Structural requirements for bisphosphonate binding on hydroxyapatite: NMR study of bisphosphonate partial esters

Supporting Information

Elina Puljula,[*,†] Petri Turhanen,[†] Jouko Vepsäläinen,[†] Maelle Monteil,[‡] Marc Lecouvey[‡] and Janne Weisell[†]

[†] University of Eastern Finland, School of Pharmacy, Biocenter Kuopio, PL-1627, 70210 Kuopio, Finland

[‡] Université Paris 13, Sorbonne Paris Cité, CSPBAT, CNRS UMR 7244, 74, Rue Marcel Cachin F-93017 Bobigny, France

[*]elina.puljula@uef.fi

Experimental Procedures

General ^{31}P NMR spectra were recorded at 202.4 MHz on a Bruker Avance 500 DRX (Germany) spectrometer. Etidronic acid or medronic acid (12 mM) in D_2O were used as internal standards depending on the chemical shift of the studied BP. Approximate relaxation times (T_1) were evaluated for clodronic acid and alendronic acid. They were approximately 1.8 seconds. The relaxation delay d1 was set to 15 seconds, since it is recommended to be over 5 times greater than T_1 when quantitative data is desired. 90 degree pulse was determined to be 28.5 µs. The probe was manually tuned for each sample. All spectra were measured with proton-decoupling and 40 scans were measured per sample. Alendronate was synthesized by using the Kieczykowski method.[1, 2] Etidronic acid was synthesized as previously described by Turhanen & Vepsäläinen 2004.[3] Clodronic acid was received as a donation from Star Oy. All compounds were synthesized by methods reported previously; compounds **2**, **4**, **5**, **6**, **7**, **9**, **10** and **11**[4], compound **3**[5], **13** and **14**[6], **16** and **17**[7] and **18**[8]. All values are presented as the mean±SD from 2 determinations. Statistical analysis was done by using the t-test. Differences were considered statistically significant at $P < 0.05$.

BP binding to HAP BP stock solutions were prepared in Tris-buffer (50 mM, pH 7.4). The concentrations varied from 3.6 mM to 10.7 mM for the BP esters and diacetylated compound **18** and from 3.2 mM to 3.7 mM for the BP acids. 1 ml of the BP solution was incubated with HAP (60 mg for BP esters and compound **18**, 30 mg for BP acids) for 1 hour in a sample mixer in room temperature. Samples were centrifuged and a 800 µl sample of supernatant was taken.

NMR The NMR samples consisted of 300 µl of supernatant or BP stock-solution where 150 µl of $CaCl_2$ solution (10 mg/ml of 2 M HCl) and 100 µl of internal standard were added. The

BP concentration was determined according to the known concentration of internal standard. The amount of bound BP was determined by subtracting the BP concentration in the supernatant from the BP initial stock solution concentration.

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