JOURNAL OF MATERIALS SCIENCE **38** (2003) 2087–2093

Infrared spectroscopy and X ray diffraction study on the morphological variations of carbonate and phosphate compounds in giant prawn (*Macrobrachium rosenbergii*) skeletons during its moulting period

D. S. SOEJOKO*
Physics Department, Faculty of Mathematics and Sciences, University of Indonesia, Depok 16424, Indonesia
E-mail: djarwani@indo.net.id; djarwani@fisika.ui.ac.id

M. O. TJIA
Physics Department, Faculty of Mathematics and Sciences, Bandung Institute of Technology, Ganesha 10, Bandung 40132, Indonesia

A systematic study has been carried out on the quantitative and morphological variations of carbonate and phosphate compounds in giant prawn (*Macrobrachium rosenbergii*) skeletons during the moulting period on the basis of infrared spectroscopy and X ray diffraction (XRD) analyses. Skeletons samples were prepared from adult giant prawns, extracted from the intact skeletons of the prawns at the ages of 4, 8, 12, 16, 20, 24, 30 days after moulting, as well as from the exuviae skeletons. Their phosphate bands were compared to those of synthetic hydroxyapatite (HAP) and human enamel, while their carbonate bands were compared to those of coral (*oculina sp*) and sea urchin (*psammechinus miliaris*) skeletons. It is well known that mineral compounds in human enamel consist mainly of calcium phosphates with a small amount of carbonates, while those found in coral and sea urchin skeletons consist mainly of calcium carbonates, coexisting with significant amount of magnesium. In contrast to those compositions, the spectroscopic data presented in this work display a strong indication that comparable amounts of calcium carbonates and calcium phosphates do exist in giant prawn skeletons during most of their moulting period. Based on the infrared analysis of the carbonate bands it is further suggested that calcium carbonates experience partial conversion from the amorphous to the crystal phase toward the end of the moulting cycle, as confirmed by similar trend exhibited in XRD data. On the other hand, the phosphate bands in giant prawn skeletons were found to be attributed to a mixture of amorphous and microcrystal phases without a clear contribution from apatite phase throughout the moulting period. This is also consistent with the pattern displayed by the XRD profiles. The lack of evidence for the presence of apatites could be understood on the basis of interfering and competing effects induced by the presence of various ions other than calcium phosphate ions, as well as the relatively high susceptibility of calcium phosphates to the associated substitutional effects.

1. Introduction

Hard tissues are generally characterized by the dominant presence of minerals in the forms of phosphate and carbonate compounds. In bones, the minerals are mainly deposited in the form of calcium phosphate compounds with the great majority existing as apatite and only a small amount of them are carbonate containing apatites [1–3]. In contrast to this, calcium carbonates in comparable amount with calcium phosphates were found in crustacean skeletons [4]. Deposition of those mineral compounds occurs generally in aqueous medium by way of calcification. Basically, this process involves precipitation of calcium salts, followed by the formation and subsequent growth of the crystals.

*Author to whom all correspondence should be addressed.

In prawn skeletons, calcification is a cyclical process, which takes place in conjunction with each moulting cycle undergone by the prawn. In order to understand the calcification mechanism in skeletons, it is necessary to have a clear identification of the specific morphological forms of the carbonate and phosphate compounds involved in the process within the skeletons. In addition, one also needs to have a clear picture of the variation of relative amount of the two compounds at different stages of the cycle, as well as information on the formation and growth of the associated crystals.

In the report of an early study on morphology of calcium carbonate as cited in reference [4], it was suggested that the compounds occurred in the form of vaterite as well as calcite. A recent article [5] reported that calcium carbonate in the white spots on pink shrimp skeletons after a long freezing process was composed of two crystal forms of calcite and relatively small amount of vaterite. In another study [6] it was reported that stable amorphous calcium carbonate existed in arthropodan skeletons and was suspected as a precursor of crystalline calcium carbonate polymorphs. However, no study has so far been conducted on the possibility of various incorporated forms of carbonate in calcium phosphate compounds in the skeletons. Regarding the phosphate compounds, a result quoted in reference [4] also claimed that the calcium phosphate found in the skeletons was in the form of hydroxyapatite (HAP). This conclusion has not been fully confirmed in view of the lack of detailed studies on the full range of samples of crustacean. In addition to that, carbonate and other ions which are ubiquitous in hard tissues are known to interfere with the formation of calcium phosphate compounds [7]. Hence, the resulting apatite crystals even if ultimately formed in the skeletons, are not likely to share the same chemical composition with the simple hydroxyapatite crystal. In this connection it is important to call attention to the result of a recent study [8] which showed that phosphate acid containing nonapatitic crystals such as octacalcium phosphate (OCP) were produced as intermediate phases prior to the formation of bone minerals. To the best knowledge of the authors only calcium phosphates in hard tissues vertebrates have been widely investigated, not much has been unraveled on these compounds as well as the composition of mixture of calcium phosphates and calcium carbonates in hard tissues of invertebrates. Much less is known about their morphological and quantitative variations within the moult cycle.

