

Apatite and Octa-Calcium Phosphate: Effects of Carbon Dioxide and Halogens on Formation

Abstract. *Octa-calcium phosphate forms instead of apatite under a high partial pressure of CO₂ in solutions, almost neutral in pH, containing chloride ions or wholly lacking halogens. However, in solutions containing fluoride ions, apatite forms under the same conditions. Fluoride also increases the crystallinity of the formed apatite.*

Carbon dioxide, presumably in the form of carbonate ions, has been shown to occur commonly in the apatite structure (1, 2). Because of such solid solution, variations in the total system in the partial pressure of CO₂ in the gas phase, or some species of carbonate ions in the solution, will affect the stability of apatite and other calcium phosphates. I now present data on such effects.

A method of forming apatite by reaction of calcite with a sodium phosphate solution has been described (1, 3); its advantages are that: (i) the re-

action is slow, a feature conducive to formation of an equilibrium product; (ii) the crystals tend to be coarser in grain than those formed by a precipitation process; and (iii) the solution can be maintained at nearly constant concentration and having known chemical properties.

For the first series of experiments 4 g of finely divided calcite, contained in each of two flasks, reacted at 25°C in 600 ml of 0.2M phosphate solution made basic with sodium hydroxide; initial pH of the solutions was about 10.5. Then the solutions were flushed

with CO₂ before the flasks were sealed. In order to keep the solutions at nearly constant composition, they were frequently replaced and refushed with CO₂.

This procedure, along with intermittent agitation, was continued for 3 months. The pH of the initial solutions differed, and, after being flushed and standing with the reacted solid for several weeks, one solution was of pH ~ 8.1, the other, pH ~ 7.4. The solid formed in each experiment was octa-calcium phosphate (OCP), probably mixed with poorly crystallized apatite. The flasks were resealed, and after 13 months the pH's of the solutions were 9.06 and 8.96 ± 0.02, respectively; the solid products remained OCP. Heating, from 25° to 900°C, resulted in 20- to 27-percent loss of weight for the solid; analysis showed that the unheated material contained less than 1 percent CO₂.

In similar experiments, but with unflushed solutions and in systems vented to air, apatite was the only solid product in solutions of pH's 7.5 and 9.1.

Octa-calcium phosphate, which was formed under a high partial pressure of CO₂ at pH ~ 7.4, was put under a sodium phosphate solution of pH ~ 10.4; OCP was present after 40 days, probably with poorly crystallized apatite, but within 1 year the material entirely recrystallized to apatite. At 40°C, OCP in the solution in which it formed slowly decomposed to apatite.

The results of these experiments indicate that formation of OCP is enhanced by a high partial pressure of CO₂ at the ionic strength and sodium concentration used. It is possible that the concentration of sodium ions in the solution also affects formation of OCP. There is no proof that the OCP is in equilibrium with the solution, or that OCP has a stability field. However, the crystals did persist for more than 1 year in contact with a solution flushed with CO₂.

In order to determine quantitatively the effect of the partial pressure of CO₂ on formation of apatite and OCP, I made a series of experiments under controlled atmospheres. Phosphate compounds were formed at 25°C by reaction of calcite and 0.2M sodium phosphate solutions of various pH values. During the entire time that the crystals were forming and in contact with the solution, the system was aerated with these controlled mixtures

