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ORIGINAL PAPER

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Production characteristics of interferon-α using an L-arabinose promoter system in a high-cell-density culture

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Abstract Using high-cell-density culture of *Escherichia coli* under the control of an L-arabinose promoter (P_{araB}), several factors affecting the production of recombinant protein and the formation of inclusion bodies were studied. The inducer, L-arabinose, showed a maximal induction level above 10.7 mM in the final concentration. The concentration of inducer also affected the partition of interferon-α (IFN-α) into the soluble form and inclusion bodies. Induction kinetics of the rate of accumulation of IFN-α on the P_{araB} promoter showed a slower rate than those of other promoter systems, for example T7, *lac* or *tac*. These innate characteristics of P_{araB} enabled cells to grow continuously in spite of the metabolic burden induced by the expression of foreign protein. The duration time of induction could control the expression of both soluble and insoluble protein. The ratio of yeast extract to glycerol (N/C ratio) in feeding media significantly affected both the production level of recombinant protein and inclusion body formation. The reason for decreasing specific bioactivity during induction can be explained by the increased proportion of inclusion bodies in the total expressed IFN-α.

Introduction

Escherichia coli has been popularly used as a host for the production of recombinant proteins because it is one of the most thoroughly studied microorganisms, regardless of the fact that it has many problems in the expression of complex or rather large proteins (Lee 1996). Many inducible promoter systems in *E. coli* such as *tac*, *lac*, *trp*, P_L, and P_R have usually been adapted for the commercial production of recombinant proteins. These are all strong promoters and thus promote protein synthesis abruptly, which may inhibit cell growth. Therefore, induction was executed generally after obtaining enough cell mass for high-cell-density culture. However, there was a 1–3 times difference in the order of magnitude in its expressed interferon-α (IFN-α) activity per liter of culture volume for each expression system (Fieschko and Ritch 1986; Lee et al. 1989; Miyake et al. 1985).

In contrast to the above promoters, a high level of expression tended not to arrest cell growth in the L-arabinose induction system (Guzman et al. 1995). This was a strong point for the mass production of recombinant proteins because high-cell-density culture was more accomplishable than with other promoter systems. The L-arabinose promoter is an inducible promoter related to pentose (L-arabinose) metabolism, as the *lac* operon is to lactose. The operon that related to L-arabinose metabolism was composed of three genes, namely *araB*, *araA*, and *araD* (*araBAD*), which regulate its transcription by the same promoter (P_{araB}). *araC* is a regulatory gene located beside the *araBAD* cluster. It is distinctive in that the translational product of *araC* can act as either an activator or repressor of P_{araB} according to environmental conditions unlike *lac* or *gal* repressors (Lee et al. 1981). If glucose was present above a certain concentration in the culture media, P_{araB} was repressed by the decrease in cAMP concentration, even with the addition of L-arabinose (Ullman and Danchin 1980). Because of the above distinguishing features, the use of P_{araB} was advantageous for studying the effect of specific genes by null mutation (Guzman et al. 1995; Murphy and Beckwith 1994; Phillips and Silhavy 1992) and was applicable for studying the sensitivity of specific genes to in vivo variation of regulator protein that can be varied by controlling the L-arabinose concentration in media (Chen and Newman 1998; Nishihara et al. 1998). There are several reports of using the L-arabinose promoter for the expression of different proteins, for example HB gene II protein, HIV-1 protease, leader peptidase, and Lpp-HMT fusion protein, although their expression

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levels were very low (Dalbey and Wickner 1985; Jacobs et al. 1989; Johston et al. 1985; Nilsson and von Heijne 1991; Taylor et al. 1992). Moreover, there were few reports about the application of the L-arabinose promoter to mass protein production, and there was no reports that made use of a high-cell-density culture using an L-arabinose promoter system. In this paper, we designed a fermentation process using L-arabinose for the mass production of IFN-α, and characterized its properties in high-cell-density culture. We applied the L-arabinose induction system to a dissolved oxygen (DO)-stat fed-batch culture under various conditions. For this purpose, the basic features of the L-arabinose promoter and proper media composition for cell growth and the expression of IFN-α were investigated.

Materials and methods

Strain

The expression vector under the control of P_{araB}, ΔpMA (KCCM-10054), was constructed in our Institute (Fig. 1) and treated with the restriction enzyme from *Nde*I-*Hind*III at a multi-cloning site before it was ligated with an INF-α gene. The resulting construct, ΔpMAIFNα (KCCM-10053), was transformed into *E. coli* MC1061 (F^-*araD139 Δ(ara-leu) 7696 galE15 galK16 Δ(lac)X74 rpsL (Str*^{r}*) hsdR2* ($r_k^- m_k^+$) *mcrA mcrB1*) that cannot metabolize L-arabinose as a carbon source (Son et al. 1994).

