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Effects of mutations altering SOS regulation on a nalidixic acid-inducible system for the production of heterologous proteins in *Escherichia coli*

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SUMMARY

The major leftward early promoter of phage λ p_L, has frequently been used to drive expression of heterologous genes in *Escherichia coli*. p_L is typically maintained fully repressed by the lambda cI protein. When induction of heterologous protein synthesis is desired, one of several potential mechanisms of destroying cI function is employed and the expression of the foreign gene commences. One method of derepressing p_L involves exposing cells to nalidixic acid, which results in the "activation" of RecA protein and the subsequent RecA-mediated proteolytic cleavage of cI. Activated RecA also mediates the cleavage of the *E. coli* LexA protein, resulting in induction of the SOS regulon (at least 15 *E. coli* genes, including *recA*). We have examined the effect of two chromosomal mutations on the productivity of nalidixic acid inductions. One of the tested mutations (*recA* o) increased the intracellular concentration of RecA prior to induction; the other (*lexA*ind$^-$) resulted in a mutated lexA protein insensitive to RecA-mediated cleavage. These mutations were introduced into a strain carrying a cI$^+$ defective lysogen. Synthesis of two heterologous proteins, human α1-antitrypsin and a fusion protein partially derived from the *Plasmodium falciparum* circumsporozooite surface antigen, was examined in the wild-type and mutant strains. The maximum α-1 antitrypsin concentration achieved was improved by 50% when the *recA* o strain was used rather than the wild type; however; only smaller changes (20% or less) in the maximum concentration of the malaria fusion protein were observed. Use of the *lexA*ind$^-$ strain resulted in a decrease in the maximum concentration attained for both heterologous products.

INTRODUCTION

Exposure of *Escherichia coli* to agents which damage DNA or interfere with DNA replication results in a range of physiological effects, collectively referred to as the SOS response (for reviews, see Little and Mount [17], Walker [34], and Walker [35]). One intensively studied aspect of the SOS response is the induction of temperate lambdoid prophage [24]. Upon treatment of cells with an SOS-inducing agent, the *E. coli* RecA protein is converted to an activated form in response to an unidentified intracellular signal [38]. Prophage induction results from the activated RecA-mediated proteolytic cleavage of the phage repressor protein (in the case of λ, the cI protein) [24,25]. In addition to mediating the cleavage of phage repressors, activated RecA also mediates the cleavage of the *E. coli* LexA protein, which acts as a repressor for at least 15 chromosomal genes, including *lexA* and *recA* [34].

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The SOS regulatory apparatus has been utilized in an inducible system for the expression of heterologous proteins in *E. coli* [14,22,29]. This system makes use of the λ p_L promoter for expression of genes of interest. In most p_L-based expression vector systems, the plasmid carrying the heterologous gene (downstream for p_L) is maintained in a cell expressing a thermolabile cI protein [26,29,30]. When expression of the product is desired, the thermolabile cI protein is rendered non-functional by an increase in the culture temperature. Although this type of temperature-inducible system has allowed high-level accumulation of many heterologous proteins, the levels of expression of some proteins in the thermally induced system have been extremely low. A subset of these proteins can be expressed at higher levels if activated RecA-mediated proteolytic cleavage, rather than a temperature shift, is used to remove cI function (refs. 22, 22, 29; and A. Shatzman, personal communication). While a variety of SOS-inducing agents could conceivably be used to induce this system, nalidixic acid has been used most frequently because it is inexpensive, can be used practically at large scale, and is less hazardous than many of the other potential agents. Furthermore, in several direct

comparisons with other inducing agents, nalidixic acid has provided the highest product yields (ref. 22; and A. Shatzman, personal communication).

Treatment of cells with nalidixic acid results in many physiological effects. Some of these effects, including cell filamentation and cessation of respiration, are manifestations of the SOS response [17,34]. However, nalidixic acid treatment elicits some physiological changes which are not typically observed in cells exposed to other types of SOS-inducing agents. These changes include the immediate cessation of DNA synthesis [2,10,37], a reduction in the level of negative supercoiling of the chromosome [20], changes in the levels of transcription from some promoters (including a negative effect on λ p_L) [16,20,27], alterations in membrane permeability [6], and induction of the heat shock response [15].