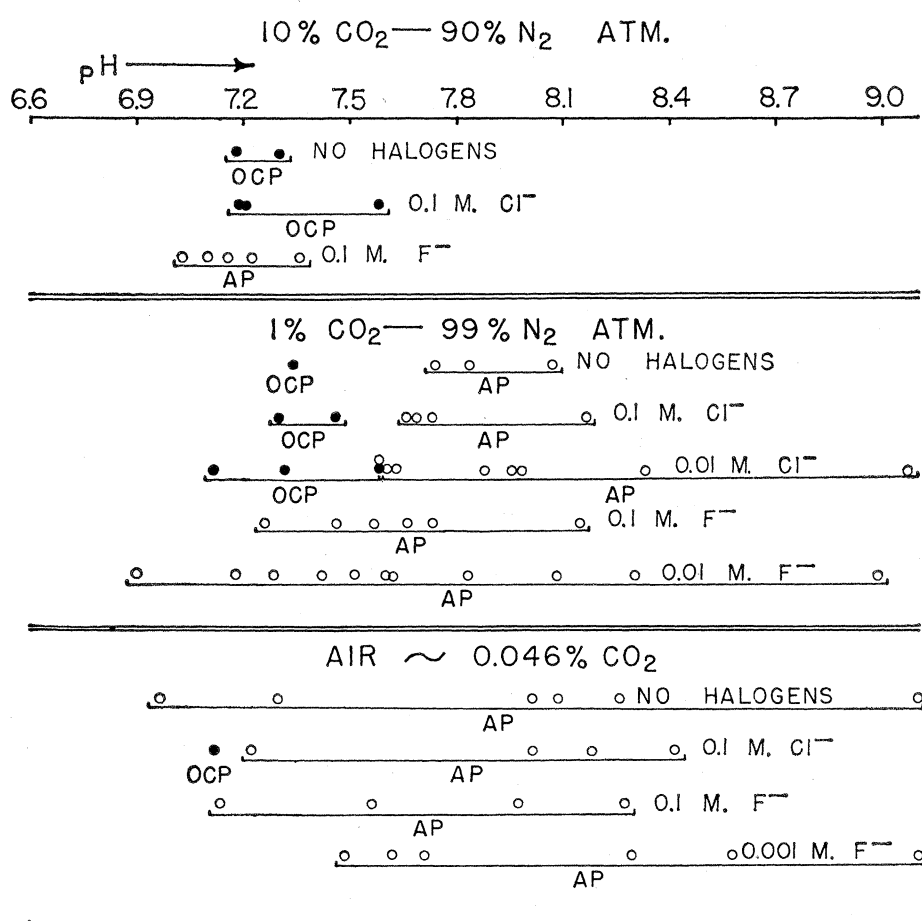


Fig. 1. Effects of halogens and carbon dioxide on the formation of apatite and octa-calcium phosphate. Compositions of aerating gases are in percentages by weight. Each pH value is the final pH of the solution in which the phosphate compound formed. Circles and solid circles indicate samples; Ap, apatite; OCP, octa-calcium phosphate, possibly with apatite.

(by weight) of gases: (i) 10 percent CO₂, 90 percent N₂; (ii) 1 percent CO₂, 99 percent N₂; and (iii) air. Initial pH of the solutions ranged from 7.2 to 10.5; however, because of buffering by the partial pressure of CO₂ and the solid phase, the final pH was more restricted in range.

Effects of halogens on the formation of apatite and OCP were determined by the addition of 0.1M, 0.01M, or 0.001M fluoride or chloride to the solution; the results are summarized in Fig. 1. Under a gaseous mixture containing 10 percent CO₂, only OCP formed in solutions containing the chloride ion or lacking any halogens. In the fluoride solution, however, apatite formed in the same pH range; under these conditions, the presence of fluoride enlarged the field of formation of apatite.

In a system aerated with 1 percent CO₂ the effect was similar. In a solution at pH ~ 7.34, OCP formed in the absence of halogens, while apatite formed at pH ≥ 7.74. With solutions bearing 0.01M chloride, the boundary between the fields of formation of apatite and OCP is at pH 7.58 ± 0.03.

The data do not establish whether or not chloride ions affect the field of formation of apatite. Solutions bearing 0.1M and 0.01M fluoride ions (190 ppm F) have enlarged the field of formation of apatite by more than 0.6 pH units. Furthermore, in the 1-percent CO₂ atmosphere and under acid conditions, apatite can form only from a fluoride solution.