Culture

Seed culture was performed in a 500-ml baffled flask (with a culture volume of 100 ml) at 37 °C on a shaking incubator for 12 h. The main culture was carried out in a 2.5-l fermentor, BiofloIII (New Brunswick Scientific, USA) with an initial volume of 1.0 l. The composition of the initial culture medium used in DO-stat fed-batch culture was designed based on terrific broth (TB) (Sambrook et al. 1989). The feed media used weight ratios of 0.5, 0.75, and 1.0 of yeast extract: glycerol (N/C ratio) and totaled 630 g/l and 10 g/l of anhydrous magnesium sulfate. The pH was maintained at 7.0 using phosphoric acid (10%, v/v) for acid, and ammonia solution (10%, w/v) for base. A dissolved oxygen level was sustained at or above 20% by controlling the agitation speed and mixing the pure oxygen into the inlet air flow, using the computational command of Advanced Fermentation Software (New Brunswick Scientific). After all carbon sources in the initial culture medium were depleted, the feed medium was introduced into a fermentor repeatedly by a DO-stat fed-batch mode, which turned on the feed pump when the DO level increased above 50% of saturation and turned it off when the DO level decreased below 50%.

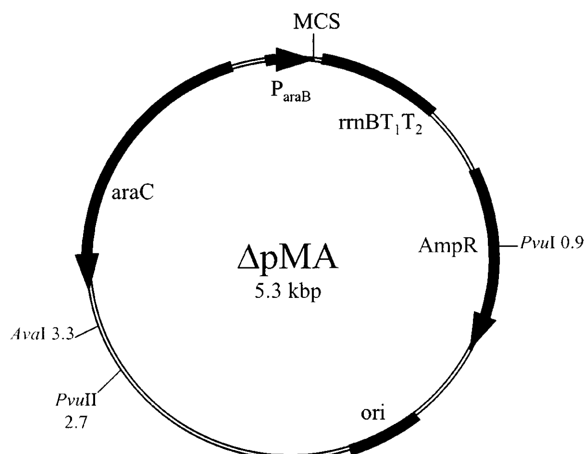

Fig. 1 Schematic diagram of vector, ΔpMA, for production of IFN-α. *MCS* Multi-cloning Site

Assay

IFN-α bioactivity was measured using a cytopathic effect-reduction assay (Familletti et al. 1981). To prepare samples for the assay of IFN-α bioactivity, recombinant *E. coli* containing soluble and/or the inclusion body form of IFN-α sampled at a certain time was dissolved with 8M guanidium hydrochloride and then diluted tenfold with Dulbecco's modified Eagle's medium (DMEM). This sample was applied to MDBK cells (ATCC CCL-22) spread previously on a 96-well plate (Costar, No. 3596, flat-bottom/tissue culture treated, USA). A vesicular stomatitis virus (VSV) was used to challenge MDBK cells that had been incubated with the sample for 15 h. After incubation at 37 °C in a CO_2 incubator for 12 h, the MDBK cells were stained with crystal violet and the absorbency at 550 nm read using a microplate autoreader (BIO-TEK Instruments, EL311, USA). Human recombinant α_2-interferon kindly donated by the National Institute of Allergy and Infectious Disease (NIAID) in the National Institutes of Health, USA (cat.no. Gx 01-901-535) was used as a reference.

The amount of expressed IFN-α was also measured by scanning the Coomassie blue-stained SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel of the total cell lysate followed by image analysis (Lim and Jung 1998). In gel scanning, the degree of overlap of IFN-α with other cellular proteins having similar molecular weight was not serious and was almost undetectable, in contrast to the band of IFN-α showing strong intensity after induction. Thus, it was not necessary for us to load total cell lysate before induction for an use as a negative control to SDS-PAGE gels.

Cell mass was observed using a spectrophotometer (LKB Biochrome, Ultrospec II, UK) at a wavelength of 600 nm. An inclusion body was prepared for running on SDS-PAGE by disrupting a lysozyme-treated cell with a sonicator (LAB-LINE Ultratip labsonic System, LAB-LINE Instruments, USA) for 1 min three times and then separating it into a soluble and insoluble fraction by centrifugation at 17,000 *g* for 30 min.

