Lactate dehydrogenase enhances immunoglobulin production by human hybridoma and human peripheral blood lymphocytes

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Abstract

Lactate dehydrogenase (LDH) derived from rabbit muscle enhanced IgM production by human–human hybridoma HB4C5 cells 12.4-fold at 320 $\mu\text{g/ml}$ under serum-free conditions. LDHs from pig muscle and pig heart also accelerated IgM production 8.4- and 6.4-fold, respectively. The immunoglobulin production stimulating activity of LDH was not accompanied by activation of cell proliferation. LDH from rabbit muscle facilitated IgM and IgG production by human peripheral blood lymphocytes. This means LDH stimulates immunoglobulin production not only by the specified hybridoma cell line, but also by unspecified immunoglobulin producers. LDH from rabbit muscle enhanced IgM production of transcription-suppressed HB4C5 cells treated with actinomycin D. The immunoglobulin production-stimulating factors (IPSFs) effect of LDH was slightly weakened by sodium fluoride (translation inhibitor) treatment of HB4C5. Moreover, the amount of intracellular IgM of monensin-treated HB4C5 cells was obviously enhanced by LDH. This result means that the IPSF effect of LDH is irrelevant to the post-translation activity of target cells. It is expected from these findings that LDH from rabbit muscle accelerates the translation step to enhance immunoglobulin productivity. The immunoglobulin production-stimulating activity of LDH was inhibited by colchicine, endocytosis inhibitor. This fact suggests that it is necessary for LDH to be taken by target cells to act as an IPSF.

Abbreviations: Act D – Actinomycin D; ELISA – Enzyme-linked immunosorbent assay; FITC – Fluorescein isothiocyanate; GAPDH – Glyceraldehyde-3-phosphate dehydrogenase; Ig – Immunoglobulin; IPSF – Immunoglobulin production stimulating factor; LDH – Lactate dehydrogenase; MAb – Monoclonal antibody; PBL – Peripheral blood lymphocytes; PBS – Phosphate buffered saline

Introduction

A monoclonal antibody (MAb) has high specificity to an antigenic substance. For this reason, MAbs are applied to various fields, such as medical applications and biochemical research. On the other hand, many researchers have attempted to improve the productivity of hybridoma cells for the sufficient supply of MAbs. There are two strategies for

the efficient production of MAbs. One is the high-density cultivation of hybridomas, and the other is the enhancement of cellular productivity by molecular biological and/or cell physiological ways.

Fetal bovine serum is a common supplement for animal cell culture, because the serum contains many kinds of stimulating factors. In addition to stimulating substances, there is some possibility that the serum from bovine involves the pathogenic

substances, such as prion protein that induces bovine spongiform encephalopathy. In the case of therapeutic usage of MAbs, the MAb-producing cells should not be cultured in serum-containing medium to avoid contamination of the final products with such pathogenic substances. However, the MAb productivity of hybridoma cells in serum-free media is obviously inferior to that in serum-supplemented media. To enhance cellular productivity, we have screened immunoglobulin (Ig) production-stimulating factors (IPSFs), which enhance Ig production by hybridoma cells and peripheral blood lymphocytes under serum-free conditions. As the result of investigations, several substances were identified as IPSF (Sugahara et al. 1995, 1997; Miyazaki et al. 1998).

Lactate dehydrogenase (LDH; EC 1.1.1.27) from rabbit muscle also enhanced antibody production of human hybridoma cell line, HB4C5 cells in serum-free medium. LDH is a tetramer of A and B subunits, and some isozymes exist in organism (Stolzenbach 1966). Sperm-specific LDH, which exists only in testis, plays important role in the immune system (Gupta and Chaturvedi 2000). In addition to enzymatic activity, LDH has the DNA binding property (Calissano et al. 1985; Grosse et al. 1986; Sharief et al. 1986; Kaiserman et al. 1989). Moreover, LDH is localized in nucleus of several cells (Cattaneo et al. 1985). It is supposed from these facts that the enzyme plays some roles in the regulation of cellular functions. However, there is no report concerning about such functions of this enzyme. Consequently, it is suggested that the regulation of antibody production activity of hybridomas is a novel function of LDH.

Here we report the characterization of LDH as IPSF, and the putative mode of actions as IPSF was also investigated.