In order to clarify the calcification process in prawn skeletons, a systematic study of carbonate and phosphate compounds in giant prawn skeletons is carried out by means of infrared spectroscopy. For this study, samples are prepared from prawns at various stages of their moult cycle. Their infrared spectra are compared with those of well known synthetic hydroxyapatite and other hard tissues for qualitative identification. The spectral intensities of the carbonate and phosphate bands are used for the estimate of quantitative changes of the relative amount of these two compounds at various moult stages. These spectra will also be used to examine the existence of conversion process of amorphous calcium phosphate into apatite crystal in the skeletons. Furthermore, X ray diffraction (XRD) measurements were also performed for comparison.

2. Material and method

Skeletons samples were prepared from adult giant prawns (*Macrobrachium rosenbergii*) reared in the laboratory aquaria. The aquarium which measured 60 cm $\times$ 60 cm $\times$ 30 cm, was occupied by two prawns weighing about 15–25 grams each. Their moult cycles varied from 24 to 30 days. Different indicators were tagged on the two prawns for their separate identifications. The samples were extracted from the intact skeletons of the prawns at the ages of 4, 8, 12, 16, 20, 24, and 30 days after moulting, as well as from the exuvial skeletons. Only the body segments of the skeletons were extracted for this study. After being peeled off from the bodies, these parts of the skeletons were then shredded, sun dried, and ground into powder (250 mesh). To remove the organic materials, the samples were treated with analytical ethylenediamine for 7 days, filtered subsequently and finally air dried at 110°C for about 6 hours.

As reference materials for phosphate absorption bands, samples of human enamel was extracted from adult molar tooth, while sample of synthetic HAP was acquired from Merck Chemical Company. Additionally, coral (*oculina sp*) and sea urchin (*psammechinus miliaris*) skeletons samples were obtained from Institut für Geowissenschaftung und Lithosphärunforschung, at Giessen, to provide reference spectra of carbonate absorption bands. All the samples were pulverized by filing, and purified with ethylenediamine following the same procedure applied to the skeletons samples.

Each specimen used for infrared spectroscopic analysis was prepared according to standard procedure by mixing about 2.50 mg of powder sample with 250 mg of KBr, which was subsequently pressed into pellet. All the spectra were measured by using HITACHI infrared spectrometer, which covers the wave number range of 4000–400 cm^{-1}, except the spectra of coral and sea urchin which were measured by means of Varian infrared spectrometer. Special effort was made to ensure that all the data were obtained under more or less the same experimental condition. Background contribution from water content in KBr was taken into account by means of simultaneous measurement of a KBr pellet with the reference beam. Each recorded infrared spectra are backed up by the corresponding numerical data showing all the maxima occurring in the spectra. However we counted only the major maxima identifiable with those found in the published literature [9–12], and correspond to perceptible peaks in the recorded spectra. This criterion is expected to be adequate for our analysis.

For XRD measurements, the samples were prepared from skeletons extracted from the body segments, pulverized and pressed into pellet. The measurements were performed by using X ray diffractometer of Rigaku Denki with Cu K_α radiation ($\lambda = 1.5409$ A). The XRD profiles were accompanied by the print-out data, which allow peaks assignment according to published references [13, 14].

3. Results

The free tetrahedral PO_4^{3-} ion has four infrared vibration modes at the fundamental wave numbers of 980 cm^{-1} (υ_1), 363 cm^{-1} (υ_2), 1082 cm^{-1} (υ_3), and 515 cm^{-1} (υ_4) [15]. These vibration modes give rise to the two characteristic infrared absorption bands of phosphate compounds located at 1200–900 cm^{-1} and 650–550 cm^{-1} in the wave number range above 400 cm^{-1}, corresponding respectively to the υ_3 and υ_4 vibration modes. The free carbonate CO_3^{2-} ion also has four infrared vibration modes at the fundamental wave numbers of 1063 cm^{-1} (υ_1), 879 cm^{-1} (υ_2), 1430 cm^{-1} (υ_3), and 680 cm^{-1} (υ_4) [15]. The three characteristic infrared absorption bands of the carbonate compounds at 900–850 cm^{-1}, 1600–1400 cm^{-1}, and 720–680 cm^{-1} are associated with the υ_2, υ_3, and υ_4 absorption bands of CO_3^{2-} respectively.

The results of measurements on the HAP, human enamel, exuvial skeletons, coral, and sea urchin skeletons are collected in Fig. 1 for convenient comparison of the phosphate and carbonate absorption bands. It is readily observed upon comparison with the reference spectra, that the spectrum of exuvial skeletons in Fig. 1 contains the υ_3 and υ_4 phosphate bands and as well as υ_2, υ_3, and υ_4 carbonate bands. In addition to the phosphate and carbonate bands, all samples except the coral and sea urchin skeletons exhibit considerable water contents as indicated by a broad band in the 3700–2500 cm^{-1} region. The characteristic absorption bands of OH^- at 3576 cm^{-1} and 632 cm^{-1} appear only on the HAP spectrum.