Results

High-cell-density culture using a DO-stat in a P_{araB} promoter system

To establish the high-cell-density culture of IFN-α-producing *E. coli*, the batch culture medium was first designed, based on TB. Batch cultures, in which the glycerol concentration of TB increased from 5 g/l to 40 g/l, were conducted to find the glycerol concentration at which the maximum cell growth did not increase further (Fig. 2). Above the glycerol concentration of 20 g/l, there was no further increase in the maximum cell growth of the batch culture; that is, some medium ingredient other than glycerol was nutritionally limiting. Therefore, TB was supplemented with M9 salts containing a glycerol concentration of 40 g/l to examine whether the maximum cell growth of a batch culture increased. Consequently, it was observed that the maximum cell growth increased the OD_{600nm} from 29.5 to 47.5. To confirm the nutrient balance of the TB supplemented

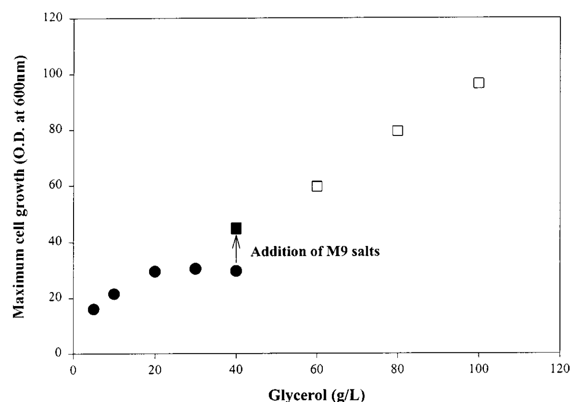

Fig. 2 The maximum cell growth in batch culture with various initial glycerol concentrations. At 5–40 g/l glycerol, batch cultures were carried out using terrific broth (TB, ●). At 40 g/l glycerol, batch culture was supplemented with M9 salts (indicated by *arrow* ■) At 60–100 g/l glycerol, all ingredients were proportionally increased as glycerol concentration increased (□)

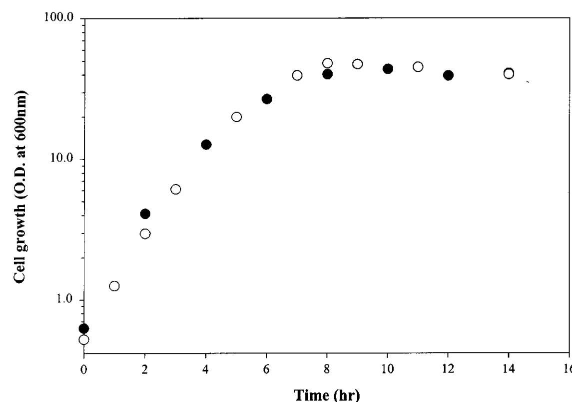

Fig. 3 Comparison of cell growth during batch culture with TB containing 40 g/l glycerol supplemented with M9 salts (●) and with yeast extract (○) as a substitute for M9 salts and TB, using only glycerol and phosphate buffer

with M9 salts (TB + M9), batch cultures were carried out with the ingredient concentrations of that medium proportionally increasing as the glycerol concentration increased to 60, 80, and 100 g/l. It was shown that, up to 100 g/l glycerol, the maximum cell growth in each experiment proportionally increased along the same interpolated line as the maximum cell growth obtained with TB containing a glycerol concentration of 5–20 g/l. Finally, it was demonstrated that balanced batch culture medium where the maximum cell growth could be controlled at OD_{600nm} 16.0–96.5 by changing the concentration of ingredients could be designed based on TB + M9. However, exceeding 40 g/l glycerol resulted in the production of acetic acid in an amount inevitably greater than 2.0 g/l (data not shown), and a supplement of pure oxygen during the batch culture was necessary to control the dissolved oxygen tension. Therefore, we decided that the optimal glycerol concentration was about 40 g/l in our culture conditions.

In general, the simplification of medium is convenient for large-scale cultivation. Because the yeast extract was known to contain inorganic salts and trace metals as well as amino acids and vitamins, the substitution of all ingredients of TB + M9, except for glycerol and phosphate buffer, was tried with yeast extract. As shown in Fig. 3, it was found that yeast extract was a good substitute in terms of maximum cell growth. In the case of a glycerol concentration of 40 g/l, the optimal concentration of yeast extract was 40 g/l (data not shown). Finally, we established a balanced batch culture medium that consisted of only three ingredients (glycerol, phosphate buffer, and yeast extract) for the initial batch culture period during the fed-batch culture.

