Transcriptional co-regulation of five chitinase genes scattered on the Streptomyces coelicolor A3(2) chromosome

Akihiro Saito, Masumi Ishizaka, Perigio B. Francisco Jr, Takeshi Fujii and Kiyotaka Miyashita

Author for correspondence: Kiyotaka Miyashita. Tel: +81 298 388256. Fax: +81 298 388199. e-mail: kmiyas@s.affrc.go.jp

Streptomyces coelicolor A3(2) strain M145 has eight chitinase genes scattered on the chromosome: six genes for family 18 (chiA, B, C, D, E and H) and two for family 19 (chiF and G). In this study, the expression and regulation of these genes were investigated. The transcription of five of the genes (chiA, B, C, D and F) was induced in the presence of colloidal chitin while that of the other three genes (chiE, G and H) was not. The transcripts of the five induced chi genes increased and reached their maximum at 4 h after the addition of colloidal chitin, all showing the same temporal patterns. The induced levels of the transcripts of chiB were significantly lower than those of the other four genes. Dynamic analysis of the transcripts of the chi genes indicated that chiA and chiC were induced more strongly than chiD and chiF. Addition of chitobiose also induced transcription of the chi genes, but significantly earlier than did colloidal chitin. When cells were cultured in the presence of colloidal chitin, an exponential increase of chitobiose concentration in the culture supernatant was observed prior to the induced transcription of the chi genes. This result, together with the immediate effect of chitobiose on the induction, suggests that chitobiose produced from colloidal chitin is involved in the induction of transcription of the chi genes. The transcription of the five chi genes was repressed by glucose. This repression was apparently mediated by the glucose kinase gene glkA.

Keywords: chitin, streptomycetes, transcriptional regulation, glucose repression, chitobiose

INTRODUCTION

Chitin is the second most abundant polysaccharide after cellulose in nature. The decomposition of chitin, therefore, is important in terms of the carbon and nitrogen cycles in the biosphere. Streptomyces are saprophytic soil bacteria known as major decomposers of chitin in soil. The genes encoding chitinases (EC 3.2.1.14) – enzymes that hydrolyse chitin – have been cloned from them (Robbins et al., 1988; Miyashita et al., 1991, 1997; Blaak et al., 1993; Fuji & Miyashita 1993; Miyashita & Fujii, 1993; Tsujibo et al., 1993, 2000; Ohno et al., 1996; Saito et al., 1999). The products of these Streptomyces chitinase genes are classified, based on amino acid sequences analysis, into two distinct families of glycosyl hydrolases, families 18 and 19 (Henrissat, 1991; Henrissat & Bairroch, 1993). Family 19 chitinases also differ from family 18 chitinases in their catalytic mechanisms (Armand et al., 1994; Iseli et al., 1996). Streptomyces are unique micro-organisms in possessing family 19 chitinase genes, which are usually found only in higher plants (Ohno et al., 1996; Saito et al., 1999; Watanabe et al., 1999).

We previously cloned seven chitinase genes from the ordered cosmid library representing the entire chromosome of S. coelicolor A3(2) (Redenbach et al., 1996) and analysed their sequences (Saito et al., 1999). The genes do not form clusters but are scattered on the chromosome. Recently, the S. coelicolor A3(2) Genome Sequencing Project revealed the presence of another putative chitinase gene on cosmid 7B7. The accession and gene numbers are AL009199 and 7B7.09c, respectively. We named this gene chiH. Analysis of the deduced amino acid sequence of the gene product showed that ChiH belongs to subfamily C of bacterial family 18.
chitinases (Suzuki et al., 1999) and is presumed to consist of three domains: signal domain, catalytic domain and chitin-binding domain (Fig. 1). With the inclusion of chiH, S. coelicolor A3(2) evidently has genes encoding chitinases belonging to each subfamily of bacterial family 18 chitinases (Suzuki et al., 1999), i.e. subfamily A (chiC, chiD and chiE), B (chiA and chiB) and C (chiH), in addition to the family 19 chitinases (chiF and chiG). The multiplicity of chitinase genes in S. coelicolor A3(2) is quite high as compared with other chitin-degrading bacteria, such as Bacillus circulans (Watanabe et al., 1990, 1992) and Serratia marcescens (Watanabe et al., 1997; Suzuki et al., 1999).