Materials and methods

Materials

LDH derived from rabbit muscle was purchased from Oriental Yeast (Tokyo, Japan). LDH was dialyzed against 10 mM sodium phosphate buffer (pH 7.4) and sterilized by filtration before use. As the result of the SDS-PAGE analysis of this product, only one band derived from LDH was

detected. This means that the purity of this LDH preparation is high enough to investigate the IPSF activity of this enzyme. LDHs from pig muscle and pig heart were purchased from Roche Diagnostics (Germany).

Actinomycin D (Act D) and fluorescein isothiocyanate (FITC) were purchased from Sigma (MO, USA). Sodium fluoride (NaF), cycloheximide monensin, and colchicine were obtained from Wako Pure Chemistry (Osaka, Japan). Act D, monensin, and colchicine were dissolved in ethanol. Cycloheximide and NaF were dissolved in water.

Cells and cell culture

Human-human hybridoma HB4C5 cells producing lung cancer-specific monoclonal IgM were used for the assay of the IPSF activity. HB4C5 cell line was a fusion product of a human B lymphocyte from a lung cancer patient and a human fusion partner, NAT-30 (Murakami et al. 1985). HB4C5 cells were cultured in ERDF medium (Kyokuto Pharmaceutical, Tokyo, Japan) supplemented with 10 μ g/ml of insulin, 20 μ g/ml of transferrin, 20 μ M ethanolamine, and 25 nM selenite (ITES-ERDF) at 37 °C under humidified 5% CO₂-95% air (Murakami et al. 1982).

Human peripheral blood lymphocytes (PBL) were obtained from peripheral blood of a healthy donor as mentioned in a previous report (Sugahara et al. 1998a). After washing three times with ERDF medium, human PBL were pre-cultured in ERDF medium supplemented with 10% fetal bovine serum for 1 day to remove adhesion-dependent cells, such as monocytes and fibroblasts.

Assay of the IPSF activity of LDH

The IPSF activity of LDH was determined by measuring the amount of IgM secreted by HB4C5 cells in ITES-ERDF medium. HB4C5 cells were inoculated in ITES-ERDF medium supplemented with or without LDH at 1×10^5 cells/ml. After cultivation, the amount of IgM in each culture medium was determined by the enzyme-linked immunosorbent assay (ELISA) using anti-human IgM antibody (ICN Pharmaceuticals, USA), as mentioned in a previous report (Sugahara et al. 1994).

Assay of the enzymatic activity of LDH

LDH activity was determined in 100 mM potassium phosphate buffer (pH 7.0) containing 130 μ M NADH and 670 μ M sodium pyruvate (Stolzenbach 1966). Following enzyme reaction at 37 °C, absorbance at 340 nm was measured by Ultrospec 3000 spectroscope (Amersham Pharmacia Biotech AB, Sweden).

Inhibition of protein synthesis

Transcription activity of HB4C5 cells was suppressed by Act D, which irreversibly inhibits transcription activity (Reich et al. 1961). HB4C5 cells were treated with 4.0 μ g/ml of Act D for 2 h. Following Act D treatment, cells were washed twice by ERDF medium and inoculated at 1×10^5 cells/ml to examine the IPSF effect of LDH on transcription-suppressed HB4C5 cells.

Sodium fluoride and cycloheximide suppress protein synthesis by inhibiting the initiation complex formation and peptide chain elongation on ribosome, respectively (Colombo et al. 1965; Ravel et al. 1966). HB4C5 cells were pre-cultured for 6 h in ITES–ERDF medium containing 4.0 mM NaF or 10.0 μ g/ml of cycloheximide before the IPSF assay. After pre-cultivation, the cells were washed twice with ERDF medium and inoculated in ITES–ERDF medium with or without each inhibitor for evaluation of the IPSF activity of LDH under the translation-suppressed conditions. The inhibiting effects of these two inhibitors are reversible. Hence, the translation activity of the cell is recovered when the inhibitors are removed from the culture media.

Monensin was employed to suppress the post-translational process and secretion of HB4C5 cells. Monensin inhibits intracellular processing of newly synthesized proteins in the Golgi body (Oda et al. 1983). HB4C5 cells were pre-cultured for 6 h in ITES–ERDF medium containing 10.0 μ M monensin. After pre-cultivation, the cells were washed twice with ERDF medium and inoculated ITES–ERDF medium with or without monensin for the IPSF assay.

Investigation of the uptake of LDH by HB4C5 cells

Colchicine, which causes microtubule depolymerization, was used as an endocytosis inhibitor

(Biswas et al. 2000). Colchicine was dissolved in ethanol. Prior to investigation, HB4C5 cells were treated with 100 μ M colchicine for 6 h in ITES–ERDF medium to inhibit endocytosis.