To prepare feed medium for the fed-batch culture, concentrated feed medium with concentrations of glycerol and yeast extract at 315 g/l and 315 g/l, respectively, was designed by using the same ratio of yeast extract and glycerol in the balanced batch culture medium. Magnesium sulfate was added to the feed medium to minimize precipitation with potassium phosphate salts in the batch culture medium (Yee and Blanch 1993).

Using an initial concentration of 40 g/l glycerol and yeast extract with phosphate buffer, DO-stat fed-batch culture was carried out, as shown in Fig. 4. The oxygen limitation observed at a cell density with OD_{600nm} at 25 was overcome by the addition of pure oxygen mixed into the inlet airflow manually. Induction was executed at the early exponential growing phase in a batch culture period with excess L-arabinose (43.2 mM) (Hwang et al. 1992). Cell growth was not severely inhibited after induction and reached a cell density with OD_{600nm} about 160. Acetic acid accumulated slightly towards the end of the batch period but was fully consumed before the feeding in DO-stat mode was started. During the DO-stat fed-batch culture, little acetic acid was detected.

The effect of L-arabinose concentration on IFN-α production and inclusion body formation

To evaluate the saturated concentration of inducer for IFN-α production using P_{araB} promoter, five consecutive

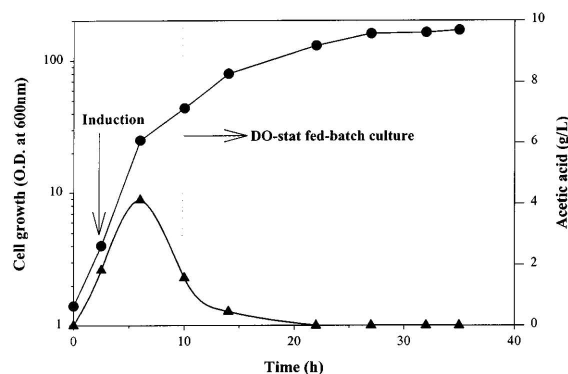

Fig. 4 Time profiles of cell growth with initial glycerol concentraion of 40 g/l in high-cell-density dissolved oxygen (DO)-stat fed-batch culture

high-cell-density DO-stat fed-batch cultures were performed, each induced at the same point in the early exponential growing phase, with 2.7, 5.4, 10.8, 21.6, or 43.2 mM L-arabinose in the culture. After 8 h fermentation time, all the carbon sources in the batch culture medium were exhausted and the dissolved oxygen level in the fermentor started to increase. The induction lasted for 22 h. In all experiments, the maximum content of IFN-α was achieved at around 10 h after induction and this level was sustained for another 12 h while cells were growing. All cultures reached nearly the same final cell density with OD_{600nm} about 160. There was an increase in the IFN-α production (the percentage of IFN-α to total cellular protein) with concentrations of L-arabinose up to 10.8 mM. Above a concentration of 10.8 mM L-arabinose, IFN-α production did not increase. At a concentration of 2.7 mM L-arabinose, IFN-α production reached only about 30% of the full induction level accomplished by using 10.8 mM L-arabinose (Fig. 5A).

To understand the effect of L-arabinose concentration on inclusion body formation, the cells induced with L-arabinose were ruptured and then separated into a soluble part and inclusion bodies. The proportion of inclusion bodies in the total induced IFN-α was estimated by image analysis of a scanned SDS-PAGE gel containing the total cell extract separated into a soluble form and inclusion bodies. As shown in Fig. 5A, the total induced IFN-α increased with increasing L-arabinose concentration. The percentage of IFN-α formed as inclusion bodies also increased with increasing inducer concentration, as shown in Fig. 5B. With 2.7 mM L-arabinose, about 80% of the total induced IFN-α was produced as a soluble form although the proportion in the total cellular protein was very low, less than 5%. With increasing L-arabinose concentration, production of IFN-α resulted in the accumulation of a large amount of IFN-α as inclusion bodies, more than 30% of the total cellular protein.

