pinacol esters, *para*-substituted with hydroxyl, acetamide or amine groups.

2. Experimental procedure

Phenylboronic acids, the corresponding pinacol esters, and the other main reagents were from Sigma-Aldrich (Milan, Italy).

The phenylboronic pinacol ester stock solutions (100 mM) were made in anhydrous ethanol, diluted to 0.5 mM in deionised water or 50 mM sodium phosphate pH 7.4 buffer, incubated for different times at room temperature, and then analysed by reverse-phase HPLC with a Supelcosil LC-18-T (25×0.46 cm, 5 µm particle size) column (Supelco). The eluents were water/methanol, both supplemented with 0.1% (v/v) formic acid. The gradient elution program was: 20% methanol for 5 min, from 20% to 70% methanol in 5 min, 70% methanol for 5 min, from 70% to 20% methanol in 1 min, and then equilibration for 14 min (flow rate: 1.2 mL min^{-1}). For each analysis 250 µL of sample were loaded. The elution was performed at room temperature and was detected at 280 nm. The retention times were (mean±standard deviation, n=10): 6.3±0.07 min for *para*-hydroxy-phenylboronic acid, 7.8±0.2 min for *para*-acetamido-phenylboronic acid, and 5.3±0.4 min for *para*-amino-phenylboronic acid.

3. Results and discussion

The conversion of phenylboronic pinacol esters to the corresponding boronic acids in aqueous solvents, whose reaction is schematised in Fig. 1A, was monitored in time by HPLC analysis, until completion of hydrolysis. The kinetics of hydrolysis for the three compounds in water (Fig. 1B) and in a 50 mM sodium phosphate buffer at pH 7.4 (Fig. 1C) are shown.

Hydroxyl- and acetamide-substituted compounds have a similar rate of hydrolysis in water: the half-time of this reaction is about 10 min, and the hydrolysis is complete in 1 h. Conversely, the amine-substituted ester display a slower rate of hydrolysis: the half-time is of 3 h and the hydrolysis is complete within 8 h. The observed differences could be justified by the mechanism proposed for the hydrolysis of boronic

esters, which occurs as a nucleophilic attack to boron by water: electron-donor substituents on the aromatic ring decrease the electrophilic character of the boron, by accumulating a partial negative charge on the boron itself, thus disadvantaging the reaction [12]. In fact, the amine group has a higher electron-donor character than the hydroxyl or acetamide groups, as indicated by the sigma values of Hammett (amine: -0.66, hydroxyl: -0.37, and acetamide: -0.15) [13].

Similar to what observed in water, in a buffered system at pH 7.4 the kinetics appears to be influenced by the same electronic effects, but the reaction is much faster: for the amino-substituted ester the half-time of hydrolysis is 5 min, and the reaction is complete in 1 h, while for the hydroxyl- and acetamide-substituted compounds the reaction is complete within 5 min.

The kinetics of hydrolysis is also influenced by pH: at pH values above the pKa of the boronic acid the reaction is favoured. The theoretical pKa values of boronic acids analysed here are: 9.0 for acetamide, 9.5 for hydroxyl, and 10.1 for amine [14]. The pH of the solutions obtained by dissolving the various compounds in water was found to be approximately 5.4, thus explaining the lower rates observed in the unbuffered, slightly acidic solutions.

4. Conclusions

In conclusion, we have shown that the rate of hydrolysis for different phenylboronic pinacol esters is slower when strong electron-donor groups, rather than mild electron-donor groups, are linked in *para* position to the boronic moiety on the aromatic ring, and that at physiological pH the reaction is markedly faster. These factors should be taken into consideration when designing new phenylboronate derivatives for use in medicinal chemistry as boron-carriers for BNCT or for other purposes.

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