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The Effect of Bendroflumethiazide and Hydrochlorothiazide on the Rate of Dissolution of Calcium Hydroxyapatite

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Summary. Inhibition of dissolution of calcium hydroxyapatite in the presence of bendroflumethiazide, Centyl®, and of hydrochlorothiazide has been investigated. The former compound has a pronounced larger inhibitory effect than the latter. The trifluormethyl group in bendroflumethiazide has been shown to hydrolyze, with the release of fluoride and hydrogen ions, presumably forming a carboxylic acid. The inhibitory effect of hydrolyzed bendroflumethiazide is found to be similar to the effect of a potassium fluoride solution with the same fluoride ion concentration, as measured by a fluoride selective ion electrode.

Key words: Bendroflumethiazide — Hydrochlorothiazide — Calcium hydroxyapatite — Hydrolysis — Osteoporosis.

Thiazide diuretics (TZ) are known to cause renal calcium retention. Other effects of long-term treatment of patients with TZ, for example, the effect of TZ on serum calcium concentration and on skeletal turnover, are subject to controversy [1]. Jørgensen et al. [2] reported that rats treated with bendroflumethiazide (BFT, see Fig. 1a) show increased bone incorporation of previously administered ^{45}Ca. Transbøl et al. [3] found that BFT treatment causes an initial decrease in bone mineral loss, but no long-term decrease in bone resorption, in normal postmenopausal women. Increased bone mineral content has, however, been observed by Wasnich et al. in elderly male patients treated with hydrochlorothiazide (HCT, see Fig. 1b) [4].

Although thiazide diuretics have been known since 1959 to affect calcium metabolism [5], no fundamental studies of the molecular effect of thiazides on calcification have been made. The aims of the present investigation were to demonstrate that BFT and HCT have markedly different inhibitory effects on the rate of dissolution of calcium hydroxyapatite (HAP), and to investigate the stability of the trifluormethyl group in BFT.

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Theory

Adsorption of small molecules or ions onto HAP crystals can often be described by a simple Langmuir adsorption isotherm:

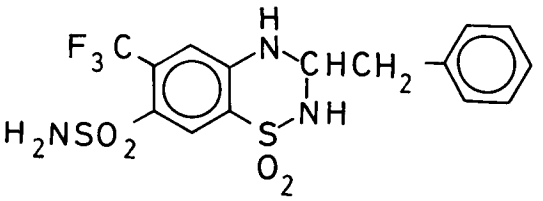
$$K_L = \frac{x}{(1 - x)C_L} \tag{1}$$

in which K_L is the adsorption constant, x the mole fraction of the adsorption sites occupied by adsorbate, the concentration of which in the aqueous solution is C_L.

If the fraction of the surface available for dissolution is equal to 1-x, the fraction of adsorption sites not occupied by adsorbate, one obtains

$$\frac{J_O}{J_L} = \frac{A}{A - A_L} = \frac{1}{1 - x} = 1 + K_L C_L \tag{2}$$

in which J_o and J_L are the rates of dissolution without and with an inhibitor, absorbate, being present, both rates being determined for the same value of all parameters influencing the rate, when no inhibitor is present in the system. A and A_L are the total surface area of the crystals and the area covered by the inhibitor. As the rate of dissolution of HAP is controlled by a surface nucleation process, [6], the effect of the presence of an inhibitor



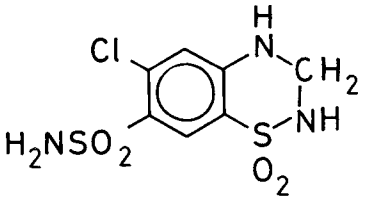


Fig. 1. Formulae for the bendroflumethiazide (BFT) **(top)** and the hydrochlorothiazide (HCT) **(bottom)** molecules.

increases as the dissolution affinity approaches zero, [7,8]. This leads to values of K_L in eq. (2) depending on the solution composition. Eq. (2) should thus be replaced by

$$\frac{J_O}{J_L} = 1 + K_{kin}C_L \qquad (3)$$

in which K_{kin} is a kinetic constant, only constant if all parameters influencing J_o are constant.

Christoffersen et al., [9] have recently shown that the inhibitory effect of fluoride ions on the rate of dissolution of HAP can be described by an equation of the type of eq. (3), but with K_{kin} increasing with decreasing values of pH. To our knowledge, no other inhibitor of the HAP dissolution process shows this effect, which is a consequence of the structural similarity of HAP and calcium fluorapatite (FAP) enabling fluoride ions to substitute for hydroxyl ions in the crystal surface of HAP, even in solutions that are unsaturated with respect to FAP. Around pH 8, the solubility of HAP and FAP are nearly identical, whereas the solubility of HAP is about three times that of FAP at pH 7, the difference in solubilities increasing with decreasing pH. This is mainly due to the fact that aqueous solutions at low pH can have a high concentration of fluoride ions, but cannot have a high concentration of hydroxyl ions.