Previous studies have shown that the transcription of the family 18 chitinase genes from Streptomyces is induced in the presence of chitin and repressed in the presence of glucose plus chitin (Delic et al., 1992; Fujii & Miyashita, 1993; Miyashita & Fujii, 1993). We recently demonstrated that the transcription of chiA, a family 18 chitinase gene of Streptomyces lividans, was induced not only by colloidal chitin but also by chitin oligosaccharides, among which N,N’-diacetyctchitobioside (chitobiose) was the most efficient inducer (Miyashita et al., 2000).

Although the molecular mechanisms governing the regulation of expression of chitinase genes in Streptomyces are still unclear, a pair of direct repeat sequences present in the promoter region of chi63, a family 18 chitinase gene of Streptomyces plicatus, was shown to be involved in the induction by chitin and the repression by glucose of the gene expression (Delic et al., 1992; Ni & Westpheling, 1997). On the other hand, the glkA gene encoding a glucose kinase (Angell et al., 1992) was shown to be involved in the glucose repression of chitinase production in S. lividans (Saito et al., 1998). It was also demonstrated that the glkA gene is involved in the glucose repression of several catabolic enzymes, including agarase (Angell et al., 1992), glyceral kinase (Seno & Chater, 1983) and α-amylase (Virolle & Bibb, 1988). It was thus inferred that the glkA gene plays a central role in the glucose repression of gene expression in Streptomyces. However, Ingram & Westpheling (1995) reported that glkA is not required for the glucose repression of the chi63 promoter in an S. coelicolor A3(2) ccrA1 genetic background.

The current study was done to elucidate the regulation of the expression of the chitinase system in S. coelicolor A3(2) M145, by investigating the induction and repression of the transcription of each chitinase gene in this strain. The promoter regions of the genes were deduced after the determination of their transcriptional initiation sites. The expression level of each chitinase gene is discussed in relation to the structure of its promoter region.

METHODS

Bacterial strains and media. The wild strain Streptomyces coelicolor A3(2) M145 and its glkA mutant M480 were used (Hopwood et al., 1985; Angell et al., 1994). Escherichia coli XL-1 Blue (Sambrook et al., 1989) was used as the host for gene manipulations. LB medium (Sambrook et al., 1989) and LB supplemented with 50 μg ampicillin ml−1 were used for the
culture of *S. coelicolor* A3(2) and *E. coli* transformants, respectively. The expression of chitinase genes in *S. coelicolor* A3(2) was induced by growing the cells in YE (Saito et al., 1998) or an inorganic salts medium (Miyashita et al., 1991) with or without 0.05% (w/v) colloidal chitin (Lingappa & Lockwood, 1962) or chitobiose (1 mM or 250 µM) (Seikagaku Kogyo).

**Culture conditions.** Spores (approx. 6 × 10^7) of *S. coelicolor* A3(2) strains formed on SFM agar medium (van Wezel et al., 1997) were inoculated into 30 ml LB medium in a 100 ml flask with a spring (Hopwood et al., 1985) and grown for 20 h at 30 °C on a rotary shaker at 150 r.p.m. Mycelia were harvested by centrifugation, washed with either YE or inorganic salts medium, resuspended, and divided into several aliquots. After centrifuging the aliquots, the mycelia were resuspended in the corresponding medium supplemented with various carbon sources, i.e. 0 05% (w/v) colloidal chitin, 1 mM or 250 µM chitobiose, or 0 05% (w/v) colloidal chitin with 1 0% (w/v) glucose. The culture was further grown at 30 °C on a rotary shaker at 150 r.p.m. Samples of 5 ml, taken periodically, were centrifuged to separate the supernatants and the mycelia and stored at −80 °C.

**Recombinant DNA techniques.** Plasmid preparation and restriction enzyme digestion were done as described by Sambrook et al. (1989). Dephosphorylation, blunting and ligation of DNA fragments were done using bacterial alkaline phosphatase (Toyobo), a DNA blunting kit (Takara) and a DNA ligation kit (Takara), respectively, according to the manufacturers’ instructions.

**Northern-blot hybridization.** The mycelia of *S. coelicolor* A3(2) harvested from a 5 ml culture sample by centrifugation were disrupted by using alumina type A-5 (Sigma) and a pellet was collected at 17000 g for 10 min. The supernatant was mixed with 0 1 ml 0 25 M EDTA (pH 8 0), 75% (v/v) acetonitrile containing 100% (v/v) formamide at 40 °C, and an automated DSQ 2000L Laser Fluorescent Sequencer (Shimadzu).

**Chitinase assay.** Chitinase activity in the culture supernatant was measured as described previously (Miyashita et al., 1991), using the fluorescent substrate 4-methylumbelliferyl-N',N'-diacetylchitobioside or 4-methylumbelliferyl-N,N',N'-triacetylchitotrioside (Sigma).