In order to facilitate further comparison, the υ_3, υ_1, and υ_4 phosphate bands of HAP, human enamel, and exuvial skeletons in Fig. 1 are extracted and presented in inverted form in Fig. 2. The spectral intensities of these bands, measured under the same conditions, indicate that exuvial skeletons appears to have the lower content of phosphate compounds among those samples. In general, the υ_3 phosphate band (1200–900 cm^{-1}) can be divided into two overlapping components, one component is located in the high wave number region at 1200–1050 cm^{-1} and the other component is found in the low wave number region at 1050–900 cm^{-1}. The high wave number component in the human enamel spectrum is relatively less intense than that of the low wave number component, while these two components are almost of the same intensity in the spectrum of the exuvial skeletons. The human enamel spectrum has two maxima at 1090 and 1030 cm^{-1}, as indicated by the associated numerical data. These two maxima are shifted to 1070 and 1028 cm^{-1} in the spectrum of the exuvial skeletons. Apart from those differences in spectral positions, the υ_3 absorption band of the exuvial skeletons appears to be qualitatively distinct from the HAP and human enamel. In particular, an additional maximum at about 1153 cm^{-1} is clearly visible in its spectrum shown in Fig. 2.

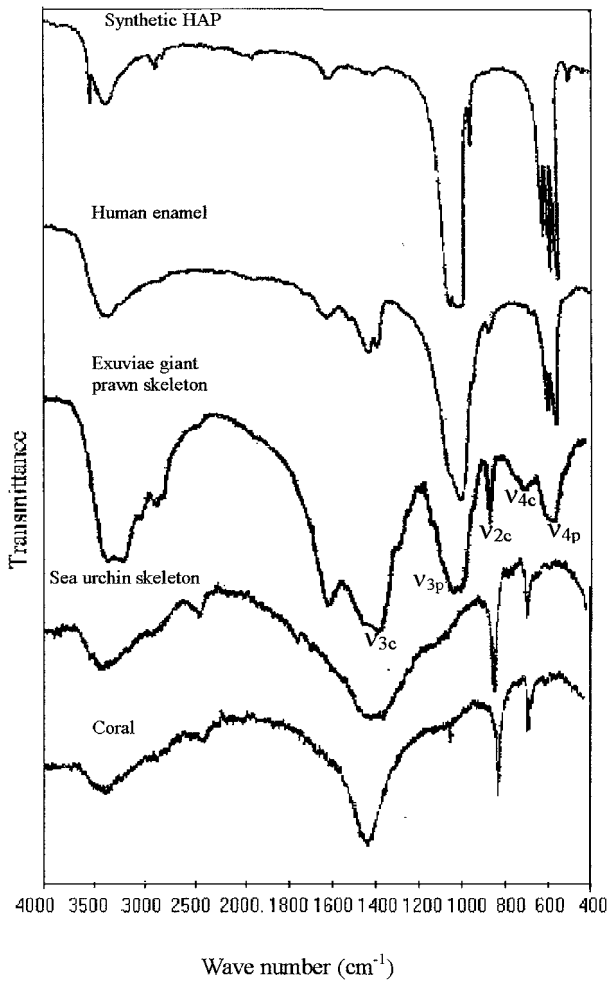

Figure 1 Infrared spectra of the synthetic HAP, human enamel, exuviae giant prawn skeleton, sea urchin skeleton, and coral. Subscript c and p are referred to carbonate and phosphate.

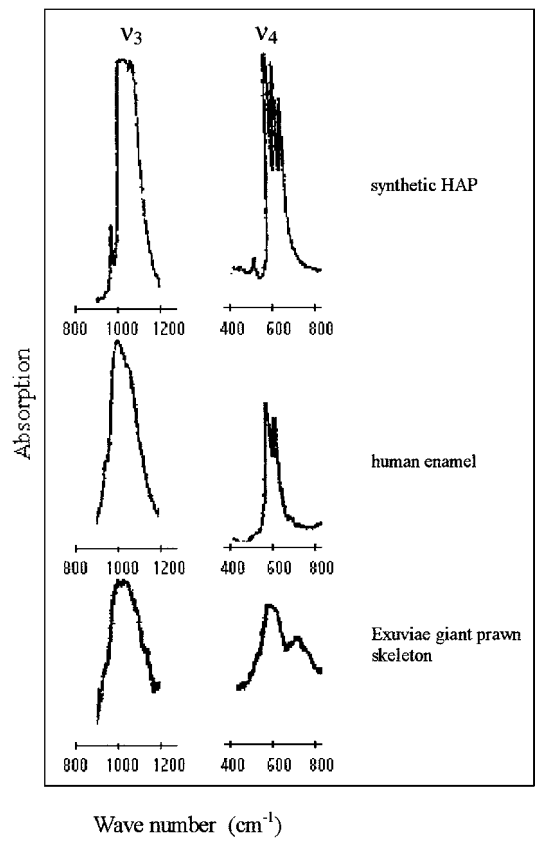

Figure 2 The υ_3 and υ_4 phosphate bands of synthetic HAP, human enamel, and exuviae giant prawn skeleton as extracted from Fig. 1 and presented in inverted form. The low wave number component of the υ_3 band of HAP is truncated due to the limited intensity range of the measurement.