As part of ongoing investigations to determine which aspects of cell physiology might impact the productivity of this complex induction system, we have examined the effects of two mutations that alter specific aspects of SOS regulation. One of the mutations examined is an alteration in the operator of the *recA* gene, which results in an increased basal level of RecA [33]. Cells carrying this *recA* o mutation have an increased intracellular concentration of RecA in the absence of SOS-inducing treatments [9]. In experiments with a ϕ80 p_L-*trp* fusion, an increased pre-induction concentration of RecA has been shown to decrease the length of time before the onset of induction and to increase the maximum extent of induction caused by some chemical inducers [31]. However, in other studies it was found that an increased pre-induction concentration of RecA did not sensitize λ prophage to induction by low UV doses [23]. It was therefore of interest to determine what effects, if any, the *recA* o mutation would have on the productivity of this nalidixic acid inducible system.

While it seemed possible that an increased initial concentration of RecA might improve the productivity of this nalidixic acid inducible system, it had yet to be established whether the post-induction derepression of *recA* and other LexA-controlled genes was in fact required for optimal productivity in our system. Previously, it has been shown that prophage induction can occur in *lexA* ind$^-$ strains, which produce an altered LexA protein insensitive to activated RecA-mediated cleavage [23,28], and in cells where amplification of RecA has been prevented through other means [1]. We have therefore examined the effect of a host *lexA* ind$^-$ mutation on the productivity of nalidixic acid inductions.

The effects of these mutations on expression of two heterologous proteins of potential commercial interest were examined and compared in these studies. For both of these proteins, higher yields were obtained in the nalidixic acid induction system than in a thermally induced system (ref. 14; and D. Wareheim, personal communication). One of these proteins was human α1-antitrypsin. α1-Antitrypsin is a protease inhibitor which acts against neutrophil elastase [3], and reduced levels of α1-antitrypsin activity in vivo have been linked to pulmonary emphysema [7,8]. The other product whose expression was examined was RC1, a fusion protein containing 32 copies of the *Plasmodium falciparum* circumsporozoite major surface protein repeat unit (ref. 39; and M. Gross and J. Young, personal communication). This protein represents a potential component of a human malaria vaccine.

MATERIALS AND METHODS

Plasmids. The construction of pOTSα8, which contains the coding sequence for full-length human α1-antitrypsin downstream from the λp_L promoter and *c*II ribosome binding site, has been described [32]. This plasmid also carries an ampicillin resistance determinant. The coding sequence for the malaria antigen RC1 has been introduced into a similar vector (M. Gross and J. Young, personal communication); this construct is referred to as pRC1.

Bacterial strains. The bacterial strains used in this work are listed in Table 1. For long term storage, frozen stocks of these cultures were prepared by suspending exponential phase cells in 10% glycerol, 20% skim milk (in water), freezing in a controlled rate freezer, and storing under liquid nitrogen.

Media and buffers. The medium for all fermentations was 2XSB, which contained the following (g/l): tryptone (Difco Laboratories; Detroit, MI), 24.0; yeast extract (Difco), 48.0; glycerol, 26.0; K_2HPO_4, 15.3; KH_2PO_4, 1.7. The flask seed medium, 1XSB, contained the following (g/l): tryptone, 12.0; yeast extract, 24.0; glycerol, 13.0; K_2HPO_4, 15.3; KH_2PO_4, 1.7. The pH of both media was adjusted to 7.0 prior to sterilization. Ampicillin was prepared as a 5 mg/ml stock, filter sterilized, and added after the medium had cooled. Phosphate buffered saline (PBS) contained, per liter: NaCl, 8.0 g; KCl, 0.2 g; Na_2HPO_4, 2.16 g; KH_2PO_4, 0.2 g.

