Acid and alkaline phosphatase heterogeneity in liver, heart and intestine of the adult chick

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Summary. The molecular heterogeneity of acid and alkaline phosphatase from chick liver, intestine and heart is demonstrated by polyacrilamide disc gel electrophoresis. Liver and heart show the same number of molecular forms of acid and alkaline phosphatases. In contrast, the intestine homogenate shows electrophoretic bands different in number and in gel position from those of the liver and of the heart.

Acid and alkaline phosphatase activities have been found in almost all the tissues studied. Their wide distribution and the presence of different molecular species during the ontogenetic processes make these enzymes suitable, as convenient molecular probes, for the differentiation studies. Wang[1,2] and Strittmatter[3,4] reported the biochemical heterogeneity of acid phosphatase of liver, intestine and metanephros of chicken at various stages of embryonic development. Chang and Moog[5] described 2 isozymes of alkaline phosphatase in the intestine of the late embryos and of the hatched chicks. However, literature reports on acid and alkaline phosphatases of the adult chicken, to which one must refer in studies of embryonic development, are almost missing. In this paper, we describe the molecular heterogeneity of these enzymes in the liver, heart and intestine of 1-year-old chicks.

Materials and methods. Specimens of liver, heart and intestine of 1-year-old chicks (White Leghorn) were homogenized in 5% Triton X-100. The homogenates were allowed to stand for 30 min at 4 °C and successively centrifuged at 100,000 × g per 60 min. The clear supernatants were used as enzyme sources. Electrophoretic separation of acid and alkaline phosphatases was carried out on polyacrylamide gels whose composition was: 10% acrylamide, 0.18% Bis-acrylamide, 0.056% temed in 10 mM histidine-NaOH buffer pH 8.5. The polymerization was accomplished at room temperature in 60 min after the addition of ammonium persulfate (0.25%). The electrode solution was histidine-NaOH buffer 10 mM pH 8.5. In the case of the intestine alkaline phosphatases, the gel and the electrode buffer were at pH 7.5. Electrophoretic separation was carried out at 3 mA per tube for 90 min at 4 °C. Acid phosphatase bands were stained according to Allen and Gockerman[6]. Alkaline phosphatase bands were stained as described by Hochberg and Sargent[7] using β-glicerophosphate as substrate.

Results and discussion. In Figure 1 are reported the electrophoretic patterns of acid phosphatases of liver, heart and intestine of adult chick. 3 fractions are present in liver and heart, while 2 are found in the intestine. The spread of the electrophoretic bands prevents a comparison of the mobility of the liver and heart fractions. The 2 bands of intestine, however, are clearly different from the others. Identical results, though with more diffuse bands, are obtained when the electrophoretic bands are detected by the β-glicerophosphate and lead nitrate technique[7].

Acid phosphatase activity has been studied mainly in the liver of the adult chick. Moore and Angeletti[8] have shown the presence of 2 chromatographically distinct molecular forms in the liver homogenate. It is likely that 2 molecular species are eluted simultaneously from the column.

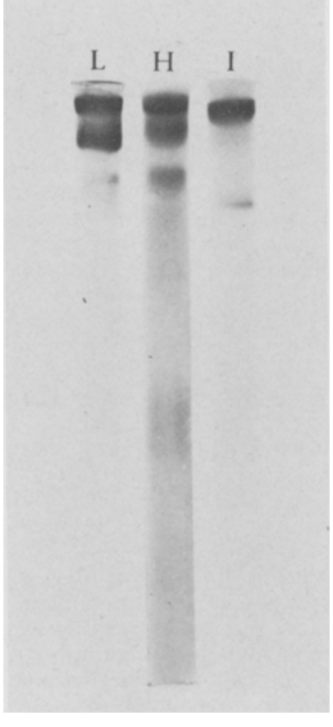

Fig. 1. Electrophoretic separation at pH 8.5 of acid phosphatases from chicken liver (L); heart (H) and intestine (I).

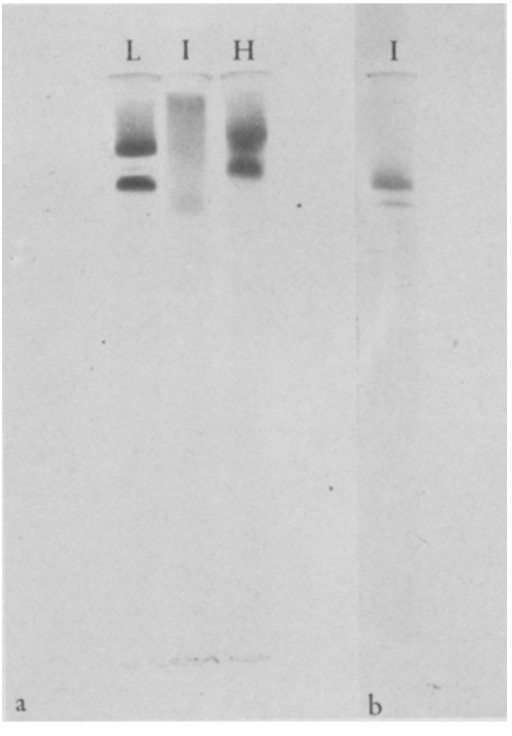

Fig. 2. *a* Electrophoretic separation at pH 8.5 of alkaline phosphatases from chicken liver (L), intestine (I) and heart (H). *b* electrophoretic separation at pH 7.5 of alkaline phosphatases from chicken intestine (I).

