Phagocytosis by amoebocytes in *Apostichopus japonicus*

Xing Kun,[1,2] Hongsheng Yang[1]

Abstract

The present study is a quantitative analysis of phagocytosis by amoebocytes of *Apostichopus japonicus*. Zymosan A was used as a marker. It was mixed *in vitro* with the coelomocytes and the rate of phagocytosis was analysed by counting cells under a light microscope. The phagocytic rates (expressed as percentages) of ingested zymosan A by amoebocytes were quantified when the marker was placed in contact with amoebocytes at different temperatures (4, 10, 15, 20 and 30°C) and for different periods of incubation (15, 45 and 60 min). It was found that the phagocytic activity varied in relation with the temperature and the exposure time. Amoebocytes are highly efficient at cleansing zymosan A.

Introduction

Most of the immune responses are performed by phagocytes (reviewed by Gross et al. 1999), and phagocytes may also function in the cellular encapsulation of foreign materials and microbes through clotting, in which the phagocytes intermesh with each other (Hillier and Vacquier, 2003). Characterisation of the coelomocytes of *A. japonicus* has been demonstrated directly by using a light microscope and an electron microscope (Eliseikina and Magarlamov 2002; Xing et al. 2008). Six types of coelomocytes were identified: lymphocytes, morula cells, amoebocytes, crystal cells, fusiform cells and vibratile cells (Xing et al. 2008). In echinoderms, there have been some quantitative analyses of phagocytes. Beck and Habicht (1993) demonstrated that *in vitro* phagocytosis in the sea star *Asterias forbesi* was positively correlated with incubation time. Xing and Chia (1998) used a flow cytometric method to study the quantitative characteristics of amoebocytes in *Holothuria leucospilota*; phagocytosis was abundant and positively correlated with the bead/cell ratio. The goal of the present study was to quantify the rate of phagocytosis of *A. japonicus* amoebocytes using zymosan A.

Materials and methods

Coelomic fluid collection

Healthy sea cucumbers *Apostichopus japonicus* (body length: 10 to 15 cm; weight: 100 to 150 g) were obtained from aquatic farms in Qingdao, Shandong Province, China. The sea cucumbers were transferred to the laboratory in a 500 L PVC tank supplied with 10°C sand-filtered and aerated seawater, which was renewed daily. All animals were acclimatised for at least four weeks prior to the experiments. About 5 to 10 mL of coelomic fluid (CF) were drawn from the right lateral side of the body (Santiago-Cardona et al. 2003) using a 25 gauge needle, and diluted with an equal volume of artificial seawater (ASW, pH 7.4); EDTA fixative (6×10^{-3} M EDTA, 0.01 M phosphate buffered saline [PBS], ASW, pH 6.0) (Noble 1970) was used as a disaggregating agent. The CF was stored in sterile 5 mL centrifuge tubes at 4°C.

Phagocytosis of zymosan A

The CF was placed in sterile 1.5 mL centrifuge tubes and then centrifuged at 2000 r min^{-1} for 10 min at ambient temperature. Zymosan A (BSA, Sigma — Aldrich, Steinheim, Germany) was then added to the extracted serum two hours prior to the phagocytosis assays at a concentration of 0.1 g mL^{-1} at ambient temperature. In order to study the phagocytosis of the zymosan A, 0.2 mL of filtered CF diluted with the sterile ASW (proportion 1:1) was extracted and mixed with the diluted activated zymosan A. The assays were performed at 4, 10, 15, 20 and 30°C for 15, 30 and 60 min in sterile centrifuge tubes. Subsequently, the percentage of phagocytising cells was estimated after observing 100 cells randomly in Opton image system at magnifications of 400x.

Results

Amoebocyte morphology

The amoebocytes possessed various pseudopodia radiated from the cytoplasm. All amoebocytes possessed petaloid or filiform pseudopodia (Figs. 1

[1] Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China, Email: xingkun84@gmail.com, corresponding author: hshyang@126.com
[2] Graduate School, Chinese Academy of Sciences, Beijing, 100039, China

and 2) that radiated in different directions from the central endoplasmic mass of the cells. Amoebocytes were capable of clotting with filiform pseudopodia (Fig. 2).

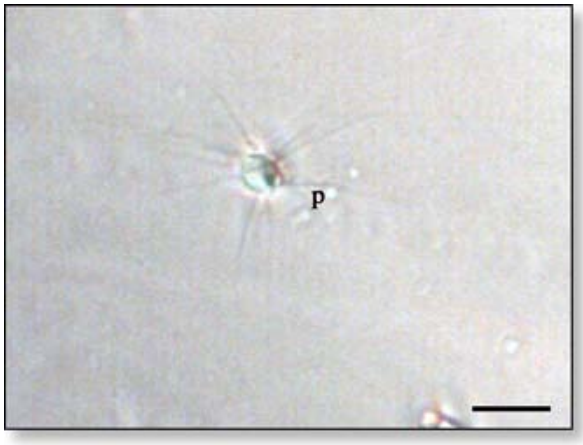

Figure 1. Light micrograph showing petaloid amoebocytes; p: pseudopodia (bar = 10 µm).