Construction of bacterial strains. P1 transductions were carried out by the method of Miller [21]. P1*vir* was obtained from E. Witkin. Tetracycline was used for selection at a concentration of 35 μg/ml. Selection of spontaneous tetracycline-sensitive mutants of *Tn* 10-carrying stains was performed by the method of Maloy and Nunn [19]. Presence of the *lexA* ind$^-$ allele was confirmed by screening potential transductants for very high sensitivity (as compared to the *lexA*$^+$ parent) to killing by UV light. The *lexA* ind$^-$ allele used in these studies was *lexA*102,

TABLE 1

Bacterial strains

Strain	Genotype	Source/reference
N99	*galK*	lab collection
IV55	*lexA* 102, *malE*::Tn10, *uvrA* 155, *trp*, *lon*, *sfiA*	E. Witkin
MV2	*recA* o 281, *srl*::Tn10, *uvrA* 155, *trp*, *lon*, *sfiA*	E. Witkin
AR120	N99, λ, Φ (P_L-*lacZ*) lysogen, *nadA*::Tn10	Mott et al. [22]
MFG001	N99, *lexA* 102, *malE*::Tn10	P1 transduction from IV55
MFG002	N99, *lexA* 102, *malE*	Tetracycline-sensitive isolate from MFG001
MFG003	N99, *recA* o281, *srl*::Tn10	P1 transduction from MV2
MFG005	N99, *recA* o281, *srl*	Tetracycline sensitive isolate from MFG003
ECO277	MFG002, λ Φ (P_L-*lacZ*) lysogen, *nadA*::Tn10	P1 transduction from AR120
ECO278	MFG005, λ Φ (P_L-*lacZ*) lysogen, *nadA*::Tn10	P1 transduction from AR120

originally isolated and characterized by Hill and Simson [12] as the *exrA* lesion in strain B_{s-2}. Presence of the *recA*o281 (*recA* o; ref. 33) allele was confirmed by preparing P1 stocks on potential *srl*::*Tn*10 *recA* o transductants, transducing into a *lexA* ind$^-$ strain, selecting for tetracycline-resistant transductants, and screening for a high frequency of cotransduction (greater than 50%) between tetracycline-resistance and increased UV resistance. The basis for this screen is the observation that *recA* o *lexA* ind$^-$ strains are more UV resistant than otherwise isogeneic *recA*$^+$ *lexA* ind$^-$ strains [9]. Presence of the defective lambda lysogen was confirmed by screening for resistance to infection by *imm*$^\lambda$ phage.

Seed development for fermentations. Baffled 2.8-l Fernbach flasks containing 1 l of 1XSB with 50 μg/ml ampicillin (Sigma Chemical Co., St. Louis, MO) were inoculated with 100 μl of thawed cell stock. These cultures were then incubated 14–16 h at 37 °C with 200 rpm shaking in a Gyrotory shaker (Model G-26, New Brunswick Scientific, Inc., Edison, NJ).

Nalidixic acid inductions. All induction experiments were performed in 15-l, stirred-tank fermentors (LSL Biolafitte, Inc.; Princeton, NJ) containing 9.5 l of 2XSB with 50 μg/ml ampicillin and 0.05% polypropylene glycol 2000 (Dow Chemical Co; Midland, MI). The fermentors were inoculated from the shake-flask seeds to an optical density (650 nm) of 0.5. Culture absorbance was monitored with a Gilford Stasar II spectrophotometer (Gilford Instrument Co; Oberlin, OH); samples were diluted to give an optical density (650 nm) of 0.02 to 0.5. The fermentors were under direct digital control (D/3 system, Texas Instruments; Hunt Valley, MD). This system controlled temperature, aeration rate, and agitation rate. pH was not controlled. The temperature, back pressure, and aeration rate were maintained at 37 °C, 7 PSIG, and 5 SLPM, respectively. Dissolved oxygen concentration was monitored with a polarographic oxygen sensor (Ingold Electronics, Wilmington, MA). The agitation rate was initially set to 300 rpm. As growth of the culture progressed, the dissolved oxygen concentration was allowed to fall to 20% of saturation. The dissolved oxygen concentration was then controlled at 20% by automatic adjustment of the agitation rate. When the culture attained an optical density of 4.0, nalidixic acid (sodium salt; Sigma) was added to the final concentrations specified.