The v_1 phosphate band appears as a small band at 960 cm^{-1} in the HAP spectrum, as a weak shoulder at 960 cm^{-1} on the v_3 phosphate band of human enamel, and occurs as a faint shoulder at 952 cm^{-1} on the same band of exuvial skeletons. This 952 cm^{-1} band is located between a characteristic band of amorphous calcium phosphate (ACP) at about 945 cm^{-1} [12] and that of crystalline calcium phosphate at about 962 cm^{-1} [13]. Comparison of the spectral intensities of the v_4 bands (650–550 cm^{-1}) also indicates that giant prawn skeletons has the lowest content of phosphate compounds among all the samples. It is also important to note that the v_4 phosphate bands of HAP and human enamel display the general feature of split structure and has two well resolved maxima at about 602 and 564 cm^{-1}. The additional maximum at 632 cm^{-1} in the HAP spectrum is attributed to OH^-, and not to be associated with the v_4 phosphate band. It has been suggested that the splitting fraction of this v_4 band is indicative of the crystal content of the associated compound in the samples [16]. In contrast to those observations, the v_4 band of the exuvial skeletons of giant prawn does not indicate the presence of split structure. Instead it has a relatively smooth form with a broad maximum at about 582 cm^{-1}, similar to the infrared spectral band of ACP [11].

Calcium compounds in coral and sea urchin skeletons are mainly carbonates, as indicated by the absence of phosphate absorption bands in their spectra shown in Fig. 1. The v_2, v_3, and v_4 carbonate bands of coral, sea urchin skeletons, and exuvial skeletons in Fig. 1 are extracted and presented in inverted form in Fig. 3. The v_3, v_2, and v_4 carbonate bands are located at about 1430, 860, and 710 cm^{-1} in the spectrum of coral sample, and located at about 1430, 870 and 710 cm^{-1} in the spectrum of sea urchin sample. These bands are similar to characteristic v_2 and v_4 carbonate bands of aragonite located at 860 and 713 cm^{-1} and its very broad v_3 band at around 1600–1350 cm^{-1} [17], as well as of calcite at those of calcite at 1430, 873 and 712 cm^{-1} [10]. In the spectrum of exuvial skeletons however, the band at about 872 cm^{-1} is likely to have a significant additional contribution from HPO_4^{2-} ions which has one of its characteristic bands at 875 cm^{-1} [18]. In the same vein, the v_3 carbonate band in skeletons at 1429 cm^{-1} is also inevitably contaminated by the protein absorption bands due to the presence of CH and Amide groups which were not effectively eliminated by the sample treatment with ethylenediamine [19]. Therefore one can not rely on these two bands alone for the elucidation of carbonate compounds in the skeletons. On the other hand, the v_4 carbonate band is free from those complications and will hence be employed as a major signature along with the complementary roles of v_2 and v_3 bands for the following analysis. It is clearly observed that the v_4 carbonate band in the exuvial skeletons spectrum at 712 cm^{-1} is not as sharp as those in the coral and sea urchin skeletons spectra. It is also smaller, but it has a maximum closely matches the reference v_4 bands.

Fig. 4 consists of the infrared spectra obtained from the intact giant prawn skeletons at various stages within a moult cycle and that obtained from the exuvial skeletons. The maxima of the v_3 and v_4 phosphate bands are presented in Table I in terms of their respective averages obtained from a number samples as noted in the table. This table shows that most of the maximum positions of all those bands do not experience significant changes with respect to advancing age of the skeletons.

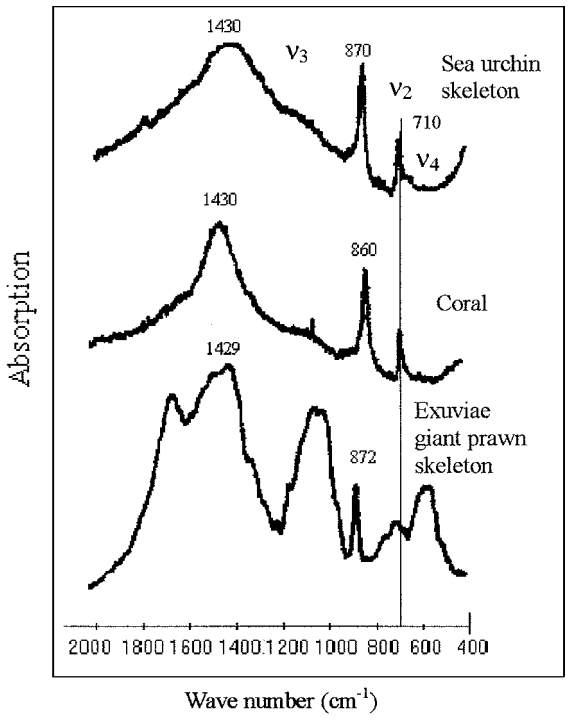

Figure 3 The v_2, v_3, and v_4 carbonate bands of the sea urchin skeleton, coral, and exuviae giant prawn skeleton as extracted from Fig. 1 and presented in inverted version.