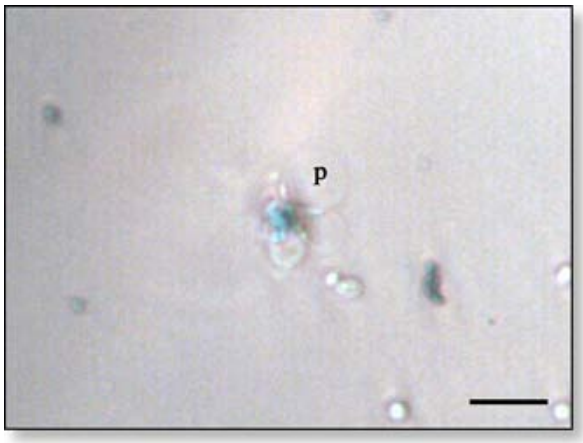

Figure 2. Light micrograph showing filiform amoebocytes; p: pseudopodia (bar = 10 µm).

Phagocytosis of zymosan A

Light microscopy revealed that amoebocytes showed a phagocytic response to the zymosan A (Fig. 3) when incubated in suspension. Amoebocytes were shown to be actively phagocytic. The results suggested that the phagocytic ability of amoebocytes was high: phagocytising cells represent from 30 to 70% of the counted cells. The relationship between the phagocytising ability and temperature was consistent (Fig. 4). The phagocytising ability was efficient when zymosan A was added and soon after the CF was extracted from the sea cucumber; after 1 hour the coelomocytes had clotted at 30°C.

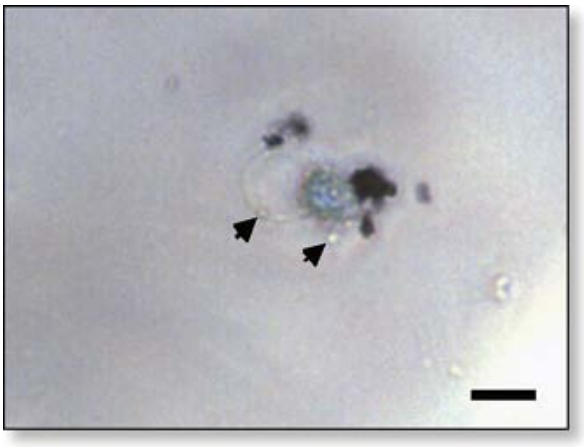

Figure 3. Light micrograph showing phagocytic responses to the zymosan A; arrowhead: zymosan A (bar = 5 µm)

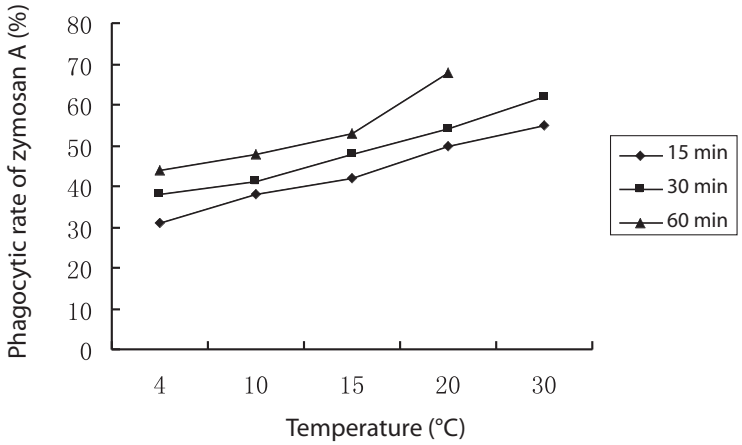

Figure 4. Quantitative analysis of phagocytic rate of zymosan A by amoebocytes in *Apostichopus japonicus*.

Discussion

Of the six types of coelomocytes of *A. japonicus*, the amoebocytes were the second most abundant cells, the first being the lymphocytes (Xing et al. 2008). Amoebocytes can take up dyes and other particles from the gut contents, and filiform amoebocytes participate in wound healing and clotting. On the whole, amoebocytes carry out echinoderm cellular immunity as they are capable of phagocytosis and nutrition and are active agents in the clotting reaction. Amoebocytes are recognised as containing heterogeneous materials of various sizes. These cells frequently have plenty of pseudopodia, and amoebocytes loading with pseudopodia often clump together. In the holothurian species studied, as well as in other echinoderms, the pseudopodia of the amoebocytes were petaloid and filiform (Edds 1993). The amoebocytes present the morphological changes that occur prior to and during cell aggregation. Careful observations of the fresh preparations reveal that all amoebocytes possess ectoplasmic pseudopodia that radiate in different directions from the central perinuclear cytoplasm of the cell. Petaloid-shaped phagocytes were capable of direct transformation into amoebocytes with filiform pseudopodia, but the transformation of filiform pseudopodia into petaloid pseudopodia has never been observed (Hetzel 1963). Amoebocytes were sensitive to induced stress. Amoebocytes also undergo a stress-induced petaloid-filopodial transition in response to UV-B radiation (Matranga et al. 2006). The changes of the petaloid pseudopodia into filiform forms were accelerated and the cell aggregation was apparent after the cells were mixed with zymosan A *in vitro*.

The quantitative study of phagocytosis showed that there is a consistent relationship between phagocytising ability and temperature. Both of the petaloid and filiform amoebocytes reacted; after phagocytosis the amoebocytes transformed from petaloid form to filiform form, other types of coelomocytes showed limited phagocytising ability. The amoebocytes showed the ability to agglutinate *in vitro*. In fresh preparations the petaloid pseudopodia appear to collapse and a redistribution of the cytoplasm produces branching filiform pseudopodia of considerable length, often intermeshing with the filiform pseudopodia of other amoebocytes. As the temperature increased and time elapsed, the amoebocytes clotted with morula cells and lymphocytes, which would reduce the effectiveness of phagocytosis.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (grant no. 40576073) and the National Key Technology Research and Development Program (grant no. 2006BAD09A02/09). Prof. Conand provided suggestions and assistance with this paper; we are grateful for her help and information.

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