Role of Acid Phosphate in Hydroxyapatite Lattice Expansion*

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Summary. Questions remain about which subcomponents of human tooth enamel (TE) are responsible for its crystallographic a axis being nearly 0.02Å longer than that of pure hydroxyapatite (OHAp) and contracting to that of OHAp on heating. From infrared spectroscopic and X-ray diffraction studies of a synthetic OHAp containing HPO_4 and "structural" H_2O, it has been concluded that HPO_4 expands the a axis at the rate of ~ 0.0015Å/ wt % but that this accounts for substantially less than one-half of the total observable contraction. The remaining, more than one-half of the a axis change, may be only partially ascribable to "structural" H_2O and partially to P_2O_7 (formed from the HPO_4), coming out of solid solution in the apatite. Some 90% of the HPO_4 observed with infrared is lost in the 160–240° temperature range and more than one-half of the P_2O_7 observed as a separate phase is developed above that temperature and continues to increase all the way up to the 500°C, the limit of the experiments. The loss of HPO_4 is accompanied by reduction of disorder or variety in the structural OH ion sites, consistent with the view that initially some of the PO_4 groups neighboring the OH ions were actually HPO_4 groups.

Key Words: Human tooth enamel — a axis — HPO_4 — Structural H_2O.

The a lattice parameter of the unheated dense (sp g > 2.95) portion of human tooth enamel (TE) is ~0.02Å larger than that of stoichiometric hydroxyapatite (OHAp). When the specimen is heated beyond 300–400°C, the a parameter decreases irre-

versibly to essentially that of pure OHAp (9.419Å). The accompanying internal changes in atomic positions and amounts present probably have important effects on diffusion of ions [1] along the X-ion channels parallel to the c axis at $x = y = 0$, where X = OH, F, Cl, among others [2]. "Structural H_2O" has been most strongly suggested as the cause of the a-axis expansion (i.e., of TE compared to OHAp) [3, 4]. However, the mechanism of the a-axis expansion (and subsequent reduction during heating) cannot be credibly known until the effects on lattice parameters are known both separately and collectively for all species that undergo significant concentration changes in the temperature range being considered. The principal ones are CO_3 in A sites, CO_3 in B sites, "structural" H_2O, and HPO_4.

The separate effects of the two types of CO_3, present at 2–4 wt%, on the lattice parameters of synthetic apatites are well known (5–7) and the temperature dependences of their concentrations and of structural water, initially present at 3–5 wt% in TE, have been reported [4]. A substantial part of the a-axis expansion has been attributed to H_2O [3, 4] but no complete quantitative measure of its effect on lattice parameters has been reported. In fact, the very concept of "structural H_2O" remains somewhat blurred because its location(s) in the apatite crystal structure has not been determined. HPO_4 has been reported present in TE at 5 wt% [8], but no published report of its effect on lattice parameters is known to us.

For a first determination of the effect of HPO_4 on lattice parameters, a hydroxyapatite ("reflux OHAp") prepared by hydrolysis of $CaHPO_4$ is preferrable to TE. Of the four possible major contributers to a-axis changes in the region below 400°C, reflux OHAp contains only HPO_4 and structural H_2O. This fact both simplifies data analysis and permits the changing HPO_4 content to be followed with the principal HPO_4 infrared band at 875 cm^{-1} which in TE is lost in a CO_3 band.
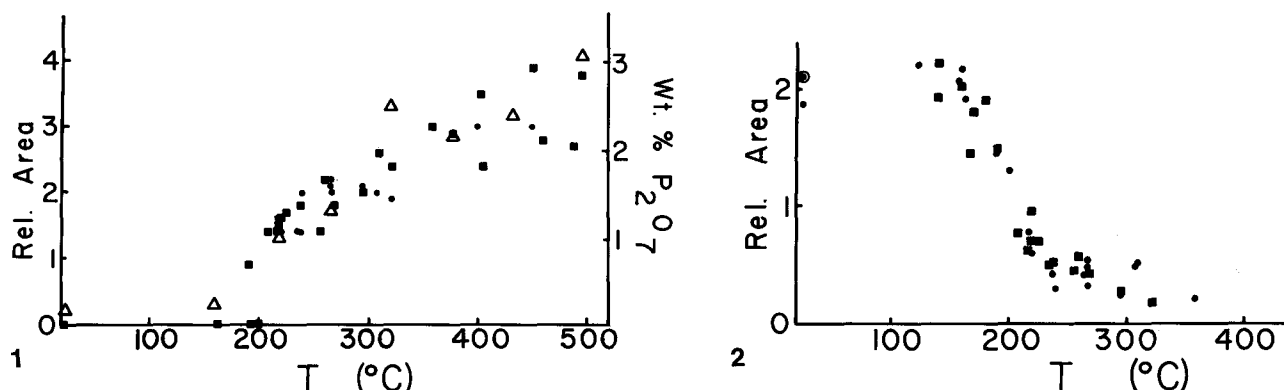