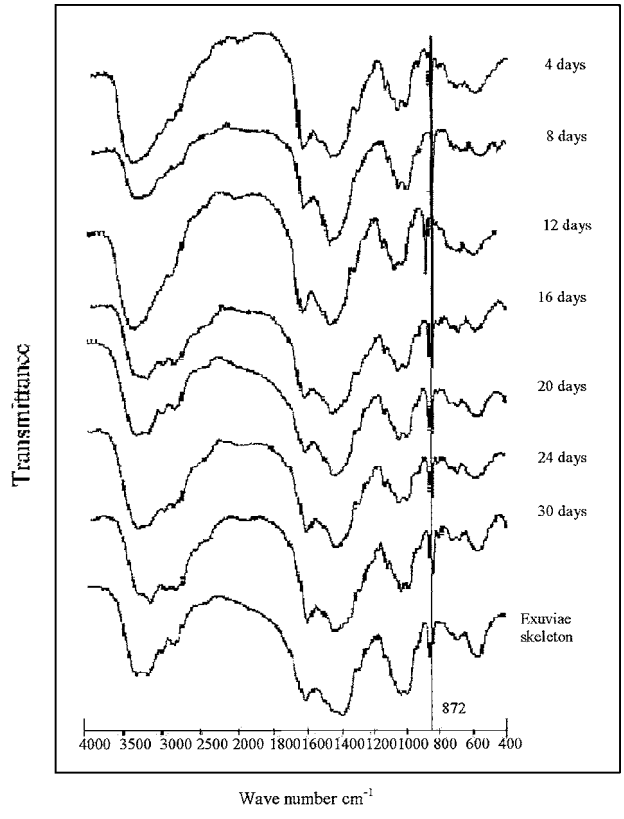

Figure 4 Infrared of giant prawn skeleton during 1 moulting cycle.

TABLE I Maxima in phosphate bands of giant prawn skeletons at various stages in the moult cycle

Age (days)	No. of samples	v_3 (cm^{-1})			v_4 (cm^{-1})
4	4	1024	1070	1154	582
8	3	1025	1070	1153	583
12	4	1026	1070	1153	581
16	3	1025	1070	1153	583
20	2	1024	1073	1153	583
24	2	1026	1072	1153	583
30	2	1028	1068	1153	580
Exuviae	3	1028	1070	1153	585

TABLE II Maxima in carbonate bands of giant prawn skeletons at various stages in the moult cycle

Age (days)	No. of samples	v_4 (cm^{-1})	v_2 (cm^{-1})	v_3 (cm^{-1})	
4	4	702	872	1434	1484
8	3	700	872	1435	1486
12	4	701	874	1430	1490
16	3	697	872	1434	1481
20	2	702	872	1436	1483
24	2	697	872	1428	1481
30	2	702	872	1429	1483
Exuviae	3	712	872	1429	1474

The shift from around 1025 cm^{-1} to 1028 cm^{-1} in the v_3 band does not represent a significant deviation from the 1030 cm^{-1} peak of human enamel spectrum cited earlier. The v_1 phosphate band appears in all spectra at about 952 cm^{-1} as a shoulder on the v_3 band. It is to be noted that the v_3 and v_4 phosphate bands of the exuvial skeletons appear with the highest intensity. The recorded maxima of the v_2, v_3, and v_4 carbonate bands are listed in Table II. The maximum positions of these three carbonate bands for the intact skeletons are unchanged with respect to advancing age. However the maximum of the v_4 band at 700 cm^{-1} and that of the v_3 band at 1484 cm^{-1} observed in the spectrum of the intact skeletons are respectively found to be up shifted to 712 cm^{-1} and down shifted to 1474 cm^{-1} in the spectrum of exuvial skeletons. In contrast to the phosphate bands, the v_4 carbonate band does not show significant variation throughout and toward the end of the moulting cycle. This is more or less consistent with the observation on the v_2 and v_3 carbonate bands.