LDH was directly labeled with FITC. LDH was treated with 1 mg/ml of FITC in 100 mM carbonate bicarbonate buffer (pH 9.0) at room temperature. After 2 h, FITC-conjugated LDH was separated from free FITC by PD-10 column (Amersham Pharmacia Biotech AB). HB4C5 cells treated with colchicine were inoculated in ITES–ERDF medium supplemented with FITC-conjugated LDH. After cultivation, the cells were washed three times with cold PBS, fixed at room temperature for 10 min with 99.5% of ethanol, and finally mounted on slides using Permafluor (Immunotech S.A., France) containing 50% of glycerol. The slides were visualized by Olympus IX70 inverted fluorescence microscope system (Japan) (Okamoto et al. 2001).

Results and discussion

The effect of LDH on IgM production by HB4C5 cells

Human–human hybridoma HB4C5 cells producing lung cancer-specific monoclonal IgM were used for the assay of the IPSF activity. HB4C5 cell line was a fusion product of a human B lymphocyte from a lung cancer patient and a human fusion partner, NAT-30 (Murakami et al. 1985). HB4C5 cells were inoculated in ITES–ERDF medium supplemented with various concentrations of LDH at 1×10^5 cells/ml. After cultivation for 6 h, the amount of IgM in each culture medium was measured by ELISA. As demonstrated in Figure 1, the Ig production-stimulating effect of LDH from rabbit muscle was observed above 2.6 μ g/ml, and the IPSF effect was increased in a dose-dependent manner. The amount of IgM produced by HB4C5 cells reached 129.1 ng/ml by the addition of 320 μ g/ml of LDH. The IgM production of the control medium was 10.4 ng/ml. This means that the IgM production of this hybridoma line was enhanced 12.4-fold by this enzyme. In addition to LDH from rabbit muscle, the IPSF activities of LDHs from pig muscle and pig heart were investigated. As the result of that, it was revealed that

Figure 1. The effect of LDH on IgM production by HB4C5 cells. HB4C5 cells were inoculated at 1×10^5 cells/ml in ITES-ERDF medium supplemented with various concentrations of LDHs from rabbit muscle (●), pig muscle (○) and pig heart (△), and cultured at 37 °C under humidified atmosphere of 5% CO₂-95% air. After 6 h cultivation, the amount of IgM in each culture medium was measured by ELISA. Results are represented as the mean $\pm$ SD of three independent measurements.

LDHs from pig muscle and pig heart also facilitated IgM production of HB4C5 cells 8.4- and 6.4-fold, respectively (Figure 1).

Time-course effect of LDH from rabbit muscle on IgM production was examined to characterize its IPSF effect. HB4C5 cells were inoculated at 1×10^5 cells/ml in ITES-ERDF medium with or without 500 µg/ml of LDH from rabbit muscle, and the amount of IgM produced in culture medium was measured at each culture period. As summarized in Figure 2, IgM production by HB4C5 cells was enhanced 11.0-fold at 24 h after inoculation, and the IPSF effect was maintained throughout 72 h. The IPSF effect was remarkable at the beginning of cultivation.

In contrast to the effect of LDH on Ig production, there was no significant effect on cell growth and viability (Figure 2B). Doubling time of HB4C5 cells during the entire cultivation period was 22.1 h in LDH-supplemented medium, and that in control medium was 22.8 h. This result means that Ig

production-stimulating effect is not accompanied by growth promotion. It is supposed from this fact that LDH contributes to stimulation of protein synthesis of HB4C5 cells to facilitate Ig production. This prediction is also supported by the findings that the IPSF effect is observed soon after inoculation.

Effect of LDH on Ig production by human PBL

Effect of LDH on Ig production by human PBL was examined under the serum-free conditions. Human PBLs were inoculated at 1×10^6 cells/ml in ITES-ERDF medium supplemented with 500 µg/ml of LDH from rabbit muscle, and cultured for 4 days. IgM production by human PBL was facilitated by LDH up to 3.1-fold as summarized in Table 1. The enzyme also stimulated IgG production by human PBL 1.9-fold. Cell proliferation and viability of human PBL were not affected by LDH (data not shown). These results suggest that LDH facilitates specific Ig productivity of Ig producing human PBL, as well as that of HB4C5 cells. This fact also suggests that this enzyme acts as IPSF, not only for the specified hybridoma cell line but also for unspecified Ig producers.

