DISTRIBUTION AND ACTIVITY OF CHITINOLYTIC ENZYMES IN THE DIGESTIVE TRACT OF BIRDS AND MAMMALS

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ABSTRACT

The extraction and purification of chitinolytic enzymes devoid of any lysozymic activity, taken from the gastric mucosa of mammals, indicated that these enzymes are true chitinases, rather than lysozymes with chitinolytic activity.

A close relationship was found between chitinase secretions in the digestive tract and the nature of the normal diet of the species considered. Changing the diet of an animal (mouse, rat, hamster, guinea pig) for a few weeks did not modify its ability or inability to secrete chitinase.

In the digestive tract of birds, chitinase is secreted only by the gastric mucosa. All the more or less insectivorous birds so far studied, including chickens, do secrete gastric chitinases. No chitinase secretion was detected in the pigeon or the parrot.

In mammals, gastric chitinases were found in omnivorous and insectivorous species belonging to the orders Insectivora, Chiroptera, Carnivora, Rodenta and primates. In the pig, chitinases are secreted by the gastric mucosa and by the pancreas.

The digestibility of chitin in both a purified and a natural form was estimated in feeding experiments with mice, Japanese nightingales and chickens. From 19% to 58% chitin was digested by mice and chickens that were fed a diet containing pure chitin, and by Japanese nightingales that were fed mealworm larvae.

INTRODUCTION

Since the discovery by Jeuniaux (12) of a secretion of chitinases in the digestive tract of some vertebrates, these enzymes, whose glandular origin was clearly demonstrated by Dandrifosse et al. (7), have been sought in a wide series of species (9, 13, 14, 21, 22).

In order to explain the erratic occurrence of chitinase in the vertebrate species so far studied, it was suggested that the secretion of chitinase by the gastric mucosa or by the pancreas was corrected with the feeding habits of the species, an adaptative correlation that was the result of a regressive evolution (14, 16). This statement was confirmed by the study of a wide series of fish, amphibians and reptiles (21). A correlation between chitinase secretion and diet was also pointed out in the case of mammals belonging to the order Carnivora (4).

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Owing to the wide distribution of lysozymes (E.C. 3.2.1.17) (11, 17) and to the fact that these muramidases are able to hydrolyze chitin and some of its derivatives (2, 3, 18, 20), the question arose as to what extent the "chitinases" so far identified in the digestive tract of vertebrates were truly specific for chitin hydrolysis or were lysozymes with chitinolytic activity. It was observed that some pancreatic or gastric extracts of vertebrates with high chitinolytic activity were devoid of any significant lysozymic activity (6, 8). Moreover, Cornelius et al. (5) were able to purify a chitinase devoid of any lysozymic activity from the gastric mucosa extracts of a primate (Perodicticus potto). This enzyme showed both chitinolytic and lysozymic activities. It concluded that the chitinolytic enzymes observed in the digestive tract of vertebrates are "true" chitinases (E.C.3.2.1.14).

It is likely that the secretion of chitinases by some birds and mammals allows the digestion of chitin in the diet to some extent. The ability to digest chitin in vivo, however, has never been indicated. This ability will depend mainly on the chitinase concentration in the gut, on the optimum pH of these enzymes, on the physical and chemical state of the chitin provided with the diet, on the duration of the intestinal transit, and perhaps on the presence or absence of chitobiase (E.C.3.2.1.29). The aim of this paper is to sum up the quantitative data concerning the distribution and activity of chitinases and chitobiases in the gut of birds and mammals, and to bring out some preliminary experimental results dealing with chitin digestibility.

METHODS

The organs used were dissected, washed, dried on filter paper, weighed ("fresh tissue"), and then homogenized in a mortar with sand and distilled water. The suspension was allowed to stand overnight at 4° C, then centrifuged. The supernatant ("enzyme extract") was kept at -20° C until an enzyme assay could be made.