**Determination of chitobiose concentration.** Culture supernatant obtained by centrifugation was boiled for 10 min, cooled on ice, and filtered through a cellulose acetate membrane with pore size of 0 20 µm (Millipore). A 0 25 ml aliquot of the filtrate was mixed with 0 75 ml acetonitrile containing 100 ng ml^-1 of *p*-nitrophenyl-N',N'-diacetylchitotrioside (Sigma) was used as the internal standard and centrifuged at 17000 g at 4 °C for 10 min. The supernatant was injected into an HPLC-tandem mass spectrometer (MS/MS) for quantifying chitobiose concentration. The conditions for the separation of chitobiose were as follows: HPLC system, 1090 Series II (Hewlett Packard); column, Capcell Pac NH2 UG80A 5 mm, 1 5 × 150 mm (Shiseido); guard column, Opti-Guard C18, 1 0 × 15 mm (Optimize Technologies); oven temperature, 40 °C; eluent, 75% (v/v) acetonitrile; and flow rate, 0 1 ml min^-1. The eluent was mixed with 75% (v/v) acetonitrile containing 200 mM acetic acid at the flow rate of 0 1 ml min^-1 and then led into an API300 triple-quadrupole mass spectrometer equipped with a TurboIonSpray electron spray ionization interface (PE Sciex, Canada) under multiple reaction monitoring conditions. The monitored MS/MS transitions of chitobiose and PNP-(GlcNAc) were *m/z* 425 (*M+H^+*) → 204 and *m/z* 749 (*M+H^+*) → 204, respectively. The ratios between peak area of the sample product and those of the internal standard were determined, and the concentrations of samples were then calculated in relation to the internal standard. The concentration of each sample was measured in...
duplicate and the mean was shown as the result. The detailed conditions of the HPLC-MS/MS for the quantitative analysis of chitooligosaccharides will be reported elsewhere.

RESULTS
Expression of chitinase genes in the presence of colloidal chitin

Chitinase production in \textit{S. coelicolor} A3(2) was investigated in the presence or absence of colloidal chitin. Cells of \textit{S. coelicolor} A3(2) M145 grown in LB were transferred to the inorganic salts medium with or without 0.05\% (w/v) colloidal chitin, and chitinase activity in the culture supernatant was measured periodically using synthesized fluorescent substrates. Significant chitinase activity was detected 24 h after exposure to colloidal chitin whereas no activity was detected when cells were cultured without colloidal chitin (data not shown). This indicates that chitinase production in \textit{S. coelicolor} A3(2) was induced by colloidal chitin.

The transcription of the eight chitinase genes (\textit{chiA}, \textit{chiB}, \textit{chiC}, \textit{chiD}, \textit{chiE}, \textit{chiF}, \textit{chiG} and \textit{chiH}) in the presence or absence of colloidal chitin was then investigated periodically. Total RNAs used for Northern-blot hybridization were prepared from the cells grown with or without colloidal chitin after preculture in LB medium. In the presence of colloidal chitin, transcripts of 1.8, 2.0, 2.4, 1.4 and 1.1 kb were detected with \textit{chiA}, \textit{chiB}, \textit{chiC}, \textit{chiD}, \textit{chiF} and \textit{chiG} probes, respectively, whereas no transcript was detected by either the \textit{chiE} or \textit{chiH} probe (Fig. 2), even when 10 times more RNAs (20 µg) were subjected to analysis (data not shown).

\textbf{Fig. 2.} Transcripts of chitinase genes in \textit{S. coelicolor} A3(2) strain M145 in the presence or absence of colloidal chitin (CC). Northern-blot hybridization was performed using labelled anti-sense RNAs of \textit{chiA} (a), \textit{chiB} (b), \textit{chiC} (c), \textit{chiD} (d), \textit{chiE} (e), \textit{chiF} (f), \textit{chiG} (g) and \textit{chiH} (h) as probes. Three micrograms of total RNA was loaded per lane. The approximate sizes (kb) of the transcripts are indicated to the right of each panel. Cultures grown in LB were divided into two aliquots, and the mycelia in each aliquot were collected by centrifugation and resuspended in YE medium with or without 0.05\% (w/v) colloidal chitin. Total RNAs were prepared from the mycelia grown in YE medium supplemented with colloidal chitin for 1, 2, 4, 6, 12 and 24 h, and from the mycelia cultured in YE medium for 0, 2, 6 and 24 h.
Regulation of chitinase genes in S. coelicolor