Correlation between the IPSF and enzymatic activities of LDH

The important point for discussion about the mode of action of LDH is whether the enzymatic activity takes part in its IPSF activity. The time-course effect of trypsin digestion of LDH on its IPSF and enzymatic activities was investigated. Rabbit muscle LDH was treated with 500 units/ml of trypsin for up to 60 min at 36 °C, and the digestion was terminated by the addition of 5000 units/ml of soybean trypsin inhibitor at various timing. Following trypsin digestion, each reaction mixture was evaluated for its IPSF activity. As shown in Figure 3, the enzymatic activity was gradually lost in consequence of fragmentation. In striking contrast to this result, the IPSF activity of this enzyme was enhanced about 2.0-fold at 10 min of digestion. This fact indicates that the IPSF and enzymatic activities are independent functions of LDH, and the IPSF activity is a novel function of this enzyme. This result also suggests that the IPSF activity was

Figure 2. Time-course effect of LDH on IgM production by HB4C5 cells. HB4C5 cells were inoculated in ITES-ERDF medium with (●) or without (○) 500 µg/ml of LDH at 1×10^5 cells/ml. After cultivation, the amount of IgM secreted in culture medium was measured by ELISA (A: IgM production), and the cell number and viability were determined using of hemocytometer after trypan blue staining (B: cell growth). Results are expressed as the mean $\pm$ SD of three independent measurements.

Table 1. The IPSF effect of LDH on Ig production by peripheral blood lymphocytes.

	Ig production (ng/ml)	
	IgM	IgG
Control	11.5 $\pm$ 0.7	62.0 $\pm$ 0.3
LDH	35.2 $\pm$ 3.6	120.4 $\pm$ 5.6

Human PBL were freshly obtained from peripheral blood of a healthy donor. After pre-culture, human PBL were inoculated at 1×10^6 cells/ml in ITES-ERDF medium supplemented with or without 500 µg/ml of LDH from rabbit muscle. The amount of IgM and IgG in the culture medium was measured by ELISA at 4 days after inoculation. Results are represented as the means $\pm$ SD of three independent measurements.

enhanced by partial digestion. It is expected that LDH has an enzymatic domain and AN IPSF domain. The enzymatic domain was easily cleaved by trypsin and the enzymatic activity was lost. On the other hand, the IPSF domain may be exposed

to target cells by partial digestion, and finally the domain was also destructed by further digestion.

The IPSF effect of LDH on transcription-suppressed HB4C5 cells

The effect of LDH on protein synthesis of HB4C5 cells was investigated. At first, the IPSF effect of LDH on transcription-suppressed HB4C5 cells was determined. HB4C5 cells were treated with 4.0 µg/ml of Act D for 2 h. Following this treatment, HB4C5 cells were inoculated in ITES-ERDF medium supplemented with 500 µg/ml of LDH.

As shown in Figure 4, IgM production by Act D-treated HB4C5 cells was less than 2/5 of control cells. This means that the inhibitor sufficiently suppressed the transcription activity of HB4C5 cells enough to determine the IPSF activity on transcription-suppressed HB4C5 cells. If LDH acts on

Figure 3. Effect of trypsin digestion of LDH on IPSF and enzymatic activities. LDH from rabbit muscle (1 mg/ml) was treated with 500 units/ml of trypsin at 36 °C. The trypsin digestion was terminated by the addition of 5000 units/ml of soybean trypsin inhibitor at various periods. HB4C5 cells were inoculated at 1×10^5 cells/ml and cultured for 6 h in ITES-ERDF medium supplemented with each reaction mixture. After cultivation, the amount of IgM in each culture medium was measured by ELISA. The data were indicated as relative IPSF (○) and enzymatic (●) activities against intact LDH. Results are represented as the means of three independent measurements.

the transcription process, the IPSF activity against Act D-treated cells would be reduced or disappeared. However, the IgM production of Act D-treated HB4C5 cells was obviously enhanced by LDH (Figure 4A). This result suggests that LDH acts on the post-transcription processes to enhance antibody production.