The XRD profiles of the giant prawn skeletons in the moulting period and its exuvial skeletons are shown in Fig. 5. Each profile consists of continuous line and several maxima, indicated that calcium compounds in the sample are in the form of a mixture of amorphous and crystalline phases. The continuous line indicates not only the presence of amorphous phase, but it may also have contribution from microcrystalline phase which is too small to give rise to sharp peaks in XRD profiles [11, 20, 21]. This is found to be in good agreement with the results of the infrared spectroscopy. The continuous line which reflects the presence of amorphous calcium phosphate phase has a maximum at about $2\theta = 30^\circ$ [10, 18], and such continuous line is readily recognized in each profile in Fig. 5. Employing the Reference 12 and 13 for the elucidation of the major peaks occurring in

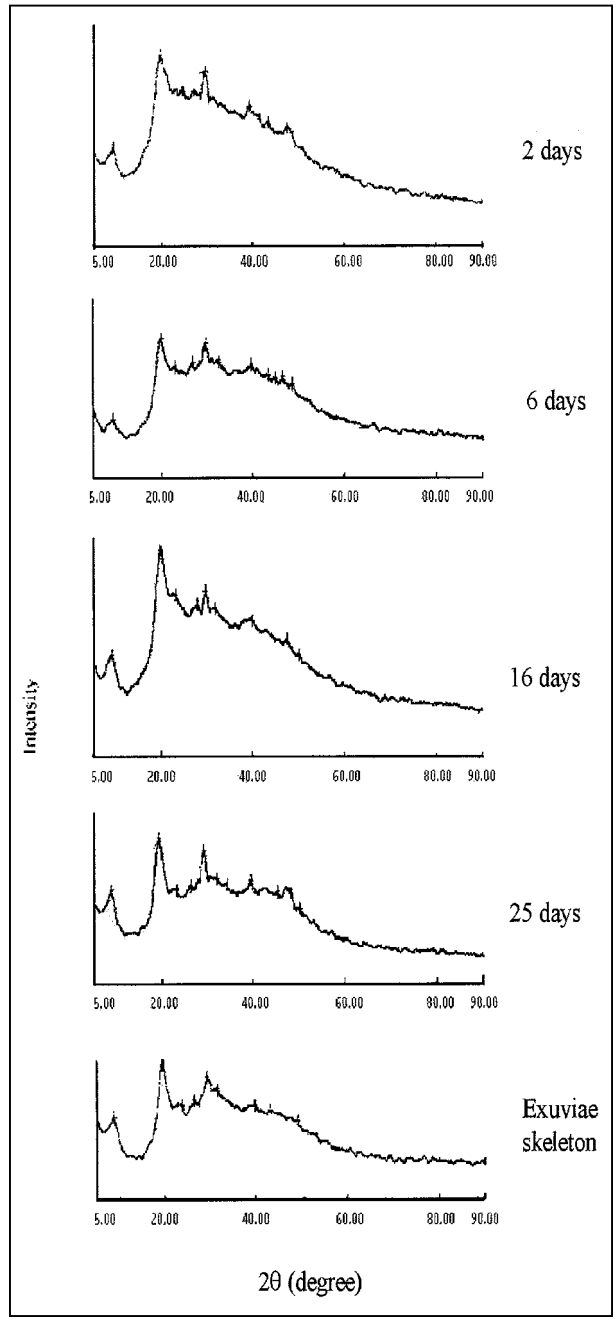

Figure 5 X-ray profiles of giant prawn skeleton during 1 moulting cycle.

the XRD profiles, we are led to the identification of OCP peaks at about $2\theta = 9.00^\circ$ and 26.00° and calcite hydrate peaks at $2\theta = 19.700^\circ$, 29.400°, and 31.800°.

4. Discussion

Calcium phosphate compounds exist in crystalline form in human enamel. In terms of its infrared spectra, the crystalline form is usually signified by the splitting structures in the phosphate bands such as the v_4 band shown in Fig. 2 for the spectra of HAP and human enamel. Further, the splitting of the v_4 phosphate band in Fig. 2 can be used to estimate the crystallinity fraction in the related samples in terms of its splitting factor [16]. The v_4 bands of HAP, human enamel, and exuvial skeletons in the figure are arranged in decreasing order of their crystal contents based on their splitting factors. It is seen that the v_4 phosphate band of giant prawn

skeletons appears smoothly without splitting, similar to the absorption band of amorphous calcium phosphate. It should be noted however, that in the form of microcrystal, which has exactly the same crystal structure except for shorter spatial periodicity, the calcium phosphate crystals may also contribute to the smooth v_4 band typical of its amorphous phase [20]. Although this argument may sound less than conclusive in view of the limited resolution of v_3 phosphate band, it is supported by our XRD data given in Fig. 5, which clearly displays the presence of this amorphous phase. In contrast to this, its v_3 phosphate band is qualitatively different from that of amorphous phase as it clearly shows the occurrence several maxima which are indicative of the presence of crystalline phase as suggested commonly [12]. The v_1 band as a weak shoulder located between the characteristic bands of amorphous and crystalline phases may suggest the presence of a mixture of amorphous and crystalline phases. Therefore, based on the combined characteristics of the v_1, v_3 and v_4 phosphate bands, we are led to the suggestion that calcium phosphates in the skeletons could exist in the form of a mixture of amorphous and microcrystals. This result is corroborated by XRD profiles of the sample presented in Fig. 5.