Under an atmosphere of air, apatite formed in systems with or without halogens over a wide pH range. Only in a sample formed in a 0.1M chloride-bearing system at pH ~ 7.1 was OCP found; this fact indicates that the presence of chloride is detrimental to formation of apatite at near-neutral pH. Under atmospheres enriched in CO₂ relative to air, the presence of fluoride ions markedly enlarged the field of formation of apatite.

X-ray diffractograms of apatite, formed at low temperatures, characteristically give poor resolution. This phenomenon may result from the crystal size or from some type of disorder in the structure. When all samples included in Fig. 1 were x-rayed, patterns from apatite formed in fluoride-bearing systems consistently showed markedly better resolution than did patterns from apatite formed in the presence

of chloride or in the absence of halogens.

In summary, the effect of high partial pressures of CO₂ is to enhance the formation of OCP at the expense of apatite; whereas, under the same conditions, addition of fluoride ions enlarges the field of formation of apatite, an effect that may be a major cause of the beneficial effect of fluoridation on teeth.

The results of this study do not demonstrate that OCP has a true stability field. However, the question of stability has no bearing on conclusions concerning the effect of halogens or of

the partial pressure of CO₂ on the formation of apatite and OCP, because, once OCP forms in an oral environment, it has its characteristic physical and chemical properties relative to attack.

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References and Notes

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Lactate Dehydrogenase Activity:

Effect in vitro of Some Pesticidal Chemicals

Abstract. *The effect of chlorinated hydrocarbon insecticides upon rabbit-muscle lactate dehydrogenase was determined by both the direct determination of diphosphopyridine nucleotide reduction and a tetrazolium salt reduction method. A comparison of the two assay procedures revealed that the pronounced stimulation of lactate dehydrogenase LDH activity was noted with the tetrazolium salt method but could not be detected with a direct photometric determination method. Incubation of lactate dehydrogenase with DDT or heptachlor prior to the addition of either diphosphopyridine nucleotide or substrate resulted in a complete inhibition of enzyme activity. The inclusion of either diphosphopyridine nucleotide or substrate in the prior incubation mixtures afforded no protection to the enzyme.*

Experiments on the mode of action of pesticides suggest that these agents function, in most cases, as inhibitors of specific enzyme systems (1). The group of compounds commonly referred to as "chlorinated hydrocarbons" has, however, been very difficult to associate with specific enzyme inhibition either in vivo or in vitro. Studies by Judah (2), who used DDT as a potential inhibitor, failed to demonstrate inhibition of aldolase, adenosine triphosphatase, glutamic dehydrogenase, choline oxidase, hexokinase, or succinoxidase. Sactor (3) presented evidence that DDT could partially inhibit cytochrome oxidase. This evidence was supported by Johnston (4), who found that both DDT and DDE inhibited rat-heart succinoxidase and cytochrome oxidase. Succinic dehydrogenase activity was not affected by the presence of either DDT or DDE. Nachlas *et al.* (5) described a colorimetric method for the measurement of lactate dehydrogenase (LDH) activity. They used phenazine methosulfate as an intermediate carrier for electron transfer from reduced diphospho-

pyridine nucleotide (DPNH) to a tetrazolium salt.

Evidence presented by O'Brien and Matsumura (6) indicates that DDT forms "charge-transfer complexes" with a component of the nerve axon. These authors suggest that this complex formation is the primary mode of action of DDT and perhaps other chlorinated hydrocarbons.

Table 1. Comparison of DPN and tetrazolium methods for LDH activity. Pesticide concentration is 1×10^{-4} M in all cases, except where otherwise indicated. (—), Inhibition of LDH activity. One hundred percent activity was determined by running the reactions with pesticide omitted.

Pesticide	Stimulation of enzyme activity (%)	
	DPN reduction	Tetrazolium
Aldrin	(—) 13	29
Dieldrin (1×10^{-3} M)	(—) 19	37
DDT	(—) 19	51
Endrin	(—) 16	59
Heptachlor	(—) 16	42
Ceresan M		(—) 20
Thiram		84