The IPSF effect of LDH on translation-suppressed HB4C5 cells

The IPSF effect of LDH on translation-suppressed HB4C5 cells was investigated. At first, the IPSF effect on HB4C5 cells treated with NaF was examined. NaF is a reversible inhibitor repressing the formation of the initiation complex of ribosomes and mRNA. After pre-culture with NaF, the hybridoma cells were inoculated in ITES-ERDF medium with or without 4.0 mM NaF to investigate the effect of LDH on translation-suppressed

conditions. As indicated in Figure 5, productivity of control cells was facilitated about 10.0-fold by 500 μ g/ml of LDH at the beginning of cultivation because of the lack of NaF. Compared with this fact, the IPSF effect of LDH on HB4C5 cells cultured in NaF-containing medium was reduced by half.

Cycloheximide was employed to suppress the peptide chain elongation of the translation process. After pre-incubation of HB4C5 cells with 10.0 μ g/ml of cycloheximide, the IPSF effect of LDH was examined in ITES-ERDF medium with or without 10.0 μ g/ml of cycloheximide. In spite of the co-existence with cycloheximide, the IgM production of the hybridoma cells was strongly enhanced by LDH (Figure 6).

The IPSF effect of LDH on HB4C5 cells suppressed post-translation activity

Monensin was used for the suppression of the post-translation process of HB4C5 cells. After pre-culture with 10 μ M monensin, HB4C5 cells were inoculated in ITES-ERDF medium supplemented with or without 10 μ M monensin to determine the IPSF effect of LDH at 500 μ g/ml under the inhibited condition. As shown in Figure 7, the IPSF effect of LDH on HB4C5 cells was obviously suppressed by monensin. The amount of IgM produced in the culture medium by monensin-treated HB4C5 cells was stimulated only 2.0-fold by LDH. The IPSF effect of LDH on control cells, that were cultured in the medium without monensin, was more than 8.0-fold. However, monensin suppresses secretion activity of the cell. Hence, it is necessary to investigate the intracellular IgM contents.

The intracellular IgM contents of HB4C5 cells treated with or without monensin were shown in Figure 8. A comparison of Figure 8A, B revealed that monensin treatment increased intracellular IgM contents in HB4C5 cells cultured in ITES-ERDF medium without LDH. This fact proves that monensin suppresses not the synthesis, but the secretion of IgM by HB4C5 cells. A comparison of Figure 8B, D suggests that the synthesis of IgM by HB4C5 cells treated with monensin was obviously facilitated by LDH. This evidence means that IgM synthesis in monensin-treated HB4C5 cells is facilitated by LDH, even though IgM secretion is suppressed by monensin

Figure 4. The IPSF effect of LDH on transcription-suppressed HB4C5 cells. HB4C5 cells were pre-cultured in ITES-ERDF medium supplemented with 4.0 $\mu\text{g/ml}$ of Act D for 2 h to suppress their transcription activity. After pre-culture, the cells were inoculated at 1×10^5 cells/ml in ITES-ERDF medium with (●) or without (○) 500 $\mu\text{g/ml}$ of LDH. (A) Act D-treated HB4C5 cells. (B) Control HB4C5 cells. IgM production is expressed as the mean $\pm$ SD of three independent measurements.

Figure 5. The IPSF effect of LDH on transcription-suppressed HB4C5 cells by NaF. HB4C5 cells were treated with 4.0 mM NaF for 6 h to suppress transcription activity. After treatment, the NaF-treated cells, were inoculated at 1×10^5 cells/ml in ITES-ERDF medium with (●) or without (○) 500 $\mu\text{g/ml}$ of LDH. (A) The effect of LDH under NaF suppression. (B) The effect of LDH under the absence of NaF suppression. The results are represented as the mean $\pm$ SD of three independent measurements.

Figure 6. The IPSF effect of LDH on transcription-suppressed HB4C5 cells by cycloheximide. HB4C5 cells were treated with 10.0 $\mu\text{g/ml}$ of cycloheximide for 6 h to suppress the transcription activity. After treatment, the cycloheximide-treated cells were inoculated at 1×10^5 cells/ml in ITES-ERDF medium with (●) or without (○) 500 $\mu\text{g/ml}$ of LDH. (A) The effect of LDH under co-existence of 10.0 $\mu\text{g/ml}$ of cycloheximide. (B) The effect of LDH under the absence of cycloheximide suppression. The results are represented as the mean $\pm$ SD of three independent measurements.