In order to acquire more detailed structural information on the calcium phosphate, we turn to the v_3 band which is more sensitive to structural variation of phosphate ions, especially its high wave number components [12]. The v_3 phosphate bands for the intact and exuvial skeletons are presented in Fig. 4 and Table I. The high wave number components are represented by the maxima at 1153 and 1070 cm^{-1} as well as a shoulder at 1112 cm^{-1}. The bands at 1153 and 1070 cm^{-1} are similar with two maxima of OCP bands at 1150 and 1076 cm^{-1}, while the shoulder at 1112 cm^{-1} has been attributed to phosphate environments in poorly crystalline apatitic crystals [12]. It must be admitted that this observation alone does not constitute a convincing evidence for the presence of OCP in view of the complex phosphate environment in biological system. Further substantiation of this suggestion is provided nevertheless by the XRD data of the sample given in Fig. 5 and explained in the text associated with the figure. The presence of nonapatitic crystals offers a way out to the ambiguity encountered earlier in the analysis of the v_4 band. It is further observed that the two bands at 1153 and 1070 cm^{-1} appear more intense in the intact skeletons spectra compared to that in the exuviae skeletons spectrum. Moreover, the shoulder observed at 1112 cm^{-1} in the case of intact skeletons disappears almost completely in the exuviae skeletons. It was reported that this 1110 cm^{-1} band did not feature clearly in well crystallized synthetic apatites [12]. We proposed that the exuvial skeletons contains more crystallized apatites compared with the intact skeletons. The implied decrease in the intensity of the bands at 1153, 1070, and 1112 cm^{-1} in the exuviae skeletons may thus provide some evidence of morphological conversion of some amount of nonapatitic crystals into apatite crystals.

The low wave number component of the v_3 band given in Table I has a maximum at about 1025 cm^{-1} in the spectra of the earlier stage intact skeletons, and at 1028 cm^{-1} in the spectrum of final stage skeletons as well as the exuviae skeletons. The characteristic phosphate band of HAP in this region is located at 1020–1030 cm^{-1}, and tends to shift toward 1020 cm^{-1} for nonstoichiometric apatites containing HPO_4^{2-} or CO_3^{2-} ions [12]. The shift of this band from 1025 cm^{-1} for the earlier stage intact skeletons to 1028 cm^{-1} for the exuviae skeletons as mentioned above does not seem to provide significant supports to the suggestion that the conversion from nonapatitic calcium phosphate into apatite crystal may actually take place in the final stages of the moult process.

Beside calcium phosphates, calcium carbonates are also found abundantly in crustacean skeletons. The small albeit distinct v_4 carbonate band at the region of 720–680 cm^{-1} associated with the skeletons as shown by Fig. 1c is obviously absent in the spectra of HAP and human enamel. This clearly suggests the presence of carbonate in skeletons. The unusually large v_3 carbonate band is due to contribution from the residual protein left over in the ethylenediamine treatment described earlier. The protein band is located at about the same position with the v_3 carbonate band, and may hence contribute to it. However, comparison of the v_3 carbonate band of skeletons with those sea urchin skeletons and coral in Fig. 1d and e appears to be consistent with that suggestion. These observations also constitute a further support to the earlier report on the comparable abundance of calcium carbonates and calcium phosphates in crustacean skeletons [4].

Turning now to Fig. 4 we note that the relatively broad spectral form of the v_4 carbonate band is most likely to be associated with the presence of amorphous or microcrystalline phases of calcium carbonates. Further, the v_4 carbonate band at 700 cm^{-1} in the spectra of intact skeletons undergoes a clear shift to 712 cm^{-1}, associated with carbonate band of exuvial skeleton which is more clearly shown in Fig. 3. An opposite shift is observed however for the v_3 bands from the Fig. 4, while no significant shift is seen for the v_2 bands. In view of these observations, no specific statement on the change of carbonate phase can be made on the basis of this analysis alone.

Apart from those carbonate compounds identified by the absorption bands discussed above, one should also consider the possibility that carbonates in giant prawn skeletons could be incorporated in calcium phosphate compounds in the form of amorphous carbonate calcium phosphate, carbonate nonapatitic crystals, and carbonate apatite crystals. It was reported that high concentration of carbonate incorporated in amorphous calcium phosphate produces infrared spectrum with maxima at 863 cm^{-1} for the v_2 band, at about 1485 and 1419 cm^{-1} for the v_3 band, and at 690 and 717 cm^{-1} for the v_4 band [11]. Whereas carbonate incorporated in nonapatitic crystal, especially in the form of carbonate octacalcium phosphate, has been known to contribute to the carbonate bands at 1470, 1430, 882, and 875 cm^{-1} [22]. The peaks revealed in the carbonate bands from the intact skeletons were recorded at about 1484, 1430, 872, and 700 cm^{-1} (Table II) as attributed

to calcium carbonates, may also have partial contributions from the amorphous carbonate calcium phosphate and carbonate nonapatitic crystals. The detailed features of those bands are not however, fully observed in Fig. 4. This is perhaps due to the relatively low concentration of the carbonate incorporated in the amorphous calcium phosphate and nonapatitic crystals, as well as a consequence of the lower purity of the compounds in the skeletons. Those same bands are distinctly absent in the spectrum of human enamel.

