removed by the protease effect of papain but not by that of ficin. Removal of sialic acid by neuraminidase also had little effect. This site is similar to that described for porcine erythrocytes and the *Nemopanthus mucronatus* lectin[7] and for bovine erythrocytes and the *Amaranthus caudatus* lectin[8].

The presence of the lectin in the pollen of the plant as well as in the seeds of *Cannabis sativa* L. shows the synthesis of the protein to occur at a very early stage in the development of the plant. The lectin was also present in higher quantities in the pollen than in the seeds.

1 Bird, G.W.G., Ann. N.Y. Acad. Sci. *92* (1969) 129.
2 Sharon, N., and Lis, H., Science *177* (1972) 949.
3 Gold, E.R., and Balding, P., Receptor Specific Proteins. Excerpta Medica, Amsterdam 1975.
4 Khanna, A.K., and Schajpal, P.K., Vox Sang. *39* (1980) 44.
5 Small, E., and Cronquist, A., Taxon *25* (1976) 405.
6 Bachrach, V., Gurevitch, J., and Zaitschek, D., J. Immun. *78* (1957) 229.
7 Tumosa, C.S., Experientia *33* (1977) 1531.
8 Bird, G.W.G., Uhlenbruck, G., and Pardoe, G.I., Proc. 12th Int. Cong. Soc. Blood Transf., Moscow 1969; Karger, Basel 1971.

0014-4754/84/070718-02$1.50 + 0.20/0

Isolation and partial characterization of cuticular collagen from the parasitic nematode *Gaigeria pachyscelis*

P. Joshi, B.P. Singh and A.K. Jaiswal

Divisions of Biochemistry and Food Science and Parasitology, Indian Veterinary Research Institute, Izatnagar 243 122, U.P. (India), 20 January 1983

Summary. The cuticle from adult *Gaigeria pachyscelis* was isolated by solubilizing the internal tissues with sodium dodecyl sulphate (SDS) at 37 °C. Cuticular protein was extracted with guanidine-HCl and β-mercaptoethanol and purified by ammonium sulphate fractionation and DEAE-cellulose chromatography. SDS-polyacrylamide gel electrophoresis of purified protein revealed 2 polypeptides with apparent mol. wts of 58,000 and 74,000. As judged from their hydroxyproline content both of them are collagenous in nature. Results of gel filtration indicate that cuticular collagen exists in two forms, a non-associated form at low concentration and an associated form at high concentration.

Nematodes are surrounded by a tough cuticle, consisting of 3 layers[2,3], which, in parasitic forms, is important in their intricate relationship with the host[4]. At present little is known about the biochemical nature of the cuticle of most nematodes. *Ascaris* cuticle is composed of 3 genetically distinct collagenous polypeptides[5] and recently the presence of 2 genetically distinct collagen chains has been reported in *Caenorhabditis*[6].

The present study reports the isolation and partial characterization of cuticular collagen from the parasitic nematode, *Gaigeria pachyscelis* (Ancylostomatid), a hookworm of sheep and goat.

Materials and methods. Adult parasites, after extensive washing with distilled water, were incubated in 1% sodium dodecyl sulphate (SDS) at 37 °C overnight[7]. This treatment selectively solubilizes the internal tissues of the worm, as could be confirmed by light microscopy.

Isolated cuticles were extracted with 8 M guanidine-HCl and 2% β-mercaptoethanol at 4 °C overnight, and centrifuged at 10,000 × g for 30 min. The clear supernatant was dialyzed against 0.2 M NaCl-0.05 M Tris (pH 7.5)-0.1% β-mercaptoethanol and made 50% with respect to ammonium sulphate. Further purification of precipitates obtained by ammonium sulphate fractionation was carried out on a DEAE-cellulose column[8]. Gel filtration studies of purified collagen, at concentrations of 500 µg/ml and 5 mg/ml, were carried out on Sephadex G-200.

Relative amount of various polypeptides and their apparent molecular weights

Polypeptide	Hydroxyproline	Percentage	Molecular weight
1	Present	42	58,000
2	Present	57	74,000
3	Absent	1	79,000

Relative amount (expressed as percentage) of polypeptides was calculated from the areas of the bands after scanning the polyacrylamide gels.

Electrophoresis was performed on 5% polyacrylamide gels[9]. After staining, gels were scanned at 580 nm and the positions of the bands were compared with those of standard proteins for molecular weight determination. Gel portions containing the bands were cut, washed with distilled water and extracted with 0.2 M NaCl-0.05% SDS at 37 °C[10]. After centrifugation, the supernatant was dialyzed against distilled water and lyophilized. Hydroxyproline was estimated after hydrolysis of the protein with 6 N HCl at 110 °C for 24 h[11]. Hexosamine was determined as described by Ashwell[12].

Results and discussion. Because of the small size of the parasite, it is difficult to isolate the cuticle by physical means. The isolation of the cuticle by solubilizing the internal tissues with SDS is simple and highly satisfactory. Guanidine-HCl (8 M) and β-mercaptoethanol (2%) solubilizes 80–90% of the cuticle. The need for a reducing agent during solubilization indicates that disulphide bonds are probably involved in the organiza-

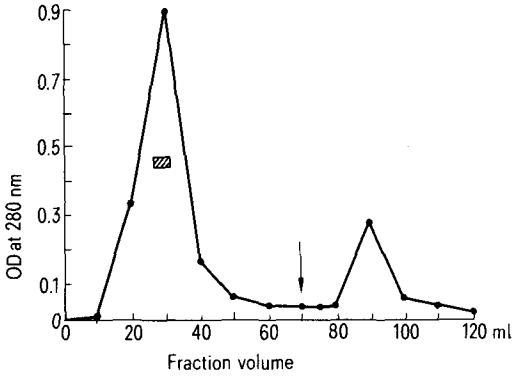

Figure 1. Ion exchange chromatography on DEAE-cullulose. Arrow indicates the elution of bound components (peak 2) with 1 M NaCl.