Processing of culture samples. Cells withdrawn from the culture were collected by centrifugation in a Microfuge B (Beckman Instruments, Inc.; Fullerton, CA), 15,000 × *g*, 1.5 min. Supernatants were decanted, and the excess medium was then removed from the pellets by blotting with a cotton swab. The pellets were stored at −20 °C.

Determination of α1-antitrypsin and RC1 concentration. α1-Antitrypsin levels were determined by a particle concentration immunofluorescence assay as previously described [5]. RC1 concentration was determined by a similar assay, utilizing monoclonal antibodies directed against the *P. falciparum* peptide repeat unit (M.D. Summers, D. Wareheim, D. Vannicolo, R. Wistar, and Z. Jonak; submitted). In all cases, product yield is defined as the fraction of total cell protein that is the product, and product concentration refers to the amount of product per volume of culture.

Determination of total cell protein. For determination of total cell protein, cell pellets were lysed by heating at 100 °C for 10 min in PBS with 0.5% SDS. Protein concentration was then determined by the method of Lowry et al. [18] using an automated robotic system [4].

RESULTS

Effects of the recA o and lexA ind$^-$ mutations on the production of α1-antitrypsin

In previous work, we have examined the effects of several parameters (including culture medium, biomass concentration at the time of nalidixic acid addition, and

concentration of nalidixic acid used) upon the production of α1-antitrypsin in 15-liter fermentors (R. Gerber, W.B. Okita, B. Del Tito, A. Shatzman, D. Zabriskie, and E.J. Arcuri, manuscript submitted for publication). To further explore factors which affect the productivity of these inductions, we investigated the effect of host *recA* o and *lexA* ind$^-$ mutations. These mutations were introduced by P1 transduction into the host strain (AR120) which had been used for all previous studies. The plasmid carrying the α1-antitrypsin gene (pOTSα8) was then transformed into the *recA* o strain (ECO278) and the *lexA* ind$^-$ strain (ECO277), and nalidixic acid inductions in these strains were compared to inductions in the *recA*$^+$ *lexA*$^+$ parent carried out under the conditions previously identified as optimal (see MATERIALS AND METHODS).

The growth of these cultures before and after nalidixic acid addition is shown in Fig. 1. Before the addition of nalidixic acid, the growth rates of the *recA* o, *lexA* ind$^-$, and *recA*$^+$ *lexA*$^+$ strains were indistinguishable. However, the maximum biomass concentration attained after nalidixic acid addition varied in the three strains. The *recA* o strain reached a maximum optical density 15% higher than that reached by the *recA*$^+$ *lexA*$^+$ strain, while the *lexA* ind$^-$ strain reached a maximum optical density 30% lower than that obtained in the control culture (Fig. 1; Table 2). These differences, although small, were highly reproducible; furthermore, similar relationships were obtained from nalidixic acid inductions of other heterologous products in these strains (Table 2; also see below).

The fact that the total cell protein concentration (per volume of culture medium) measurements followed the same trend as the optical density measurements indicates that the differences observed actually reflected changes in biomass concentration, as opposed to effects of cell morphology related differences on light scattering (Table 2). The cell morphology of these three strains during induction is fairly similar (data not shown). The similarity in cell morphology between the *lexA*$^+$ and *lexA* ind$^-$ strains is somewhat surprising, given that the cell division inhibitor