5. Conclusion

We have shown in this study that the results of infrared spectroscopy and XRD analyses of giant prawn skeletons and other hard tissues have produced an evidence indicating the coexistence of calcium carbonates and calcium phosphates in comparable amounts in the giant prawn skeletons, in contrast to what was found in other hard tissues. Based on analysis of the v_3 and v_4 phosphate bands as well as the v_3 and v_4 carbonate bands, no clear indication was observed on the morphological conversion from the amorphous to the crystalline phase of those compounds during the moulting period. The XRD data further suggest that calcium phosphates in the biochemical environment of giant prawn skeletons do not favor the formation of apatites, probably related to the presence of phosphate and carbonate mixture in the tissue.

Acknowledgement

We wish to thank Mrs. D. Satyani Lesmana, Research Installation Fresh Water Fisheries, Department of Agriculture, Depok, for rearing the prawns and preparing the dried samples. We wish to thank Prof. W. Franke, Director Institute für Geowissenschaftung und Lithosphärunforschung, Justus-Liebig -Universitaet Giessen, for providing her with the coral and sea urchin samples, as well as Institut für Biophysik at the same university for helping us in some of the measurements. This study was supported in part by research grant from the Department of Education and Culture under the contract No. 20/P4M/DPPM/3311/94/BBI/1994.

References

1. W. D. AMSTRONG and L. SINGER, *Clin. Orthop. Rel. Res.* **38** (1965) 179.
2. E. D. PELLEGRINO and R. M. BILTZ, *Calc. Tiss. Res.* **10** (1972) 128.
3. R. M. BILTZ and E. D. PELLEGRINO, *Clin. Orthop. Rel. Res.* **129** (1977) 279.
4. R. STEVENSON, in "The Biology of Crustacea," Vol. 9, edited by D. E. Bliss and L. H. Mantel (Academic Press, New York, 1985) p. 1.
5. A. MIKKELSEN, S. B. ENGELSEN, H. C. B. HANSEN, O. LARSEN and L. H. SKIBSTED, *J. Cryst. Growth* **177** (1997) 125.
6. J. AIZENBERG, G. LAMBERT, L. ADDADI and S. WEINER, *Adv. Material* **8**(3) (1996) 222.
7. J. C. ELLIOT, *Clin. Orthop. Rel. Res.* **93** (1973) 313.
8. G. R. SAUER, W. B. ZUNIC, J. R. DURIG and R. E. WUTHIER, *Calc. Tiss. Int.* **54** (1994) 414.
9. B. O. FOWLER, E. C. MORENO and W. E. BROWN, *Arch. Oral. Biol.* **11** (1966) 477.
10. W. H. EMERSON and E. E. FISCHER, *ibid.* **7** (1962) 671.
11. J. D. TERMINE and D. R. LUNDY, *Calc. Tiss. Res.* **15** (1974) 55.
12. C. REY, M. SHIMIZU, B. COLLINS and M. J. GLIMCHER, *Calc. Tiss. Int.* **49** (1991) 383.
13. JCPDS-International Centre for Diffraction Data (1995).
14. M. IIJIMA, H. KAMEMIZU, N. WAKAMATSU, Y. D. GOTO and Y. MORIWAKI, *J. Cryst. Growth* **181** (1997) 70.
15. G. HERZBERG, in "Molecular Spectra and Molecular Structure. II. Infrared and Raman Spectra of Polyatomic Molecules" (D. Van Nostrand Co, Princeton, New Jersey, 1964).
16. J. D. TERMINE and A. S. POSNER, *Science* **153** (1966) 1523.
17. G. FALINI, S. ALBECK, S. WEINER and L. ADDADI, *ibid.* **271** (1996) 67.
18. J. ARENDS and C. L. DAVIDSON, *Calc. Tiss. Res.* **18** (1975) 65.
19. J. D. TERMINE, E. D. EANES, D. J. GREENFIELD and M. U. NYLEN, *ibid.* **12** (1973) 73.
20. J. D. TERMINE and E. D. EANES, *ibid.* **10** (1972) 171.
21. E. D. EANES, J. D. TERMINE and M. U. NYLEN, *ibid.* **12** (1973) 143.
22. N. S. CHICKERUR, M. S. TUNG and W. E. BROWN, *Calcif. Tissue Int.* **32** (1980) 55.

Received 18 October 2000
and accepted 4 February 2003