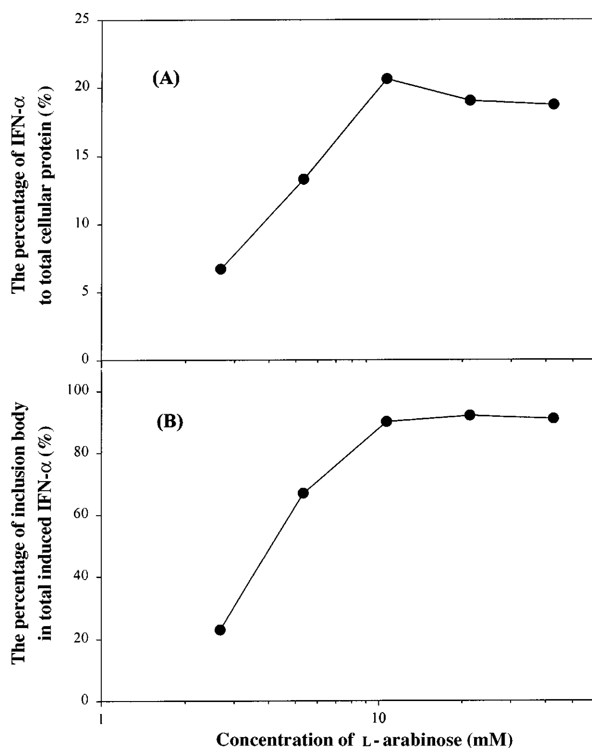

Fig. 5 Effect of L-arabinose concentration on the maximum content of IFN-α in the cell (**A**) and the percentage of inclusion bodies in total induced IFN-α (**B**) in a high-cell-density culture

Kinetics of IFN-α production and inclusion body formation

Three identical DO-stat fed-batch cultures were carried out using 10.8 mM L-arabinose to verify the induction kinetics (Fig. 6). For the first four sampling points, the specific production of IFN-α increased linearly just after induction, and thus it could be calculated that the maximum specific production rate was 3.6 mg OD^{-1} l^{-1} h^{-1}. After that point, the specific production of IFN-α sustained its maximum level, about 0.04 g l^{-1} OD^{-1}, throughout the induction period, which lasted over 25 h.

To analyze the time course of the partition of INF-α into the soluble and inclusion body forms, cells were disrupted and separated into soluble (S) and insoluble (I) parts. Each sample prepared from cells at the same OD_{600nm} was loaded into SDS-PAGE gel. The amount of IFN-α expressed as a soluble form or as inclusion bodies was calculated by scanning the band intensity. As shown in Fig. 7A and B, within 5 h after induction, over 90% of IFN-α was expressed as a soluble form. After 9 h, the band of soluble IFN-α (S2) was thicker than the band at 5 h (S1), as was the insoluble band (I2 and I1). At 16 h, the soluble part of IFN-α disappeared, whereas almost all IFN-α was shown in the form of inclusion bodies. Both L-arabinose concentration and the elapsed

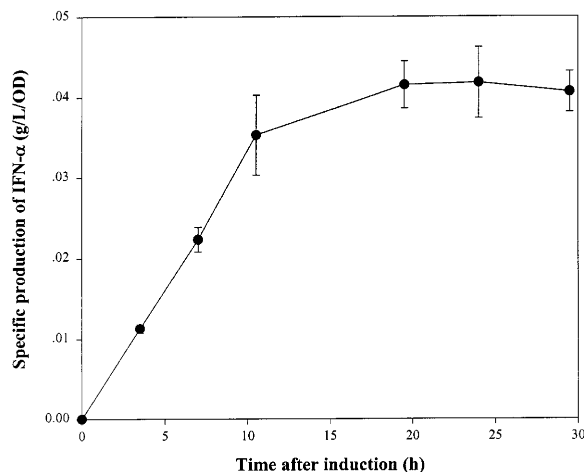

Fig. 6 Induction kinetics of IFN-α under the control of P_{araB} promoter using 10.7 mM of L-arabinose in the final concentration in a high-cell-density fed-batch culture of *Escherichia coli*

