Research Articles

The site of synthesis of hemocyanin in the crayfish, *Astacus leptodactylus*

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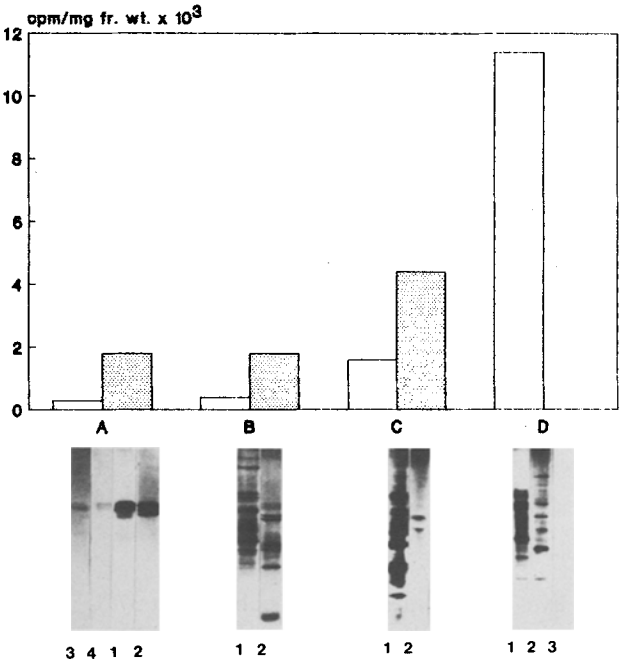
Summary. The in vitro incorporation of ^{35}S-methionine into hemocyanin was tested in the crayfish, *Astacus leptodactylus*. All organs assayed (hemocytes, heart, hypodermis, midgut gland, muscle and stomach) were able to synthesize proteins under our experimental conditions, but only midgut gland (= hepatopancreas) incorporated labeled methionine into a protein which was precipitated by an antibody against *Astacus* hemocyanin, and which comigrated with authentic hemocyanin on SDS gels.
Key words. Hemocyanin; crayfish; *Astacus leptodactylus*; biosynthesis; midgut gland (= hepatopancreas).

As a prerequisite for studies on the regulation of hemocyanin synthesis and secretion in the crayfish, *Astacus leptodactylus*, we investigated which organs of the crayfish are able to synthesize and to secrete hemocyanin. In contrast to our detailed knowledge on the structure and function of hemocyanin, relatively little is known about its biosynthesis, both with respect to the site and to its regulation; only a few species have been studied from this point of view so far[2–4]. Various organs have been described to be hemocyanin producing tissues in various arthropods, namely the inner heart wall in the tarantula, *Eurypelma californicum*[5], and the midgut gland in the lobster[6]. The identification was achieved by immunoprecipitation of radiolabeled hemocyanin. In an electron microscopic study in the crab, *Carcinus maenas*, cyanoblasts and cyanocytes from the reticular connective tissue surrounding the ophthalmic artery, the gizzard and the midgut gland were described to be the sites of synthesis[7]. Another approach has been used recently: mRNA from various tissues was extracted and translated in either a reticulocyte system or in *Xenopus laevis* oocytes. With this technique, midgut gland from *Astacus leptodactylus* and *Cancer pagurus*[8], as well as reticular connective tissue in the latter species[8] and the mantle tissue from *Limulus polyphemus*[9], were identified.

In the present study, based on an established in vitro culture system[10] and techniques described in detail elsewhere[11], all the tissues tested – hemocytes, heart, hypodermis, midgut gland (= hepatopancreas), muscle and stomach – were found to be able to synthesize and to secrete proteins in vitro (fig.). The incorporation rate of ^{35}S-methionine into proteins was highest in hypodermis and in hemocytes. Antihemocyanin-immunoprecipitable radiolabeled protein was produced only by the midgut gland. The immunoprecipitated protein comigrated with authentic hemocyanin from *Astacus leptodactylus* hemolymph (fig.).