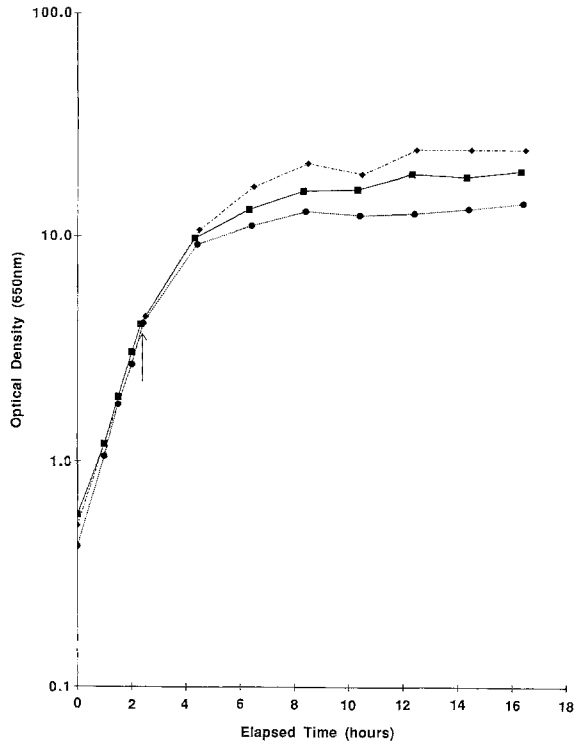

Fig. 1. Growth of AR120/pOTSα8 (—■—), ECO277/pOTSα8 (—●—), and ECO278/pOTSα8 (—♦—) before and after the addition of 165 μg/ml nalidixic acid. The arrow indicates the time of nalidixic acid addition. The data shown are derived from one representative experiment.

FtsA is the product of a LexA-controlled gene [13]. Interestingly, we have previously observed considerably less filamentation in cultures treated with nalidixic acid at relatively high cell density (as in the fermentor studies shown here) than in cultures treated at lower cell density (e.g., in shake flasks), although some increase in cell length is still detectable in high cell density induced cultures (R. Gerber, W.B. Okita, B. Del Tito, A. Shatzman, D. Zabriskie, and E.J. Arcuri, submitted). The apparent dampening of the filamentation response in high cell den-

TABLE 2

Results of nalidixic acid induction of α1-antitrypsin synthesis in wild-type and mutant strains

Host strain	Relevant genotype	OD650 at induction	Final OD650	Total cell protein (μg/ml)	α1-antitrypsin concentration (μg/ml)	α1-antitrypsin yield mg α1AT/mg TCP
AR120	wild type	4.03 ± 0.03	19.3 ± 0.15	5318 ± 135	240 ± 7.50	0.045 ± 0.003
ECO277	*lexA* 102	4.18 ± 0.05	13.9 ± 0.98	3935 ± 465	116 ± 9.20	0.030 ± 0.007
ECO278	*recA* o281	4.08 ± 0.22	22.4 ± 1.10	6803 ± 452	370 ± 20.0	0.055 ± 0.001

All data are from culture samples taken 10 h after the addition of nalidixic acid. Standard errors of the mean are derived from replicate experiments.

sity cultures may explain why some cell morphology differences that might have been expected to be dramatic (for example, between the *lexA* ind$^-$ and *lexA*$^+$ strains) were not observed. However, it is also possible that physical differences between the fermentor environment and shake flask conditions (where extent of filamentation has historically been compared) could also account for the absence of some of the expected effects.

The effects of these mutations on intracellular accumulation of α1-antitrypsin are shown in Fig. 2. In the first 4 h of induction, product accumulation proceeded at a similar rate in the AR120 and ECO278 hosts. After 4 h of induction in the *recA*$^+$ *lexA*$^+$ strain, the product yield (the fraction of the total cell protein that is the product) did not increase. However, the yield of α1-antitrypsin continued to increase in the *recA* o strain until a maximum was reached 8 h after the addition of nalidixic acid. This continued accumulation resulted in a 20% higher maximum product yield in the *recA* o strain as compared to the *recA*$^+$ strain. The *lexA* ind$^-$ strain displayed very different kinetics of product accumulation than either of the *lexA*$^+$ strains. A distinct lag in product accumulation was evident in the *lexA* ind$^-$ host. The maximum rate of intracellular α1-antitrypsin accumulation in the *lexA* ind$^-$ background occurred between 2 and 6 h of induction, after which the rate of accumulation fell off sharply. Product yield in the *lexA* ind$^-$ host always remained below the levels reached in the other two strains (Fig. 2; Table 2).