Wang[1,2] and Strittmatter[3,4] demonstrated the presence of different acid phosphatase activities in the soluble and in the particulate fractions of the liver of chicken at different development stages. These authors concluded that 2 molecular forms of acid phosphatase, at least, must exist in the liver of the developing chick. Preliminary results, obtained in our laboratory, demonstrate that the 'soluble' phosphatase constitutes the most rapid electrophoretic band, and the other 2 phosphatases are present in the particulate fraction.

The electrophoretic patterns of alkaline phosphatases of liver, heart and intestine of the chick are reported in figure 2. In the left photograph (2, a) is shown the electrophoresis carried out at pH 8.5. While 2 enzyme fractions appear in the samples of liver and heart, a very diffuse band is shown by the intestine. When the analysis is carried out at pH 7.5, this band is resolved into 2 fractions, as shown in figure 2, b. In these conditions, the alkaline phosphatases of liver and heart are not isolated on the gels. This electrophoretic behaviour demonstrates a difference between the molecular forms of alkaline phosphatase of intestine and those of liver and heart. The finding of 2 molecular forms of alkaline phosphatases in the intestine of the adult chick confirm the results of Chang and Moog[5] concerning 1-day-old chicken.

The results reported here are also in agreement with the data concerning the molecular multiplicity of acid and alkaline phosphatases of liver and intestine of other vertebrates. In all cases, no more than 3 or 4 molecular forms of acid[6,8-11] and alkaline[12-14] phosphatase have been described. Works are in progress to confirm the homogeneity of the electrophoretic fractions, to describe the function properties of each molecular form and to determine their subcellular localization.

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Formation of enantiomeric sesquiterpenes in the secretions of scale insects

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Summary. The volatile oil present in the secretion of a scale insect, *Ceroplastes ceriferus* Anderson (fam. Coccidae), has been found to consist of a number of sesquiterpenes which are enantiomeric with respect to those from *C. rubens* Maskell, which infests the same host tree. The present investigation strongly suggests that each insect possesses a specific set of C-C cyclases for the sesquiterpenoids.

The scale insects *C. ceriferus* and *C. rubens* infest over 10 species of trees and exude odoriferous waxy secretions in which they become encrusted. The secretions contain volatile oils which may serve as plasticizers of the waxy resins. We have examined the compositions of the volatile oils and found sesquiterpenes to be the major components, as shown in the table. Comparative examination of the volatile oils from *C. ceriferus* on 7 different species of host trees (*Diospyros Kaki, Liquidambar formosana, Spiraea cantoniensis, Zelkova serrata, Ilex rotunda, Camellia japonica* and *Acer palmatum*) and from *C. rubens* on 2 different species of host trees (*Camellia japonica* and *Laurus nobilis*) has shown that their constituents are independent of the species of host trees and are specific to the insects.

Materials and methods. The general procedure used will be illustrated here by means of a specific example, as follows. *C. ceriferus* (487 g) fed on the host *Diospyros Kaki* Thunb. was collected in Tokushima Prefecture, Japan, in January. The secretion was washed off with ether and was separated from insect debris (83 g). The slurry was evaporated in vacuo and the residue was steam-distilled to give the volatile oil, 2.38 g (0.59% of the secretion). On the other hand, *C. rubens* (53 g) gave volatile oil, 0.12 g, in a yield of 0.25% from its secretion. Each oil was analyzed by GC-MS, then the components were isolated by column chromatography using silver nitrate-impregnated silica gel and by preparative gas chromatography (Carbowax-20 M and Thermon-1000). The isolated compounds were identified by MS- and IR-spectrometry, and when possible, optical rotations were measured. To confirm the absolute configurations, the compounds numbered 3, 4, 14 and 15 in the table were hydrogenated to the corresponding skeletal hydrocarbons (No. 32, 33 and 34 in the table) and their optical rotations were compared with the reported values. Compounds No. 30 and 31 were dehydrated with $SOCl_2$-Py to yield compounds No. 10, 14 and 15 and then hydrogenated to selinane (No. 34).

Results and discussion. It is noteworthy that sesquiterpenes secreted by *C. ceriferus* belong to the optically antipodal series compared to those of *C. rubens* on the same host tree, as shown in the table. Moreover, our results indicate that only germacrene-D and β-bourbonene (No. 13 and 3, respectively, in the table) possess isopropyl groups epimeric to those of the rest of the compounds in both secretions. β-Bourbonene can be formed[3] very easily by photoreaction from germacrene-D, so their isopropyl groups naturally have the same configuration. Germacrene-D, with the isopropyl group epimeric to those of cadinene-, selinene- and elemene-type sesquiterpenoids, evidently cannot be a key intermediate in their formation. Therefore, 2 precursors corresponding to the epimeric isopropyl groups should exist. In addition, the sesquiterpenes other than germa-