Owing to the low chitobiase concentration in most enzyme extracts of vertebrates, the chitinase activity was measured according to the method of Jeuniaux (14, 15). This method uses a "native" chitin suspension prepared from cuttlefish bones as a substrate. A 1 ml chitin suspension (5 mg/ml) was incubated in a 1 ml of buffer at pH 5.2 and 37° C with 1 ml of the enzyme extract and 1 ml of chitobiase solution (lobster serum diluted 10 x). The N-acetylglucosamine concentration was measured (23) and the chitinase activity expressed in µg of N-acetylglucosamine (N-AG) liberated x hr^{-1} x g^{-1} of fresh tissue.

The chitobiase activity was also estimated by the above method, using as a substrate a preparation of chitobiose obtained by hydrolysis of chitin with a purified chitinase (14, 15).

The chitin digestibility experiments were performed on mice (Mus musculus, "wild" strain C 57Br, 2-4 months old), chickens (Gallus gallus, 15 days old) and Japanese nightingales (Liothrix lutea, adults). The experimental

animals were fed at regular intervals and reared in individual cages (arranged to collect all the excrement). The mice were fed (for 3 days) a mixture of starch (56%), casein (28%), corn oil (16%), 16.5 g% of purified ground-shrimp chitin, and a gelatin solution as an excipient. The chickens were fed (for 5 days) a commercial food for poultry, with the addition of 4 g% of purified ground shrimp chitin. The Japanese nightingale was fed dead mealworm larvae (Tenebrio molitor) and milk (for 2 days). The excrement was collected during the nutrition experiments for 3 to 5 days, and then for 4 more days. It was then ground, treated with NaOH 2N at 100° C for 3 hours, washed and centrifuged. The residue was hydrolyzed by HCl 11N at 40°C for 30 hours (24). After neutralization, the glucosamine and N-acetylglucosamine concentrations were measured respectively by the methods of Levvy et al. (19) and Reissig et al. (23), in order to calculate the amount of chitin by using a correction factor.

RESULTS

The location and activity of chitinase in 10 species of birds are given in Table 1.

The enzyme extracts of the liver, duodenum and intestinal mucosa of birds showed at most only very low and questionable chitinolytic activities. Chitobiase activity was absent or very low in every case (less than 200 µg N-AG x h^{-1} x g^{-1} fresh tissue), except in the caecal contents of the chickens (1160 µg N-AG x h^{-1} x ml^{-1}).

The chitinase activity in the gastric mucosa and pancreas of some mammals is given in Table 2. Only positive results are given, except for the interesting negative data concerning man. In addition, no chitinase was found in the stomach or in the pancreas of the following mammal species: sheep (Ovis aries), rabbit (Oryctolagus cuniculus), guinea pig (Cavia porcellus), cat (Felis domesticus), stoat (Mustela erminea), ferret (Mustela furo), marten (Martes foina) and sloth (Choloepus hofmanni). The chitobiase activity was very low for the mouse, except in the caecal contents (1160-1364 µg N-AG x h^{-1} x ml^{-1}).

The values of chitin digestibility in vivo are shown in Table 3.

DISCUSSION AND CONCLUSIONS

In birds as in mammals, the distribution of chitinase secretion is not a matter of systematics, but is related to the nature of the usual diet of the species. Gastric chitinases were at least occasionally found in 8 bird species that are more or less insectivorous. No chitinase was found in two strictly grain-eating birds, the pigeon and the parrot.

The same was also true for mammals. Insectivorous and omnivorous species secrete chitinases in the digestive tract, while more specialized species, both carnivorous and herbivorous, do not. However, it has been demonstrated that in rodents the secretion of chitinase was not modified by the addition of chitin to the diet for 1 to 3 months (10).