Figure 7. The IPSF effect of LDH on post-translation-suppressed HB4C5 cells. HB4C5 cells were pre-cultured for 6 h in ITES-ERDF supplemented with 10.0 μM monensin. After pre-cultivation, HB4C5 cells were inoculated at 1×10^5 cells/ml in ITES-ERDF medium containing monensin with (●) or without (○) 500 $\mu\text{g/ml}$ of LDH. (A) The effect of LDH under co-existence of 10.0 μM monensin. (B) The effect of LDH under the absence of monensin suppression. The results are represented as the mean $\pm$ SD of four independent measurements.

Figure 8. Intracellular IgM contents of monensin-treated HB4C5 cells. HB4C5 cells were pre-cultured for 6 h in ITES-ERDF supplemented with 10 μ M monensin. After pre-cultivation, HB4C5 cells were inoculated in ITES-ERDF medium with or without 500 μ g/ml of LDH. Following ethanol treatment, the cells were treated with FITC-conjugated anti-human IgM. The cells were visualized by Olympus IX70 inverted fluorescence microscope system. (A): monensin treatment (-), LDH (-); (B): monensin treatment (+), LDH (-); (C): monensin treatment (-), LDH (+); and (D): monensin treatment (+), LDH (+).

(Figure 7). We expected from all these results that LDH stimulates IgM synthesis by accelerating the translation process of the hybridomas. The precise step in the translation process at which LDH facilitation occurs should be uncovered.

The uptake of LDH by HB4C5 cells

Colchicine is a reversible endocytosis inhibitor by suppressing polymerization of microtubules (Biswas et al. 2000). The effect of LDH on HB4C5 cells treated with colchicine was investigated to reveal whether LDH acts as IPSF in the cell or not. Prior to the IPSF assay, HB4C5 cells were treated with 100 μ M colchicine for 6 h. After pre-incubation in colchicine-supplemented medium, these cells were inoculated in ITES-ERDF medium with or without colchicine to investigate the IPSF effect of LDH. As shown in Table 2, the

Table 2. The IPSF effect of LDH on IgM production by colchicine-treated HB4C5 cells.

Cultivation period (h)	IPSF activity	
	Colchicine-treated	Control
12–18	2.7	10.9
18–24	3.2	5.0

HB4C5 cells were treated with 100 μ M colchicine to suppress endocytosis activity. Following colchicine treatment, cells were inoculated at 1×10^5 cells/ml in ITES-ERDF supplemented with or without 100 μ M colchicine and 500 μ g/ml of LDH. IPSF activity represents the IPSF effect of LDH against IgM production in ITES-ERDF without this enzyme during each cultivation period.

IPSF effect of LDH was suppressed by the co-existence of colchicine. Moreover, the uptake of LDH by HB4C5 cells was determined by using FITC-conjugated LDH. As indicated in Figure 9,

Figure 9. The uptake of LDH by HB4C5 cells. HB4C5 cells were pre-cultured for 6 h in ITES-ERDF supplemented with 100 μ M colchicine. After pre-cultivation, HB4C5 cells were cultured in ITES-ERDF medium with 500 μ g/ml of FITC-conjugated LDH for 6 h.

the uptake of LDH was obviously decreased by colchicine treatment of the cell. According to these results, it is suggested that LDH is taken by target cells and acts as IPSF to enhance Ig production.

We have reported the substances that can enhance Ig production of hybridoma cells and lymphocytes. GAPDH and enolase were identified as IPSF (Sugahara et al. 1995, 1998a). The IPSF activities of these glycolytic enzymes were irrelevant to their enzymatic activities. Lysine-rich histone (H1, H2A, and H2B) and poly-lysine also stimulated IgM production by hybridomas (Sugahara et al. 1994). The common features of these proteins and peptides, including LDH, are molecular basicity and nucleic acid-binding property. We predicted that there were some relationships between IPSF activity and nucleic acid-binding activity. As expected, the IPSF activity of GAPDH was suppressed by the co-existence of DNA and polyribonucleotides (Sugahara and Sasaki 1998b). In addition, the IPSF effect of alcohol dehydrogenase was affected by co-existence of DNA (Okamoto et al. 1999). These findings suggest that the nucleic acid binding property of the proteins is strongly concerned with IPSF activity. All of the IPSFs described above stimulate transcription-suppressed HB4C5 cells. This means that these factors stimulate post-transcription process. However, the stimulating effects of each IPSF on translation-suppressed HB4C5 cells treated with

various inhibitors were different from each other. For instance, though the IPSF effect of lysozyme on NaF-treated HB4C5 cells was negligible, that of LDH was not. The mode of actions of IPSF may be uncovered by comparison of the effects of these IPSFs.

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