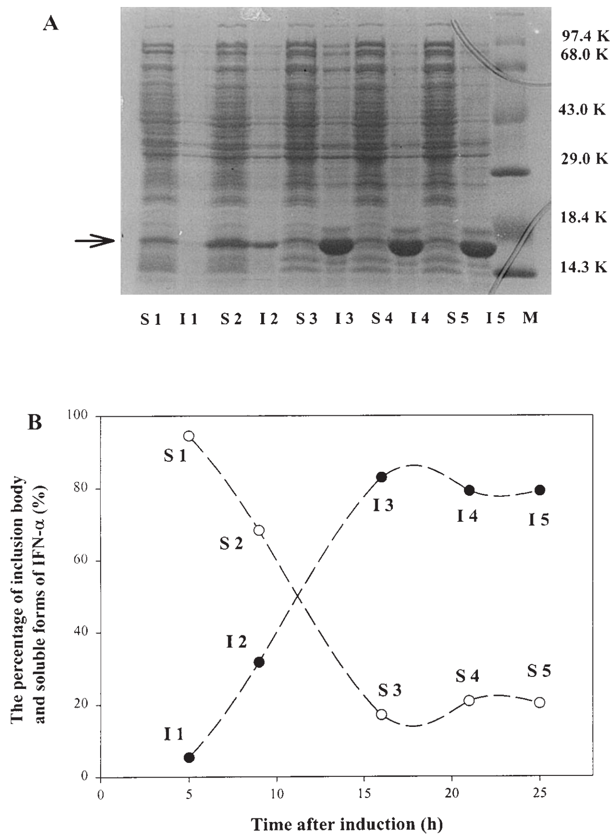

Fig. 7 A Time course of SDS-PAGE of soluble part (*S*), and inclusion body (*I*). *Arrow* indicates the expressed INF-α. Protein molecular weight standards, lysozyme, β-lactoglobulin, carbonic anhydrase, ovalbumin, and phosphorylase B were shown at the right side of the lane *M* with their reported molecular weights. **B** Percentage of inclusion bodies and soluble form of IFN-α in total induced IFN-α. Points depicted as *S1*, *S2*, *S3*, *S4*, *S5*, *I1*, *I2*, *I3*, *I4*, and *I5* are the same as the samples marked *S1*, *S2*, *S3*, *S4*, *S5*, *I1*, *I2*, *I3*, *I4*, and *I5*, respectively in **A**

time for induction jointly affected the formation of inclusion bodies.

The effects of the ratio of yeast extract to glycerol on IFN-α production and inclusion body formation

The ratio between yeast extract and glycerol (N/C ratio), which influences the cost of media for high-cell-density culture, was investigated for its effects on IFN-α production, inclusion body formation, and bioactivity of the expressed IFN-α. As shown in Fig. 8, three sets of experiments employed three different N/C ratios: 1.0, 0.75, and 0.5. Whereas the final cell mass and cell growth rate were not affected by the N/C ratio (Fig. 8A–C), the specific production and specific production rate of IFN-α were severely affected, especially at the N/C ratio of 0.5 (Fig. 8D–F). When the N/C ratio dropped to 0.5, the maximum level of specific production of IFN-α dropped to about half that for the N/C ratios 1.0 and 0.75, and the specific production rate also decreased.

The percentage of inclusion bodies in the total induced IFN-α was proportional to the N/C ratio (Fig. 8J–L). At the N/C ratio of 0.5, the rate of inclusion body formation also decreased. Although analysis by SDS-PAGE showed that IFN-α was produced at a level of 25% of the total cellular protein after induction, the specific bioactivity of IFN-α (IU/g IFN-α) in cells containing both soluble and inclusion body forms abruptly decreased before the maximal content of IFN-α in the cell was achieved. This discrepancy between expression level analyzed by bioactivity (IU/l) and by SDS-PAGE (g/l) was due to the preponderance, as induction proceeded, of a proportion of inclusion bodies that was biologically less active, being partially refolded, when it was assayed on anti-viral activity. As shown in Fig. 8G–I, at the initial point of induction, the specific bioactivity of the IFN-α produced showed a specific activity similar to the reference IFN-α (indicated by a dotted line) regardless of the N/C ratio. However, as the proportion of inclusion bodies increased, specific bioactivity decreased sharply below the value of the specific activity of the reference α_2-interferon. In our assay conditions, bioactivity was assayed with total cell lysate dissolved with 8M guanidium hydrochloride. Consequently, the refolding process of inclusion bodies proceeded only when the sample was diluted with DMEM, which resulted in the partial refolding of inclusion bodies and the exhibition of a lower specific activity of IFN-α than that of both the soluble form of IFN-α expressed during the initial part of induction and the international reference α_2-interferon.

Discussion

Complex media containing mainly yeast extract was generally used for studying the high-cell-density culture of recombinant *E. coli* because it contained numerous trace metals and vitamins in addition to amino acids. An abundance of yeast extract was advantageous in suppressing the degradation of recombinant protein expressed (Yoon et al. 1994). Glycerol was preferable to glucose as a carbon source in that it was readily sterilizable combined with a nitrogen source, and there was no need to be concerned about catabolite repression of P_{araB}. Additionally, using glycerol instead of glucose, which has a high potentiality for forming acetic acid when oxygen is limited, was advantageous for controlling the accumulation of acetic acid. In accompaniment with rich media, the DO-stat feeding mode was adopted because it was practical and feedback-controllable. This resulted in the carbon source concentration being kept very low. Consequently, the specific growth rate during DO-stat fed-batch culture was lower than 0.1 h^{-1} (Fig. 4), which could result in the repression of acetic acid formation.