Fig. 1. Pyrophosphate (P_2O_7) content of CaOHAp after being heated for 24 h at the indicated temperature. Results based on chemical analysis are indicated as △. Results indicated as ■(for independently heated) and ●(for sequentially heated) were determined from the 730cm^{-1} i.r. band **Fig. 2.** Acid phosphate (HPO_4) content of CaOHAp after being heated for 24 h at the indicated temperature. Results indicated as ■(for independently heated) and ●(for sequentially heated) samples were determined from the i.r. band 875cm^{-1}

Materials and Methods

Specimens

A batch of reflux OHAp of a size sufficient for the entire experiment was prepared by hydrolysis of $CaHPO_4$. In practice, 30 grams of reagent grade $CaHPO_4$ was put into a 3 liter boiling flask and filled with distilled/deionized water. A condenser was attached and the slurry boiled. The water was changed twice weekly for 1 month. The sample was collected and washed on a Buchner funnel, dried at 60°C, and designated Y-167. Specimens were heated for 24 h in air at one or another of various temperatures and were then examined at room temperature. The "sequential specimens" (series O, W and Y) were prepared by succesive heating of the same specimen. The "independent specimens" (series Q, X) were each heated at only one temperature.

Infrared Spectroscopic Analyses

Pellets for infrared analysis were prepared with 2 mg of specimen per 300 mg KBr. Spectra were recorded with a Perkin Elmer 580B instrument. The scale expansion feature was used to enlarge the bands of interest for better measurement of their areas (by planimeter) and breadths (full width at half maximum, FWHM). The area of the HPO_4 band at 875 cm^{-1} was taken to indicate the amount of HPO_4 present. The P_2O_7 band at 730 cm^{-1} was calibrated in terms of wt% by comparison with P_2O_7 determined in several samples by the Gee and Dietz [9, 10] method (Fig. 1).

The amount of "structural water" present was taken to be linearly related to the height, above background, of the very broad band centered near 3300 cm^{-1}, as had been done in previous work [4].

X-ray Diffraction Analysis

X-ray powder diffraction data were collected with crystal-monochromatized CuKα radiation on a standard θ-2θ diffractometer operated in a step scan mode with 0.05°(2θ) and 60 sec per step

over the range 17–84°(2θ). Each pattern thus required about 24 h of data collection time. Computer program DBW 3.2 [11] for Rietveld whole-pattern-fitting structure refinement was then used with these digital data to determine the lattice parameters (determination is incidental to the main purpose of the program), among other things. The a and c lattice parameters were thus determined with a program-calculated precision (e.s.d) of < 1 part in 10,000. However, there was an apparent sensitivity of the lattice parameter refined values to the simultaneously refined 2θ-zero correction parameter, the sensitivity being not fully reflected in these e.s.ds. The ratio a/c is much less subject to this problem and, therefore, was a more informative parameter to follow in this work.

Results and Discussion

Nearly all of the HPO_4 evidenced by the i.r. band at 875 cm^{-1} was lost between 160 and 240°C (Fig. 2). Although the reported reaction [12, 13] is