The results obtained from examining production of α1-antitrypsin in these host strains are summarized in Table 2. After 10 h of induction, the α1-antitrypsin concentration (the amount of α1-antitrypsin per volume of culture medium) was 50% higher in the *recA* o culture than in the *recA*$^+$ control. This improvement in the productivity of the fermentation was the combined result of the smaller increases in product yield and maximum biomass concentration obtained in the *recA* o strain. On the other hand, the maximum product concentration reached in the *lexA* ind$^-$ strain was 50% lower than that obtained in the *recA*$^+$ *lexA*$^+$ parent. Again, this effect on the product concentration was the combined result of smaller changes in the final biomass concentration (30% lower in the *lexA* ind$^-$ strain) and the maximum product yield attained (also 30% less in the *lexA* ind$^-$ host). The differences in product yield, product concentration, and final biomass in these strains were all statistically significant (pooled variance *t*-test, $P \leq 0.05$).

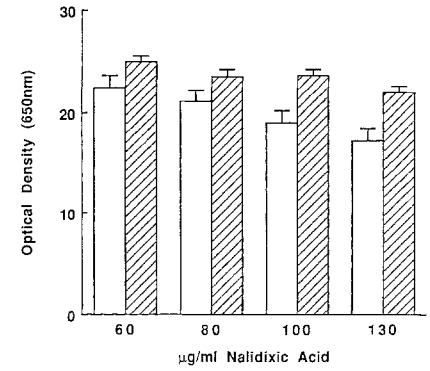

Fig. 3. Optical density achieved 10 h after addition of varying concentrations of nalidixic acid. The range bars represent the standard error of the mean of replicate experiments. Open bars, AR120/pRC1; hatched bars, ECO278/pRC1.

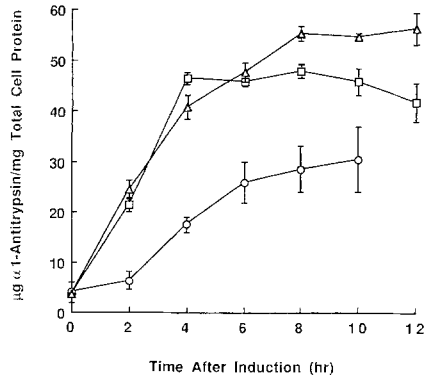

Fig. 2. Intracellular accumulation of α1-antitrypsin (as a fraction of total cell protein) during nalidixic acid induction. Time zero is the time of nalidixic acid addition. The range bars represent the standard error of the mean from replicate experiments. (—□—), AR120/pOTSα8; (—○—), ECO277/pOTSα8; (—△—), ECO278/pOTSα8.

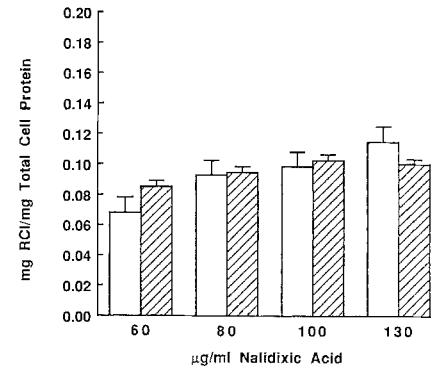

Fig. 4. Yield of RC1 (as a fraction of total cell protein) 10 h after addition of varying concentrations of nalidixic acid. Range bars represent the standard error of the mean of replicate experiments. Open bars, AR120/pRC1; hatched bars, ECO278/pRC1.

RC1 production in recAo and recA⁺ strains: evidence for product specificity and nalidixic acid concentration dependence of the recA o effects

Since the use of the *recA* o host had resulted in improved α1-antitrypsin productivity, it was of interest to determine if similar improvements could be obtained for the production of other heterologous proteins in nalidixic acid inductions. We investigated the effects of the *recA* o mutation on the expression of a fusion protein, RC1, partially derived from the *P. falciparum* major circumsporozooite surface protein. In these studies, several nalidixic acid concentrations were compared to determine if the responses of the two strains would be different.

