THE MODE OF ACTIONS OF LYSOZYME AS AN IMMUNOGLOBULIN PRODUCTION STIMULATING FACTOR (IPSF)

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1. Abstract

Lysozyme derived from hen egg white stimulated immunoglobulin (Ig) production by human-human hybridoma, HB4C5 cells producing monoclonal IgM. IgM production by HB4C5 cells was enhanced more than 13-fold by lysozyme at 380 μg/ml in serum-free medium. The mode of actions of lysozyme as an Ig production stimulating factor (IPSF) was investigated. Lysozyme enhanced Ig production by transcription-suppressed HB4C5 cells. However, the enzyme was ineffective to accelerate IgM production by translation-suppressed HB4C5 cells. In addition, the intracellular IgM content of HB4C5 cells, which were suppressed post-transcription process, was obviously increased by the addition of lysozyme. These findings suggest that lysozyme accelerates translation process to enhance Ig productivity.

2. Introduction

We have attempted to stimulate Ig production of hybridomas and lymphocytes by modification of culture media supplements under serum-free conditions for effective mass production. Then, we screened the Ig production stimulating factor (IPSF) [1, 2]. As the result of screening, it was revealed that several basic proteins and poly-basic amino acids facilitated Ig production by human-human hybridoma, HB4C5 cells [3]. These findings urged us to inquire IPSF activity of other basic proteins. Finally, we found out that lysozyme had the IPSF activity against human-human hybridoma and human peripheral blood lymphocytes [4]. Lysozyme is a very simple and stable protein, and easily separated from antibodies in culture medium by gel filtration. Moreover, this enzyme is so cheap that we can use it for mass production of monoclonal antibodies. Therefore, we investigated the IPSF activity of lysozyme.

3. Materials and method

Human-human hybridoma HB4C5 cells producing monoclonal IgM were used for the assay of the IPSF activity of lysozyme from hen egg white. HB4C5 cells were fusion
product of a human lymphocyte from lung cancer patient and a human fusion partner, NAT-30 cells. HB4C5 cells were cultured in ERDF medium (Kyokuto Pharmaceutical, Japan) supplemented with 10 μg/ml of insulin, 20 μg/ml of transferrin, 20 μM ethanolamine and 25 nM selenite (ITES-ERDF) at 37 °C under humidified 5% CO₂-95% air. The IPSF activity was determined by measuring the amount of IgM secreted by HB4C5 cells in culture media. HB4C5 cells were inoculated in ITES-ERDF medium containing 380 μg/ml of lysozyme at 1x10⁴ cells/ml. For determination of the IPSF activity of lysozyme, the amount of IgM secreted in each culture medium was measured by enzyme-linked immunosorbent assay (ELISA).

4. Results and discussion

4.1. Effect of lysozyme on IgM production

Human-human hybridoma HB4C5 cells were cultured in ITES-ERDF medium supplemented with lysozyme at various concentrations for 6 h to investigate a dose-response effect of lysozyme. IgM production by HB4C5 cells was stimulated dose-dependently by the addition of lysozyme. Lysozyme facilitates IgM production more than 13-fold at ITES-ERDF medium. The enzyme immediately started to enhance IgM production soon after inoculation, and the effect was maintained for 5 days. Lysozyme, however, showed no significant growth promoting. This suggests that lysozyme stimulates specific IgM productivity of HB4C5 cells.

4.2. Correlation between IPSF and enzymatic activities of lysozyme

The first point for discussion regarding the mode of actions of lysozyme as an IPSF is whether the enzymatic activity takes part in its IPSF activity, or not. The time-course effect of trypsin digestion on the IPSF and enzymatic activities of lysozyme was investigated. Lysozyme was treated with 250 unit/ml of trypsin, and the digestion was terminated by the addition of 5000 units/ml of soybean trypsin inhibitor. As the result of that, the IPSF activity was lost in consequence of fragmentation. On the other hand, the enzymatic activity was stable against trypsin digestion and the fragments fully retained the enzymatic activity. The cleavage sites of trypsin on lysozyme do not affect the active center of the enzyme. Hence, trypsin treatment had no influence on lysozyme activity. Our previous data also indicated that lysozyme, which lost the enzymatic activity by boiling for 30 min, stimulated IgM the same as native enzyme [4]. These results mean that the IPSF activity is not derived from the enzymatic activity. Moreover, this fact suggests that the IPSF and enzymatic activities are independent, and the IPSF activity is a novel function of lysozyme.

4.3. The IPSF effect of lysozyme on transcription-suppressed HB4C5 cells

The IPSF effect of transcription-suppressed HB4C5 cells was investigated. Following actinomycin D (Act D) treatment, HB4C5 cells were cultured in ITES-ERDF medium supplemented with 380 μg/ml of lysozyme, and the amount of IgM in the medium was