$$2HPO_4 \rightarrow\rightarrow P_2O_7 + H_2O, \qquad (1)$$
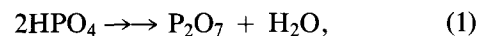
the P_2O_7 i.r. band at 730 cm^{-1} (1) does not appear as soon (in temperature) as the HPO_4 is lost and (2) it continues to grow more or less linearly with increasing temperature all the way to 400°C (Fig. 1). The P_2O_7 content determined with the method of Gee and Dietz correlates very well with the area of this infrared band (730 cm^{-1}). Thus, there is a definite "gestation" period between loss of HPO_4, as indicated by the 875 cm^{-1} infrared band, and the appearance of P_2O_7. This has been commented on by others (Bonel G, private communication). It is not clear why this occurs. Perhaps the loss of the 875 cm^{-1} band only means at first that there has been some discontinuous change in the attachment of the H to the PO_4 without, as yet, much relocation of either. Perhaps some intermediate phase is being
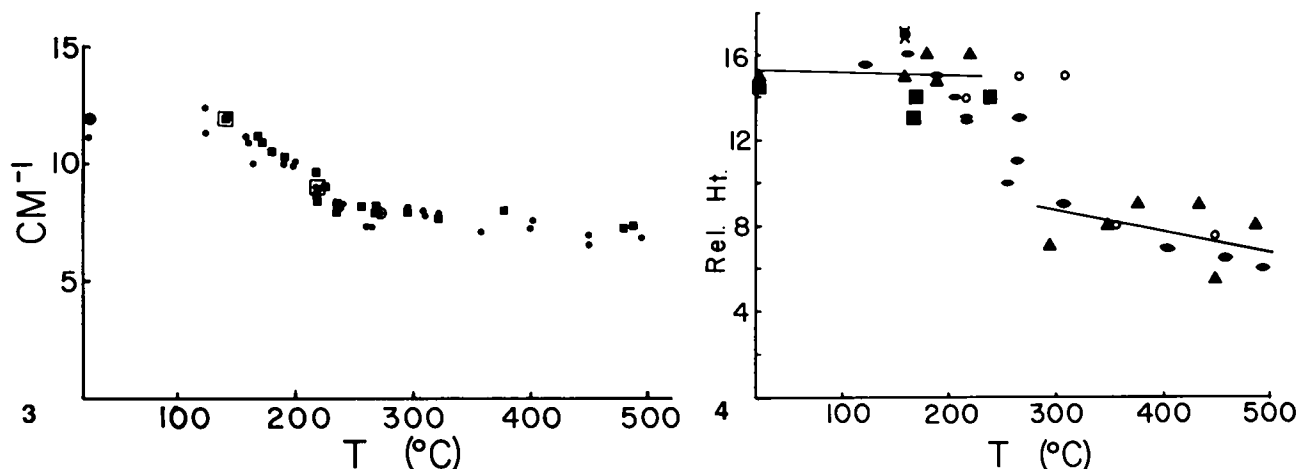
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Fig. 3. Full width at half maximum (FWHM) of the structural OH i.r. band at 3572 cm⁻¹ **Fig. 4.** Water remaining in OHAp specimen after heating to indicated temperature. Measurements were based on height-above-background of the I.R. spectrum at 3300cm⁻¹. The number of data points contributing to each plot point are O = 1, ● = 2, ▲ = 3, ■ = 4, ◢ = 5. Solid lines calculated by regression analysis of the data

formed (a "reaction intermediate"). Perhaps the initially separately formed P_2O_7 molecules simply require time and higher temperature to diffuse through the apatite structure, find, and aggregate with each other to form crystals of P_2O_7 for which the 730 cm⁻¹ band is specific. Winand and Duyckaerts [14] do suggest that the P_2O_7 is in solid solution with the apatite.

In the same temperature range, 160–240°C, in which nearly all of the initially present HPO_4 is lost, the FWHM of the 3572 cm⁻¹ band (OH stretch) decreases from 12 to 7 cm⁻¹ and remains at essentially that value with further heating (Fig. 3). This indicates that increasing ordering of the structural OH ions in the apatite structure takes place simultaneously with the HPO_4 loss (perhaps because the H of HPO_4 affects the precise positions of the phosphate O [3] atoms [15] adjacent to the H of the OH in the X-ion channels [16, 17])

Reproducibility in the structural H_2O determinations (Fig. 4) is not as good as it is for the determinations of the other quantities. However, there is little or no evidence that structural H_2O loss starts at temperatures as low as does the HPO_4 loss. Instead, it appears (Fig. 4) that the structural H_2O loss starts near 240°C, continues rather sharply to 300°C, and then continues more gradually, essentially monotonically, up to the highest temperatures investigated. In Fig. 4, one could draw in a reasonable appearing curve similar to the one reported for H_2O loss in TE [4], showing loss of about one-third of the total structural H_2O between 250 and 300°C. However, the quantitity and precision of the data preclude a firm conclusion.