In our conditions, however, the apparent saturated L-arabinose concentration lay above 10.8 mM. That was rather high compared to the L-arabinose concentration for saturating, subsaturating, or optimal induction in

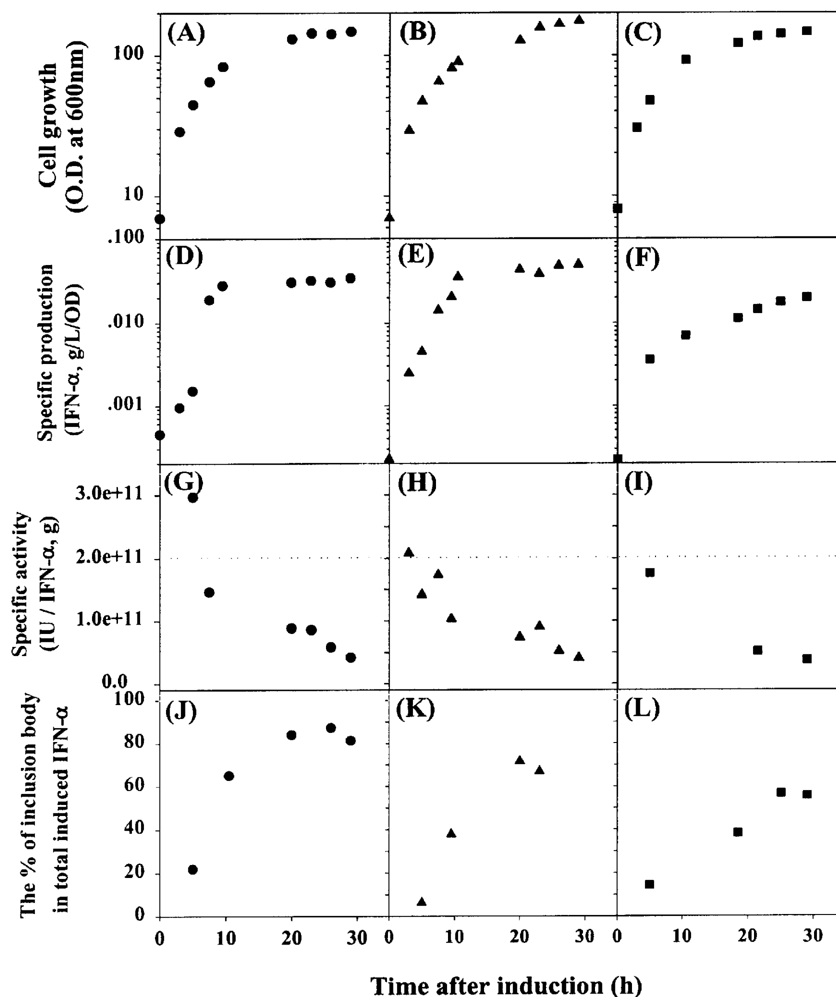

Fig. 8A–L Time profiles of cell growth using feeding medium with N/C ratios of 1.0 (**A**), 0.75 (**B**), 0.5 (**C**); the specific production of IFN-α with N/C ratios of 1.0 (**D**), 0.75 (**E**), 0.5 (**F**); the specific bioactivity of IFN-α with N/C ratios of 1.0 (**G**), 0.75 (**H**), and 0.5 (**I**); and the percentage of inclusion bodies in total induced protein with N/C ratios of 1.0 (**J**), 0.75 (**K**), and 0.5 (**L**). *Dotted line* indicates specific activity of the reference, human recombinant α_2-interferon donated by NIAID. The specific activity of the reference was known to be 2.0×10^{11} IU/g protein, using a cytopathic effect-reduction assay with MDBK cells and vesicular stomatitis virus (VSV) as a challenge

other reports, in which minimum and maximum concentrations of L-arabinose were 0.1 and 1.3 mM, respectively (Guzman et al. 1995; Johnson and Schleif 1995; Siegele and Hu 1997; Vandien et al. 1997). IFN-α expression plasmid (ΔpMAIFNα) contained araC, which can act as a repressor of the promoter P_{araB} without an inducer, or as an activator of transcription by conformational change upon binding with L-arabinose. Therefore, the required amount of L-arabinose for derepression of araC is theoretically proportional to the cell mass. From that point of view, a higher concentration of L-arabinose (10.8 mM) as an inducer, showing a plateau of IFN-α production, was not surprising in that the cell density during induction in this study was considerably higher than that of previous studies. Thus, finding the optimal amount of L-arabinose at any cell concentration was important for maximizing protein production in a high-cell-density condition.