Table 1. Chitinase in the Digestive Tract of Birds

Species	Activity: μg N-AG x h^{-1} x g^{-1} Fresh Tissue		
	Mucosa of Glandular Stomach	Pancreas	Reference
Passer domesticus (Sparrow)	8470-13360	0	13 and original
Erithacus rubecula (Robin)	4136	0	original
Liothrix lutea (Japanese nightingale)	5620-13280	0	13 and original
Sturnus vulgaris (Starling)	61560	209	original
Turdus merula (Blackbird)	3020	11	13
Corvus corone (Carrion Crow)	3680	0	original
Gallus gallus chicken	1350-4040*	0	original
adult	1780	0	13
Tyto alba (Barn Owl)	9820	0	original
Columba palumbus (Pigeon)	0	0	13
Psittacus erithacus (Parrot)	0	0	original

*Not completely free from muscular tissues

Table 2. Chitinase Activity in Gastric Mucosa and Pancreas of Some Mammals

Species	Activity: μg N-AG x h^{-1} x g^{-1} Fresh Tissues		
	Gastric Mucosa (fundus and pylorus)	Pancreas	Reference
Chiroptera			
Rhinolophus ferrum equinum	5180	0	13
Insectivora			
Talpa europaea	132-178	238-435	13 and original
Erinaceus europaeus	3350-7560	10	13
Suidae			
Sus domesticus	540-832	700-1200	13
Rodents			
Mus musculus			
young	2330 ± 139	0	10
adults	4155 ± 151	0	10
Rattus norvegicus	2254	0	10
Cricetus frumentarius	133	0	10
Carnivora			
Canis domesticus	252	0	4
Vulpes vulpes	1239	0	4
Primates			
Perodicticus potto	1245-5500	0	1
Cebus capucinus	4950	0	original
Homo sapiens*	0	0	original

*Three series of assays, with samples from 3 different individuals.

In birds, the only site of chitinase secretion seems to be the glandular stomach. The chitinase activity in the enzyme extracts of the gastric mucosa is often very high, especially in the starling. In Gallus gallus, the young chickens are as well equipped with gastric chitinases as adults are (Table 2). Chitin was actually digested by the young chickens (15 days old), when purified shrimp chitin was added to the normal food. The digestibility coefficient was 23.5-31.7% (Table 3). The chitin of dead mealworm larvae, without any previous treatment, was more easily digested by a Japanese nightingale (digestibility coefficient: 56.8%).

Chitinases are secreted by the gastric mucosa of different mammals and by the pancreas of the mole and pig. The chitinase activity in the gastric mucosa extracts was generally lower in mammals than in birds. The purified ground-shrimp chitin was digested to some extent by two mice (19% and 58.6% of the ingested chitin) in the experiment reported in Table 3, but was not digested at all by two other mice in a second experiment.

Table 3. Chitin Digestibility in Mice and in Two Species of Birds

Species	Specimen no.	Chitin Ingested (mg)	Chitin in Excrement (mg)	Chitin Digested %
Mus musculus (mouse)	1	457.5	189.4	58.6
	2	353.0	285.8	19.04
Gallus gallus (chicken)	1	2000.0	1530.0	23.5
	2	2000.0	1365.5	31.7
Liothrix lutea (Japanese nightingale)	1	656.15	283.34	56.8

It must be emphasized that no chitinase was found in man, neither in the fundic and pyloric mucosa nor in the pancreas. Gastric chitinases were found, however, in two species of primates.

Owing to the very low chitobiase concentration in the various parts of the digestive tract in all the species studied (with the exception of the caecal content in the chicken and in the mouse), the metabolic utilization of the hydrolytic products of chitin can be questioned. A study of the hydrolysis and absorption of chitobiose and chitotriose in the digestive tract of vertebrates is in progress in our laboratory.

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REFERENCES

1. BEERTEN-JOLY, B., A. PIAVAUX and M. GOFFART. 1974. Quelques enzymes digestives chez un Prosimien, Perodicticus potto. Comptes Rendus Soc. Biol. 168:140.

2. BERGER, L.R., and R.S. WEISER. 1957. The β-glucosaminidase activity of egg-white lysozyme. Biochim. Biophys. Acta 26:517.

3. CHARLEMAGNE, D., and P. JOLLES. 1972. The action of various lysozymes on chitopentose. FEBS Letters 23:275.

4. CORNELIUS, C., G. DANDRIFOSSE and Ch. JEUNIAUX. 1975. Biosynthesis of chitinases by mammals of the order Carnivora. Biochem. System. Ecol. 3:121.