Our results corroborate those of Préaux et al.[8] who used in vitro translation of RNA from various tissues. Similar findings were also reported for the lobster[6]. Thus, hemocyanin synthesis in crustaceans takes place in the midgut gland and not in the hemocytes, in contrast to the situation in the spider, *Eurypelma californicum*[5,12]. It still



Protein biosynthesis in midgut gland (*A*), stomach (*B*), hypodermis (*C*) and hemocytes (*D*) from *Astacus leptodactylus* in vitro. The organs were incubated for 5 h at 18 °C and the incorporation of ^{35}S-methionine into total proteins and into hemocyanin was measured. The upper part of the graph represents the quantitative analysis (▨ = newly synthesized proteins within the tissue and □ = secreted into the medium). For a qualitative analysis the newly synthesized polypeptides were analyzed by SDS-PAGE and subsequent fluorography (lower part). Homogenates and media of the corresponding tissues were either precipitated with trichloroacetic acid or the antihemocyanin antibodies and used for SDS-PAGE. Lane 1 always represents the pattern of newly synthesized total proteins from the cytosol of the corresponding tissues and lane 2 the pattern of secreted polypeptides. In lane 3 the immunoprecipitates of the cytosol were separated and in lane 4 those of the media. In lane D3 no immunoprecipitable peptide can be detected.

remains unknown in which cell type within the midgut gland this synthesis takes place. So far, all attempts at a more specific localization have failed. A newly available technique of separating the various cell types of the midgut gland[13] may provide a more promising approach.

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Inhibitory effects of phenolic compounds on CCl_4-induced microsomal lipid peroxidation

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Summary. The antiperoxidative effects of 35 phenolic compounds, most of them belonging to the flavonoid class, were investigated using CCl_4-induced peroxidation of rat liver microsomes. This system was rather insensitive to gallic acid, methyl gallate and ellagic acid. Nevertheless it was inhibited by flavonoids and structure/activity relationships were established. The most potent compounds were gardenin D, luteolin, apigenin (flavones), datiscetin, morin, galangin (flavonols), eriodictyol (flavanone), amentoflavone (biflavone) and the reference compound, (+)-catechin. The natural polymethoxyflavone gardenin D has shown a potency comparable to that of (+)-catechin and higher than that of silybin. Thus, it may be considered as a new type of natural antioxidant with potential therapeutical applications.
Key words. Phenolic compounds; CCl_4-induced peroxidation; TBA-reactive substances.

The toxicity of a large number of xenobiotics depends on their conversion into free radicals, which initiate the process of lipid peroxidation in cell membranes. Carbon tetrachloride (CCl_4) is probably the best-studied liver toxicant; it stimulates endogenous peroxidative changes in rat liver microsomes during incubation at 37 °C in the presence of NADPH[1].

CCl_4 is activated by the NADPH-cytochrome P-450 system of the liver endoplasmic reticulum, with formation of the trichloromethyl radical ($CCl_3\cdot$) and in aerobic conditions, of the more reactive trichloromethyl peroxy radical ($CCl_3O_2\cdot$). The latter initiates peroxidation of the polyunsaturated fatty acids, while $CCl_3\cdot$ is more important in covalent binding to both lipid and protein components of the membrane[2,3]. The onset of fatty infiltration depends upon haloalkylation, which could be involved in the pathogenesis of liver necrosis determining an increased susceptibility of the cell to oxidative stress. Therefore, covalent binding should be implicated more in the pathogenesis of cell death during chronic CCl_4 intoxication[4], whereas acute cell death seems to be mainly dependent upon lipid peroxidation[5].

Flavonoids are a widely distributed group of natural antioxidants, some of them exerting protective effects against peroxidation-induced cell damage[6]. As the stimulatory effect of CCl_4 on lipid peroxidation provides a convenient method for studying the effectiveness of potential scavengers and antioxidants[7], we have selected it to assess the activity of a number of natural phenolic compounds, most of them belonging to the flavonoid class, in order to find new antiperoxidative agents with potential therapeutical applications.

Materials and methods

Drugs. Some compounds were isolated from plants: gardenin D and 5-O-demethylnobiletin (*Sideritis mugronensis*[8]); hypolaetin-8-O-β-D-glucoside (*Sideritis leucantha*[9]); taxifolin (*Rhamnus lycioides*[10]) and methyl gallate