In our results, almost all IFN-α was expressed as inclusion bodies. As shown in recent studies, it seems that the formation of inclusion bodies is not always undesirable for industrial applications. The formation of inclusion bodies has important advantages like yielding the highest fraction of target protein, ease of isolation as an efficient first step in purification, and protection from proteolytic degradation.

The increase in the formation of inclusion bodies at a high L-arabinose concentration seemed to be due to the accumulation of partially folded intermediates because, with increasing IFN-α production, the folding process could be disrupted by a shortage of critical factors, such as chaperones (Mitraki and King 1989; Rinas 1996; Rinas et al. 1992). Therefore, the soluble expression of IFN-α with P_{araB} can be modulated by the amount of the inducer L-arabinose during the production process. One review states that the proper folding and cellular location of the expressed protein were significantly influenced by the level of isopropyl β-D-thiogalactopyranoside used for optimal induction of the *lac* promoter (Donovan et al. 1996). As another example, there is a report that the partially induced condition using a reduced inducer concentration enhanced the activity of subtilisin E secreted to the periplasm (Takagi et al. 1988). Our results also suggested that the proper folding and solubility of IFN-α benefited from the lower transcription rate resulting from a lower level of araC as activator, due to the low concentration of L-arabinose, when low L-arabinose concentration was used.

There were several reports of the P_{araB} promoter in expression kinetics. However, their results differed because of differing experimental objectives and conditions. Alkaline phosphatase expression levels traced by pulse-labeled with ^{35}S-methionine after the addition of L-arabinose increased for up to 10 min under the control of the P_{araB} promoter (Guzman et al. 1995). Using in vivo induction kinetics, P_{araB} gave a level of messenger RNA of approximately ten molecules per cell as early as 30 s after induction and reached a maximum level after 5 min (Johnson and Schleif 1995). Siegele and Hu (1997) demonstrated that reaching maximum expression level took about 5 h. In our results, the specific production of IFN-α reached a maximum level within 10 h. At 10 h after induction, almost all the cells were transformed to a fully induced state, and the induction kinetics in individual cells differed from the overall expression in high-cell-density culture (Siegele and Hu 1997). Because there are no reports related to the expression of heterologous protein using P_{araB} promoter in a high-cell-density culture, it was difficult to compare our experiments with others. However, the different situations of cells during induction, such as high cell density, DO-stat feeding, L-arabinose concentration for titration of L-arabinose to araC protein, repressor, and time taken for a population of non-expressed cells to become fully expressed cells may produce a slow rate of IFN-α expression under our culture conditions. This slow rate of expression of heterologous protein seems to lessen the burden on cell metabolism, so that cell growth was hardly arrested, as compared to other high-level expression systems. In contrast to our previous report that using a P_L promoter with various feeding strategies always resulted in the specific production rate falling below zero within 5 h, which means that a degradation of a produced recombinant protein rapidly occurred after the explosion of expression (Lim and Jung 1998), the specific production of IFN-α sustained its maximum level over 25 h when P_{araB} promoter was used.

In addition to L-arabinose concentration, the elapsed induction time also influenced the partition between the soluble form and inclusion bodies. Soluble IFN-α produced during the early part of induction seemed either to be degraded by the action of protease or to be transformed into insoluble aggregate by the hydrophobic interaction of folded intermediates. It was known that aggregation is derived from specific partially folded intermediates and not from mature natives; and there are many studies of both the refolding of denatured proteins in vitro, and of in vivo folding in maturation pathways (Mitraki and King 1989). Soluble expression was possible by controlling the duration of induction as well as by inducer concentration, although the high level of intracellular soluble expression could not be achieved under our culture conditions.

The expression level and rate under the control of P_{araB} can be controlled by the N/C ratio. It is thought that the inhibitory effect of yeast extract on the degradation of expressed IFN-α was one of the reasons for the high level expression at a high N/C ratio (Yoon et al. 1994). In our previous study, we demonstrated that the addition of a specific amino acid or the addition of complex rich compounds after induction was effective in improving protein expression (Lim and Jung 1998).

The L-arabinose promoter system was successfully applied to mass production of the heterologous protein, IFN-α, using a high-cell-density culture. Although the L-arabinose promoter is not as commonly used as *lac*-derived promoter systems, there are some differences in production during induction that may sometimes act advantageously during gradual production. Therefore, fermentation using the L-arabinose induction system may be proposed as an alternative for the mass production of heterologous proteins.

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