5. CORNELIUS, C., G. DANDRIFOSSE and Ch. JEUNIAUX. 1976. Chitinolytic enzymes of the gastric mucosa of Perodicticus potto (primate prosimian): purification and enzyme specificity. Int. J. Biochem. 7:445.

6. CORNELIUS, C., and G. DANDRIFOSSE. 1977. Substrate specificity of the β-1,4-N-acetylglucosaminidase of vertebrates. Biochem. System. Ecol. 5. In press.

7. DANDRIFOSSE, G., E. SCHOFFENIELS and Ch. JEUNIAUX. 1965. Sécrétion de chitinase par la muqueuse gastrique isolee. Biochim. Biophys. Acta 94:153.

8. FÄNGE, R., G. LUNDBLAD and J. LIND. 1976. Lysozyme and chitinase in blood and lymphomyeloid tissues of marine fish. Marine Biology 36:277.

9. FRANKIGNOUL, M., and Ch. JEUNIAUX. 1965. Distribution des chitinases chez les mamifères rongeurs. Ann. Soc. Roy. Zool. Belg. 95:1.

10. FRANKIGNOUL, M., and Ch. JEUNIAUX. 1965. Effect of chitin on chitinase secretion by rodents. Life Sci. 4:1669.

11. HANSEN, N.E. 1974. Plasma lysozyme--a measure of neutrophil turnover: an analytical review. Ser. Haematol. 7:1.

12. JEUNIAUX, Ch. 1961. Chitinase: an addition to the list of hydrolases in the digestive tract of vertebrates. Nature 192:135.

13. JEUNIAUX, Ch. 1962. Digestion de la chitine chez les oiseaux et les mammiferes. Ann. Soc. Roy. Zool. Belg. 92:27.

14. JEUNIAUX, Ch. 1963. Chitine et Chitinolyse, un Chapitre de la Biologie Moléculaire. Masson, Paris.

15. JEUNIAUX, Ch. 1966. Chitinases. In: Complex Carbohydrates, Neufeld, E.F., and Ginsburg, V. (eds.), p. 644. Methods in Enzymology, vol. 8. Academic Press, New York.

16. JEUNIAUX, Ch. 1971. On some biochemical aspects of regressive evolution in animals. In: Biochemical evolution and the origin of life, p. 304. E. Schoffeniels (ed.). North Holland Publ. Co., Amsterdam.

17. JOLLES, P. 1969. Lysozymes: a chapter of molecular biology. Angew. Chem. 8:227.

18. JOLLES, P. 1973. From lysozymes to chitinases. Rhein. Westfal. Akad. Wissenschaften 227.

19. LEVVY, G.A., and A. McALLAN. 1959. The N-acetylation and estimation of hexosamines. Biochem. J. 73:127.

20. LUNDBLAD, G., and E. HULTIN. 1966. Human serum lysozyme (muramidase). I: Viscosimetric determination with glycol chitin and purification by selective adsorption. Scand. J. Clin. Lab. Invest. 18:201.

21. MICHA, J.C., G. DANDRIFOSSE and Ch. JEUNIAUX. 1973. Distribution et localisation tissulaire de la synthèse des chitinases chez les Vertébrés inférieurs. Arch. Internat. Physiol. Bioch. 81:439.

22. OKUTANI. K., and M. KIMATA. 1964. Studies on chitinolytic enzyme present in aquatic animals. III: Distribution of chitinase in digestive organs of a few kinds of aquatic animals. Bull. Jap. Soc. Sci. Fish. 30:574.

23. REISSIG, J.L., J.L. STROMINGER and L.F. LELOIR. 1955. A modified colorimetric method for the estimation of N-acetylamino sugars. Jour. Biol. Chem. 217:959.

24. RUPLEY, J.A. 1964. The hydrolysis of chitin by concentrated hydrochloric acid, and the preparation of low molecular weight substrate for lysozymes. Biochim. Biophys. Acta 83:245.