The final optical densities attained in these inductions are shown in Fig. 3. At the lower nalidixic acid concentrations, there was very little difference in the biomass concentrations reached by the two strains. However, with increasing nalidixic acid concentration, the difference in final optical density between the two strains increased. The differences of 24% and 28%, respectively, at 100 and 130 μg/ml nalidixic acid were statistically significant (pooled variance *t*-test, $P \leq 0.05$). Similar trends were observed when total cell protein concentration measurements were compared (data not shown). It appears that increases in nalidixic acid concentration have greater detrimental effects on the *recA*⁺ strain than on the *recA* o strain.

Comparisons of the product yields obtained in the two hosts are shown in Fig. 4. At no tested nalidixic acid concentration were the differences in RC1 yield between the two strains statistically significant. However, it is of interest to note that a significant increase in product yield (70%) was obtained in the *recA*⁺ strain when the nalidixic acid concentration was increased from 60 μg/ml to 130 μg/ml. By comparison, only a 10% difference (not statistically significant) in α1-antitrypsin yield was observed between these nalidixic acid concentrations in the *recA* o strain. Therefore, RC1 yield, like final biomass concentration, varied less with nalidixic acid concentration in the *recA* o strain than in the *recA*⁺ strain.

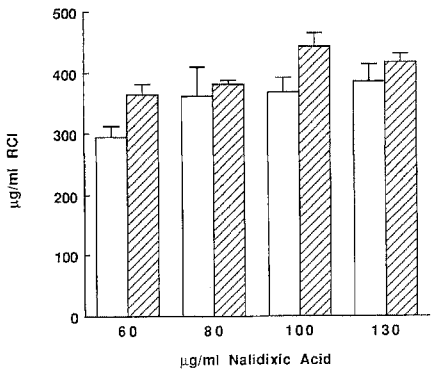

Fig. 5. RC1 concentration (per volume of culture medium) attained 10 h after addition of varying concentrations of nalidixic acid. Range bars represent the standard error of the mean of replicate experiments. Open bars, AR120/pRC1; hatched bars, ECO278/pRC1.

The RC1 concentrations achieved in inductions of the two strains are shown in Fig. 5. Significant improvements (20%) in mean product concentration were obtained in the *recA* o host at 60 and 100 μg/ml nalidixic acid. Since there was no significant improvement in RC1 yield in the *recA* o strain at the concentrations of nalidixic acid where significant improvements in final biomass concentration were obtained, the synergistic effect that had resulted in a 50% increase in maximum product concentration for α1-antitrypsin was not obtained for RC1.

DISCUSSION

The two mutations tested affected α1-antitrypsin productivity in very different ways. The *lexA* ind⁻ mutation caused reductions in both the maximum biomass concentration and the maximum α1-antitrypsin yield, resulting in a 50% reduction in the maximum α1-antitrypsin concentration. On the other hand, use of the *recA* o strain resulted in increases in both the post-induction biomass and the α1-antitrypsin yield which together provided a 50% increase in maximum product concentration. In the case of RC1, the *recA* o mutation resulted in less significant changes in the product concentrations achieved. Even though a positive effect of *recA* o on the maximum biomass concentration was observed in some RC1 inductions, intracellular RC1 concentrations remained similar in the *recA* o and *recA*⁺ strains. The α1-antitrypsin and RC1 results indicate that the effect of the *recA* o mutation on the productivity of nalidixic acid inductions varies with the gene induced and, as observed with RC1, the nalidixic acid concentration used for induction. Overall, the characteristics of RC1 inductions in the *recA* o strain varied less with nalidixic acid concentration than did inductions in the *recA*⁺ strain. The reason for yield improvements for one product and not another remain unclear. One possible explanation is that different levels of product toxicity exist which in some cases (e.g., RC1) would limit product accumulation. It is also possible that the different intracellular environments in the *recA* o and *recA*⁺ strains during induction affect the synthesis and/or stability of the two products in different ways.