The value of K_{kin} in eq. (3) depends on the solution composition and on the HAP crystal preparation. A plot of J_o/J_L for one inhibitor against J_o/J_L for another inhibitor can be used to compare the effectiveness of the inhibitors, as long as the four rates giving rise to one point are measured for the same values of all parameters affecting the rate, except that J_o is measured without inhibitor, the two values of J_L being measured with the same concentration of inhibitor. If the inhibitors are equally effective, a line with slope 1 will be obtained, otherwise the experimental points lie closer to the axis representing the strongest inhibitor.

Materials and Methods

The BFT and HCT used were kindly supplied by Leo, Copenhagen. Stock solutions of these substances were made by weight, assuming the molecular weights to be 421 and 298, respectively. HAP crystals, of the type used by Christoffersen et al. [9], were added to an aqueous solution containing, or not containing, the inhibitor. For dissolution rate experiments, the pH of the solution was kept constant by means of a pH-stat (Radiometer equipment), which controlled the addition of dilute nitric acid to the system. The rate of dissolution was determined from the rate of acid consumption [10]. Care was taken to exclude carbon dioxide from the system. The mass of crystals used was determined by acidifying the final reaction mixture, causing complete dissolution of the HAP crystals, and determining the calcium concentration by atomic absorption spectrometry and the phosphate concentration by spectrophotometric measurement of the molybdenum blue complex. Fluoride activities were measured by means of a Radiometer F1052F Selectrode®. The glass and reference electrodes were Radiometer G202C and K102, respectively. All experiments were made at 25.0 ± 0.1°C.

Results

For the dissolution of HAP, the amount of acid consumed per unit initial mass of crystals is, in Figure 2, plotted against time, for an experiment with pH = 7.15 and in which no inhibitor is present, and for experiments with the same value of pH but with either BFT or HCT present with concentration 0.013 mM. From this plot it is seen that the effect of BFT increases with the age of the solution up to about 2–3 weeks, after which little further increase is found. HCT is without effect at 2–3 weeks; a small effect is observed with an aged solution (8 weeks). A solution of potassium fluoride of concentration 0.040 mM has an effect on the rate of dissolution of HAP similar to that of aged BFT. The fluoride ion and the hydrogen ion activities in the originally 0.013 mM BFT solution were, after 2–3 weeks, found to be 0.040 mM. This indicates that the CF_3 group in BFT hydroylzes, probably to form a carboxylic acid group, with the release of hydrogen and fluoride ions to the solution. Diluting the BFT solutions causes the response of the

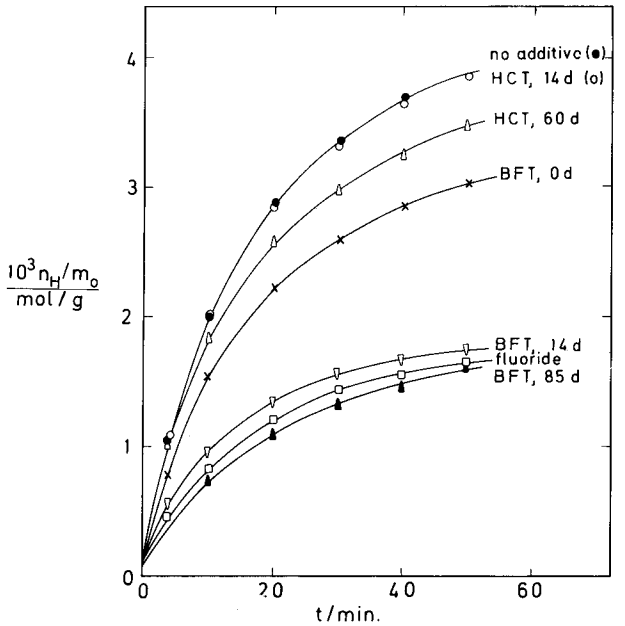


Fig. 2. The amount of acid consumed per unit initial mass of HAP for the dissolution of HAP at pH = 7.15, plotted as a function of time. $m_o/V \approx 9$ mg/liter. [BFT] = [HCT] = 0.33[F^-] = 0.013 mM. ● *no additive*, ○ *HCT*, 14 d; △ *HCT*, 60 d; × *BFT,0 d;* ▽ *BFT*, 14 d; □ *fluoride;* ▲ *BFT*, 85:d.