It is well known that the a lattice parameter is

affected much more strongly than is the c by CO_3 and many other substitutions. It is also the a parameter that is most changed by heating of the specimens. The lattice parameters calculated but not yet corrected for the sensitivity to the 2θ-zero parameter were a = 9.4389(8) and c = 6.8871(6)Å for the unheated material and, for example, a = 9.4319(7) and c = 6.8885(6)Å for sample Y167-Y12 heated at 405°C. This is a +0.023% change in c accompanying a -0.074% change in a. Therefore, three-quarters of the changes in the ratio a/c are due to changes in a. The actual reproducibility of a and c from run to run was poorer than the calculated e.s.d because of 2θ-zero correction problems, which affected both similarly. Thus, the ratio a/c is a better parameter to follow because it is relatively free of those problems. Fig. 5 verifies that the actual experimental precision in a/c is indeed < 1 part in 10,000 and that the reproducibility is sufficient to permit trends to be seen clearly.

The a/c ratio decreases sharply and nearly linearly between 160 and 240°C, in correlation with the HPO_4 loss and OH ordering increase. In the range 300–>500°C the a/c decrease is also nearly linear with temperature, but with a smaller slope. The data in Fig. 5 can be modeled rather well by two straight line regions and one transitional region in the 240–300°C range. One straight line region extends from 160–240°C. The second straight line region extends from the highest temperature to below 300°C and probably extends under the first all the way to the temperature at which P_2O_7 is first noted, ~180°.

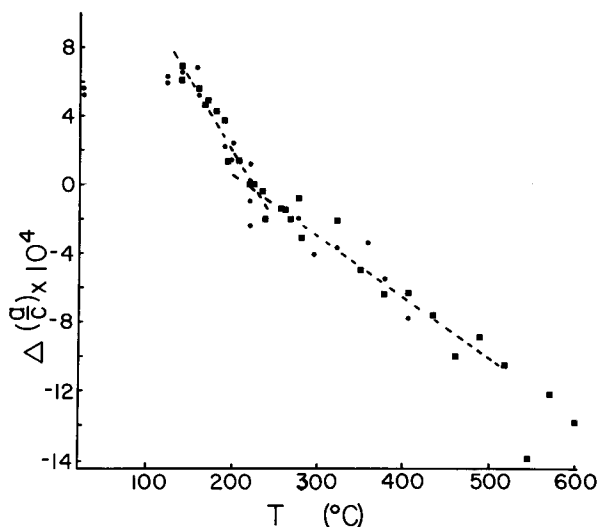We attribute the sharp changes in the 160–240° region to HPO_4 loss. The a/c ratio is thereby de-

Fig. 5. Change in the lattice parameter ratio (a/c) as a function of heating for 24 h at the indicated temperature. The base point, indicated as 0, is an a/c ratio of 1.37000. Results are for samples heated independently (■) and for a sample heated to sequentially higher temperature (●). All measurements were made at room temperature. Dashed lines approximate fit of the data to emphasize change of slope

creased from 1.3706–1.3700 by loss of most (>90%) of the HPO_4, which corresponds to an a-axis contraction of 0.0043Å resulting from a loss of 3–3¹/₂ wt% HPO_4 (deduced from the P_2O_7 determinations) or 0.0015Å/wt% HPO_4. Thus the effect of HPO_4 on the a lattice parameter is very real but is considerably smaller than is the effect of CO_3, which is +0.026Å/wt% of A type CO_3 [6] and −0.006Å/wt% of B type CO_3 [5]. It may be also concluded, therefore, that at most, only a small part of the 0.014Å a-axis contraction reported in TE [4] in the range 250–300°C could be attributed to HPO_4 loss.

The total amount of structural H_2O initially present is one-half that in TE [4]. If (1) the apparent marked decrease in structural H_2O in Fig. 4 is real and (2) the loss of H_2O is primarily responsible for the TE lattice contraction between 250 and 300°C, by analogy to the TE results [4] one might expect an a-axis decrease of 0.007Å due to H_2O loss in the range 250–300°C. This would decrease a/c by 0.0013. Figure 5 shows no evidence of any change of such magnitude in that temperature range. Thus, if structural H_2O loss is primarily responsible for the a-axis contraction TE, as was suggested [4], it would seem that the actual structural locations, or binding, or state of aggregation of the structural H_2O, or all of these, must differ between this apatite and TE

Above 300°C, the continual a/c decrease and H_2O loss correlate well with each other and with the increase of P_2O_7. Since no large change in a/c accompanied the (relatively) large H_2O loss between 250

and 300°C, one is led to the possibility that the a/c changes, at least in the region above 300°C, may be due more to P_2O_7 coming out of solid solution in the apatite [4] forming its separate phase, than to "structural H_2O" loss.

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