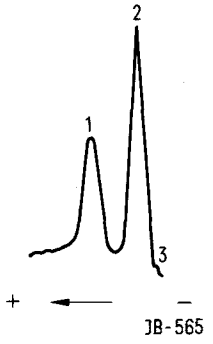

Figure 2. SDS-polyacrylamide gel electrophoresis of components not bound to DEAE-cellulose (peak 1, shadowed area of fig. 1).

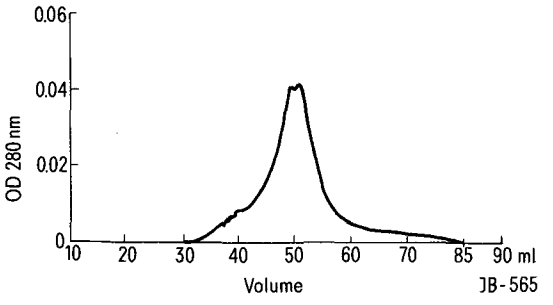

Figure 3. Gel filtration profile of cuticular collagen on Sephadex G-200 at high concentration (5 mg/ml). The protein was eluted as a single peak in the void volume.

tion of cuticle structure. Collagen does not bind to DEAE-cellulose under the experimental conditions used[8], and can be collected in the wash fraction (fig. 1, peak 1). The presence of hydroxyproline in the unbound fraction provides evidence that the protein part is collagen; it was entirely free of proteoglycans as judged by the absence of hexosamine. Bound fraction (peak 2) was non-collagenous as it failed to show the presence of hydroxyroline.

Electrophoresis of the collagen fraction revealed 2 major polypeptides with apparent mol. wts of 58,000 and 74,000 (fig. 2 and table). A 3rd, non-collagenous polypeptide was observed in many experiments. These results indicate the existence of at least 2 collagen polypeptides in the cuticle of *Gaigeria pachyscelis*. At a concentration of 5 mg/ml, the collagen was eluted in the void volume from the Sephadex column (fig. 3). The addition of a reducing agent (0.1% β-mercaptoethanol) had no effect on the elution pattern. At low concentration, (500 µg/ml), no protein could be observed in the void volume; it eluted much later from the column with a Ve/Vt value of 0.56. These results led us to believe that cuticular collagen in solution exists in two forms, a non-associated form at low concentration and an associated form at high concentration.

1 Acknowledgments. We thank Drs L. N. Singh and H. C. Tewari for providing the necessary facilities. Laboratory assistance of Mr Ram Kishore is highly appreciated.

2 Lee, D. L., in: Advances in Parasitology, vol. 4, p. 187. Ed. B. Dawes. Academic Press, New York 1966.
3 Bird, A. F., The structure of Nematodes, p. 45. Academic Press, New York 1971.
4 Mackenzie, C. D., Preston, P. M., and Ogilvie, B. M., Nature *276* (1978) 826.
5 Evans, H. J., Sullivan, C. E., and Piez, K. A., Biochemistry *15* (1976) 1435.
6 Ouazana, R., and Herbage, D., Biochim. biophys. Acta *669* (1981) 236.
7 Leushner, J. R. A., Semple, N. L., and Pasternak, J., Biochim. biophys. Acta *580* (1979) 166.
8 Miller, E. J., Biochemistry *10* (1971) 1652.
9 Weber, K., and Osborn, M., J. biol. Chem. *244* (1969) 4406.
10 Reiser, K. M., and Last, J. A., Analyt. Biochem. *104* (1980) 87.
11 Stegemann, H., Hoppe Seylers Z. physiol. Chem. *311* (1958) 41.
12 Ashwell, G., in: Methods in Enzymology, vol. 3, p. 73. Eds S. P. Colowick and N. O. Kaplan, Academic Press, New York 1957.

0014-4754/84/070719-02$1.50 + 0.20/0

A light and electron microscope study of spherical structures in the test cells of an ascidian *Ciona intestinalis* L.

C. Mansueto, G. D'Ancona Lunetta and L. Villa

Institute of Zoology, University of Palermo, Via Archirafi 18, I-90123 Palermo (Italy) and Institute of Histology, University of Palermo, Via Archirafi 20, I-90123 Palermo (Italy), 26 July 1983

Summary. Spherical structures in the test cells that surround the embryos of the ascidian *Ciona intestinalis* L. (Tunicata, phylum Chordata) were studied by both light and electron microscope. Our data support the view that these structures are microorganism-like cells living in symbiosis with the test cells. Their possible role is discussed.

The test cells surround the ascidian egg throughout embryonic development until the larval stage. They are found between the egg membrane and the follicular cells from which they are separated by the chorion. Their name is due to Kupffer[2] who maintained that their role was secreting the larvae tunic, a transparent, extracellular structure which covers the epidermis of the larva.

Although they have been under study for a long time, their function is still not clear. Many authors[3–15] agree that they furnish nutritional material to the oocyte cytoplasm during oogenesis, but their role in embryogenesis is unknown. It should be remembered that the egg develops normally after their removal. Mansueto and Villa[16] have demonstrated that they incorporate radioactive precursors of proteins and RNA during embryonic development. Moreover, the incorporation of (^{3}H)-thymidine at the end of development, i.e., when their life seems to be concluded, is a peculiar feature. In fact, many of them are lost as a result of larval movements, even though some are firmly attached to the tunic.

We have reconsidered the test cells in the light of these results. The present research is aimed at providing further information on the structure and function of these cells in *Ciona intes-*