The negative effects of the *lexA* ind⁻ mutation on the production of α1-antitrypsin in this system do not appear to be product-specific. In experiments comparing the production of several other heterologous proteins in ECO277 to their production in a *lexA*⁺ background (AR120), lags in product accumulation, decreased maximum post-

induction biomass, and decreased product yield were always observed in the *lexA* ind$^-$ host. The effects of the *lexA* ind$^-$ mutation on product accumulation are not entirely unexpected. It is possible that the lags in product accumulation observed in this strain are related to reduced rates of cI cleavage in the absence of post-induction derepression of RecA protein. Decreased yields of viable phage have been observed in UV induction of *lexA* ind$^-$ lysogens [23]. However, there may be difficulties in comparing results obtained in prophage induction systems to results from the system described here. The decreased phage yield found in the prophage induction studies appears to be independent of RecA concentration and may relate to the uninducibility of LexA-controlled DNA repair enzymes and consequent inability to repair damage to prophage DNA [23]. It is important to note that derepression of *recA* and other LexA-controlled genes does not appear to be required for all induction of product synthesis in our system, as considerable product accumulation does occur in the *lexA* ind$^-$ host. However, the possibility of some low-level induction of LexA-controlled genes in the *lexA* ind$^-$ strain cannot be ruled out.

The reduced post-induction biomass concentrations obtained in *lexA* ind$^-$ cultures are somewhat more difficult to explain. While RecA and other SOS proteins play a major role in the repair of a variety of types of DNA damage, none have been shown to have the capability of permitting DNA replication in the absence of gyrase activity or of increasing the level of nalidixic acid resistant gyrase activity. Therefore, it is not clear what role, if any, these proteins could play in furthering post-induction accumulation of cell mass. One possible protective role of the RecA protein involves its ability to protect single-stranded or gapped duplex DNA from RecBCD-mediated degradation [36]. Suggestive evidence indicates that the exoV function of the RecBCD enzyme may cause DNA degradation in nalidixic acid treated cells [11]. A role of the RecA protein in allowing maximum post-induction growth is also suggested by the greater biomass concentration attained in the *recA* o cultures.

The physiological basis of the increase in maximum intracellular α1-antitrypsin concentration in the *recA*o strain is not clear. Unlike the observations made by Smith [31] on the effects of increased pre-induction RecA concentration on p_L induction by mitomycin C, the *recA* o mutation did not appear to affect the onset time of induction or the maximum rate of α1-antitrypsin accumulation. Instead, the *recA* o mutation prolonged the period for which α1-antitrypsin accumulation occurred. Preliminary studies analyzing cI cleavage by Western blot indicate that cI cleavage was no more complete in the *recA* o strain than in the *recA*$^+$ strain at any point in the induction. It may be that the same physiological effect of the *recA* o mutation is responsible for both the slight increase in maximum biomass concentration and the observed extension of the period of product accumulation.

It would clearly have been of interest to determine whether the observed effects of the *recA* o and *lexA* ind$^-$ mutations were due to the changes in RecA protein concentration, the derepression (or lack of derepression) of LexA-controlled promoters, or both. In order to investigate these questions further, a *recA* o *lexA* ind$^-$ double mutant strain was constructed; however, it was not possible to carry out the intended experiments. The transformation efficiency of the double mutant strain was extremely low and those transformants that were obtained displayed extensive plasmid rearrangement. This phenomenon was observed with pOTSα8 and several other related plasmids carrying different heterologous gene coding sequences. No difficulties in plasmid establishment or maintenance were observed with either single mutant strain. We have not explored this phenomenon further.

Although the effects of the *recA* o mutation were product-specific, the use of this strain resulted in improvements in productivity in at least one case. It remains to be determined whether this strain will prove useful for other products produced in this induction system. However, this approach suggests the possibility of further optimization through alteration of other regulatory proteins or sites in this cascade. Genetic alteration of the regulatory mechanism may prove to be a useful component of process development for other inducible expression systems as well.

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