Enrichment of CO_2 and magnesium in early stages of biomineralization

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Summary. Simultaneous topochemical analyses of calcium, magnesium, carbonate (as CO_2) and phosphorus in a mineralizing collagen rich system (turkey tibia tendon) showed a strong enrichment of Mg and CO_2 in the prestages of biomineralization.

The content of CO_2 in hard tissues (bone, tooth, mineralized tendon) is 2-4%w/w (CO_2 of tendon calculated by data of Likins[2]). These values represent the CO_2 content when the biomineralization process is completed. In vitro experiments on early stages of apatite formation have shown that Mg^{2+} (Boskey and Posner[3]) and HCO_3^-(Blumenthal et al.[4]) - especially a mixture of both - reduce the degree of crystallinity and/or inhibit crystal growth[5].

We have demonstrated turkey tibia tendon to be an excellent model to study controlled collagen mineralization[6]. During this process alkaline phosphatase activity is observed. This enzyme was highly purified and cationic activation experiments have revealed that Mg^{2+} is an important cofactor resulting in a strong activation of this enzyme[7].

However, it is still uncertain whether carbonate and magnesium concentration is a controlling factor in the early stages of the biomineralization process. Therefore we carried out topological analyses to determine carbonate and Mg as well as the apatitic compounds phosphate and calcium.

Materials and methods. Tibia tendons of turkeys (12-17 weeks old; 8 animals) were prepared as described elsewhere[6]. The tendons were cut transversely in 1-2 mm sections starting at the proximal muscle connection and proceeding down into the fully mineralized region. The samples were treated with 1 N HCl and, by microdiffusion, the developing CO_2 gas was absorbed in $Ba(OH)_2$. The content of CO_2 was determined indirectly by flame emission spectrometry. These analyses were carried out on dentine too.

From the same sample solutions Ca, P and Mg were determined according to (Quint and Höhling[8]).

Results and discussion. Figure 1 shows the topological distribution of Ca and CO_2 for a total tendon, starting in the proximal region near the muscle connection proceeding down to the distal joint. The curve for the CO_2 content shows a characteristic wavelike shape. The waves become much smoother with age and finally disappear. Often a Ca maximum appears in the region of a CO_2 minimum and vice versa. We assume that the next step of mineralization is to be expected where a Ca minimum coincides with a CO_2 maximum. The lowest CO_2 content is about 0.3%w/w ($\approx$25 mM/kg wet wt) in the tibia tendons as well as in the physiologically non-mineralizing ulna tendons (tendons of the wings; Ca: 0.10%w/w).

The molar CO_2/Ca ratios decrease with increasing mineralization. The CO_2/Ca ratio is about 4 at the Ca enriched, unmineralized proximal border (0.1-0.25% Ca), about 2 at the front (up to 1% Ca), about 0.3 in the partly mineralized region (up to 10% Ca) and about 0.08 in the mineralized region (more than 10% Ca). For comparison, the molar ratio in fully mineralized dentine is about 0.11. In analogy with these results, the CO_2/P ratio also decreases with increasing mineralization. It is interesting that the same trend is reported by Robinson et al.[9] for the developing enamel of rat incisors.

Figure 2 shows the molar ratios of Ca/P and Mg/Ca in the mineralizing turkey tibia tendon (a) as well as in the epiphyseal plate (b). For Ca/P a continuous increase is observed in the direction from the unmineralized region into the mineralized zone. While the average molar ratio is 0.74 in the unmineralized region of the tendon (UN) and 1.22 in the proliferating cartilage (PC) of the epiphyseal plate, this ratio increases to 1.61 in the mineralized tendon (MI) and to 1.52 in the calcified cartilage (CC). The ratios for bone (BO) and dentine (DE) are about 1.5. The low Ca/P ratios in the calcifying area of the tendons can be interpreted as an enrichment of phosphorus over calcium, compared with the developing apatite.

The Mg/Ca molar ratios however show a dramatic decrease with increasing mineralization in the turkey tibia tendon as well as in the epiphyseal plate (for the epiphyseal plate Wuthier's data[10] were used). While the values are about 0.3 in the unmineralized region of the tendon (UN)

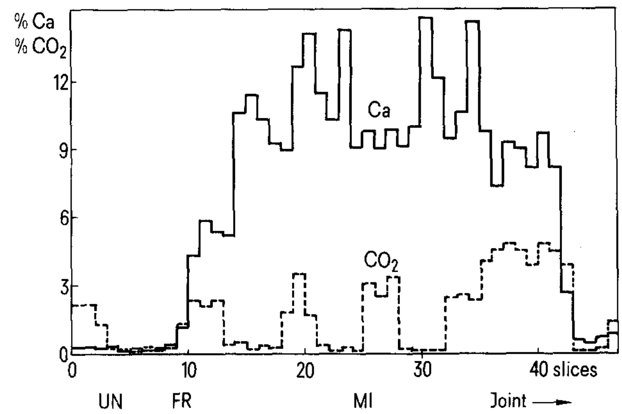

Fig. 1 The distribution of Ca and CO_2 in the turkey tibia tendon (UN: unmineralized tendon, FR: front region, MI: mineralized area).