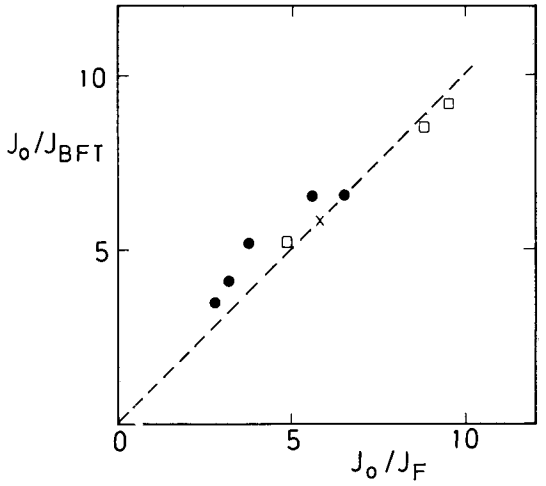


Fig. 3. J_o/J_{BFT} plotted against J_o/J_F, each point having the same response of the fluoride electrode in the two solutions containing *BFT* or fluoride ions. *BFT* solutions aged 5–8 weeks. ● pH = 7.15; □ pH = 6.75; × pH = 6.3.

fluoride electrode to follow the expression expected for dilution of a fluoride ion-containing solution. The pH of the HCT solution did not decrease with time, and the fluoride electrode did not respond to this solution.

In Figure 3, the inhibitory effect of BFT on HAP dissolution is compared with that of fluoride ions, J_o/J_L for fluoride ions, J_o/J_F, being plotted against J_o/J_L for BFT, J_o/J_{BFT}. For each point in this plot, the response of the fluoride electrode was the same in the inhibitor-containing solutions. The data in this plot have been obtained using two different crystal preparations, and values of pH between 6.3 and 7.2 have been studied. The BFT solutions were aged from 5–8 weeks. From this plot it is seen that the effect of a BFT solution with a certain response of the fluoride electrode is similar to the effect of a pure fluoride-containing solution with the same electrode response. The characteristic effect that the inhibition of fluoride increases with decreasing pH, [9], is also seen with BFT.

Discussion and Conclusion

The results indicate that the CF_3 group in BFT is not stable, but hydrolyzes to a carboxylic acid group and fluoride ions. With a daily dose of 5 mg BFT, the concentration of fluoride ions in the body fluid can be in the interval 10^{-6} M – 10^{-5} M, prior to any reaction except the hydrolysis of BFT. Such a concentration of fluoride ions effectively influences the rate of dissolution of HAP, and the rate of growth of fluoride-containing apatite, *in vitro*. The above concentration is also of the same order of magnitude of the serum fluoride level obtained, 4–7 μmol/l, in the treatment of osteoporosis with sodium fluoride [11].

Clear conclusions concerning the biological effects of the hydrolysis of the CF_3 group of BFT *in vivo* are more difficult to draw. The results of Jørgensen et al. [2] indicate that BFT in adult rats causes an increase in bone mineral content and a decrease in bone matrix resorption, but very high doses, of the order 700 mg per 70 kg body weight, were used. The results of Transbøl et al. [3] can be interpreted to show that BFT given with a dose of 5 mg per day has a positive influence on the bone mineral content of normal postmenopausal women as long as the dosage is kept up. Furthermore, some of the apparently conflicting data describing the *in vivo* effects of "thiazides" may be explained when this group of drugs no longer is treated as just "thiazides," but as different molecules with different chemical properties. HCT has here been found not to inhibit HAP dissolution *in vitro*.

The observation that the CF_3 group in BFT is not stable, as expected, but hydrolyzes, may have various applications. BFT and other molecules containing hydrolyzable fluoride groups may be potential candidates for drugs for treatment of osteoporosis, because such drugs may maintain a steady but low concentration of fluoride ions in body fluid. Even micromolar concentrations of fluoride ions in

body fluid may retard resorption of the inorganic part of bone and also enhance remineralization. In prevention of dental caries, for successful remineralization of subsurface lesions in tooth enamel, and for remineralization of surface softened enamel, fluoride ions play an important role. A steady concentration of fluoride ions in the plaque and in the liquid in partly dissolved enamel is most attractive, because these ions retard demineralization and enhance remineralization. Problems caused by precipitation of other phases such as CaF_2 can be avoided if the fluoride concentration at all times is kept so low that the solubility product of CaF_2 is not exceeded. For short-term treatment of enamel with fluoride-containing solutions with concentrations so large that the solubility product of CaF_2 is exceeded, CaF_2 need not precipitate, because precipitation of CaF_2 in pure systems shows relatively long induction times.

In the search for new drugs to be used in connection with pathological bone resorption, in prevention of dental caries, and in connection with remineralization of partly dissolved tooth enamel, it may be worthwhile investigating which chemical factors, neighboring groups, etc. in the BFT molecule are controlling the rate by which the CF_3 group hydrolyzes. Hopefully, the pharmaceutical industry may find new compounds for which the rate constant for hydrolysis of carbon-fluorine bonds can be optimized for application in connection to osteoporosis and dental caries. More speculative, such compounds could also be of importance for improving the rate of repair of bone fractures, particularly in cases where this repair process is pathologically long.

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