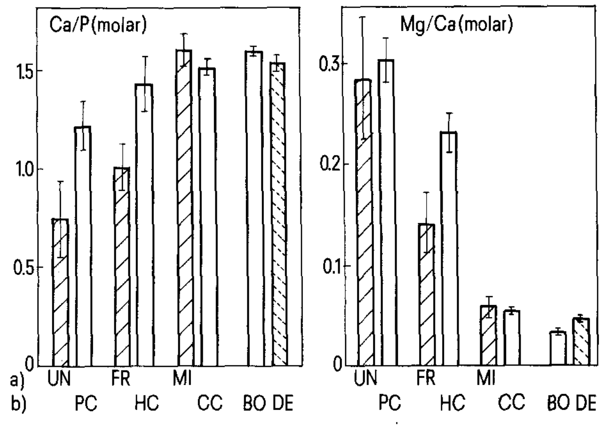

Fig. 2 The molar ratios of Ca/P and Mg/Ca for the mineralizing tendon (a, UN: unmineralized tendon, FR: front region, MI: mineralized area) and the epiphyseal plate (b, PC: proliferating cartilage, HC: hypertrophic cartilage, CC: calcified cartilage, BO: bone, DE: dentine). The cartilage values calculated by the data of Wuthier[10].

and in the proliferating cartilage (PC) we found about 0.05 in the fully mineralized areas (MI; CC). This can be interpreted as showing that the Mg content in the unmineralized regions is enriched (10-fold) in relation to the Ca content. Such a Mg enrichment in the prestages of apatite formation was also found in calcification experiments with cultures of the bacterium Matruchotii[11]. In the same region of the tendon where Mg is enriched a maximum in APase activity occurs[6]. So one may speculate that Mg has a regulating function for the enzyme activity which produces the phosphate as well as for mineralization and crystal growth[7]. CO_2 might act in the same way. Casciani[12] has found in the very first stages of enamel formation mainly CO_3^{2-} instead of phosphate. Boyde[13] assumes that the early mineral is rich in CO_3^{2-} or even contains $CaCO_3$. The fact that CO_2 is enriched in the collageneous system of turkey tibia tendon as well as in the collagen-free enamel may indicate that the same controlling functions exist in different hard tissues.

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(E,E)-10,12-Hexadecadienal: A component of the female sex pheromone of the spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae)[1]

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Summary. (E,E)-10,12-Hexadecadienal has been identified as a component of the sex pheromone of the female spiny bollworm moth, *Earias insulana,* by gas-chromatographic, electroantennographic and microchemical studies of abdominal tip extracts and entrained volatiles from female moths.

The spiny bollworm, *Earias insulana,* is an important cotton pest in Africa and the Mediterranean region, extending as far east as India and S.E. Asia. The use of traps baited with virgin female moths in monitoring attacks by this insect has already been reported[2], and identification of the female sex pheromone was undertaken to provide a synthetic attractant for this purpose.

Materials and methods. Pupae were received from Israel, Malawi and the Ivory Coast, and were incubated at 25 °C under constant light until adult emergence. Virgin female moths were then maintained at 20–25 °C on a 12 h: 12 h reversed light/dark cycle, and their abdominal tips were clipped 3 h into the scotophase on the 2nd night after emergence[3]. The excised tips were extracted with ether or hexane for 15 min at room temperature, and the extracts were filtered and concentrated before analysis. Active material was also obtained by collection on charcoal of volatiles emitted by a virgin female moth[4].

Gas-chromatographic (GC) instrumentation and packed columns were as described previously[5]. High resolution GC analysis was carried out on SGE 'D' grade support-coated open tubular (SCOT) columns as follows: Carbowax 20M (51 m × 0.5 mm inner diameter) temperature programmed from 100 to 180 °C at 20 °C/min and then isothermal; SE 30 (39 m × 0.5 mm inner diameter) programmed from 100 to 160 °C at 20 °C/min and then isothermal. GC analyses combined with simultaneous recording of electroantennographic (EAG) responses from the male moth to the column effluent were carried out as described by Moorhouse et al.[6], and EAG responses to synthetic compounds 'puffed' directly over the male moth's antenna were recorded as described previously[7].

The natural pheromone component, after GC collection from Apiezon L[8], and synthetic compounds were treated with tetracyanoethylene (TCNE) in dichloromethane at room temperature for 1.5 h and then analyzed by GC[8]. Monounsaturated alcohols were prepared by standard acetylenic routes and oxidized to the corresponding aldehydes with buffered pyridinium chlorochromate (PCC) in dichloromethane[9]. Wittig reaction of *(E)*-2-pentenal with the triphenylphosphonium salt of the tetrahydropyranyl (THP) ether of 11-bromo-1-undecanol gave *(Z,E)*- and *(E,E)*-11,13-hexadecadienal after deprotection and oxidation (Hall et al.[10]). Reaction of sorbyl acetate with the Grignard reagent from the THP ether of 10-bromo-1-decanol in tetrahydrofuran (THF), catalysed by lithium tetrachlorocuprate, gave *(E,E)*-12,14-hexadecadienal after deprotection and oxidation[11]. The 9,11- and 10,12-hexadecadienals were prepared by means of a general route to conjugated dienes outlined in the scheme.

Any of these hexadecadienals could be isomerized to a mixture of all 4 geometric isomers (ca. 61% *E,E;* 18% *Z,E;* 18% *E,Z;* 3% *Z,Z*) by heating with 0.5% thiophenol for 60 min at 110 °C[12], and the pure *E,E* isomers were obtained by LC on silica gel impregnated with 20% silver nitrate. The isomeric compositions of the hexadecadienals were most conveniently determined by GC analysis on the liquid crystal stationary phase, diethyl 4,4′-azoxydicinnamate[13]. Configurational assignments were based on a) mode of synthesis, b) selective reaction of the *E,E* isomers with