The chiD probe hybridized with a 2.0 kb extra band which was assumed to be the transcriptional product of the chiC gene (2.0 kb) as the chiD probe was highly homologous to chiC (81% at the DNA level) (Fig. 1). Furthermore, because the chiG and chiF genes share 86% similarity at the DNA level, the 1.4 kb band detected by the chiG probe was assumed to be the chiF transcript. The chiC probe hybridized with a 2.4 kb transcript in addition to the 2.0 kb major transcript. This larger transcript was presumed to be not those of chiD or chiE, since the 2.4 kb band was not detected by either the chiD or chiE probe. Considering that the chiD locus showed the highest similarity with chiC in the cosmId library representing the S. coelicolor A3(2) chromosome (Saito et al., 1999), the 2.4 kb band was assumed to include the chiC transcript. From these results, we concluded that the transcription of chiA, chiB, chiC, chiD and chiF was induced by colloidal chitin. The transcripts of the five chi genes all showed the same temporal pattern, i.e. they reached maximum at 4 h after the exposure of cells to colloidal chitin and decreased thereafter (Fig. 2). The induced levels of the chiB transcripts were significantly lower than those of the other four chitinase genes.

Dynamic analysis of induced transcription of chitinase genes

In order to compare the promoter activities of the chitinase genes, the accumulation of their transcripts was analysed dynamically. Total RNAs prepared from mycelia sampled at 1 h intervals following exposure to colloidal chitin were subjected to Northern-blot analysis. The amount of the chiC transcripts increased exponentially from 2 to 4 h after the addition of colloidal chitin (Fig. 3). The temporal patterns of the chiA, chiD and chiF transcripts showed a similar exponential increase (data not shown). These data enabled us to estimate the doubling times of transcript accumulation, which were used to compare the promoter activities of the chitinase genes irrespective of the difference in labelling efficiency of probes used in Northern-blot analysis. The doubling times of the chiA, chiC, chiD and chiF mRNA accumulations were 25, 22, 49 and 51 min, respectively. These values indicated that the promoter activities of the chiA and chiC genes were induced about twice as much as those of the chiD and chiF genes. Very low levels of the chiB transcription were detected only at 4, 5 and 6 h and the doubling time could not be calculated.

Transcription of chitinase genes induced by chitobiose

Chitin is hydrolysed to chitobiose, the dimer of N-acetylglucosamine, by Streptomyces chitinases (Blaak et al., 1993; Ohno et al., 1996). To determine whether the expression of chitinase genes is induced by chitobiose in S. coelicolor A3(2), chitinase activity in the culture supernatant and transcription of the eight chi genes were monitored. The mycelia were cultivated in the inorganic salts medium with or without chitobiose after preculture in LB medium. Significant chitinase activity was detected 4 h after the addition of chitobiose (1 mM or 250 μM), while no activity was detected in the absence of the substrate, indicating that chitinase production was induced by chitobiose (data not shown). To establish whether chitobiose induces the transcription of the chi genes, total RNAs prepared from mycelia exposed for 30, 60 and 120 min to 1 mM chitobiose were subjected to Northern-blot analysis. The results indicated that chiA, chiB, chiC, chiD and chiF were transcribed in the presence of chitobiose, and that the transcript levels of the genes increased through time (Fig. 4a). The transcript levels of the chiB gene were significantly less than those of the other four genes. No transcripts of chiE, chiG and chiH were detected even in the presence of chitobiose.

The temporal pattern of induction of the chiA transcription in the presence of chitobiose was compared with that of colloidal chitin. RNAs were taken every 2 h after the addition of the substrates. The maximum transcript level was reached at 2 h after exposure to
250 µM chitobiose and at 4 h after the addition of colloidal chitin (Fig. 4b, c). This result for chitobiose, combined with the continuous increase of the transcript levels for 2 h after exposure to 1 mM chitobiose (Fig. 4a), suggests that chiA transcription reached the maximum level at 2 h following exposure to chitobiose. It was thus evident that chitobiose induced the chiA transcription significantly earlier than colloidal chitin. The same results were obtained for chiB, chiC, chiD and chiF (data not shown). The experiments were carried out twice and similar results were obtained. These results indicate that chitobiose has a more immediate effect on the transcriptional induction of the chitinase genes than colloidal chitin.

**Chitobiose production from colloidal chitin in the culture supernatant**

Because of the immediate effect of chitobiose on the transcriptional induction of the chitinase genes in *S. coelicolor A3(2)* (Fig. 4) and in *S. lividans* (Miyashita et al., 2000), it was postulated that chitobiose is a direct inducer of chitinase gene transcription in these bacteria. If this is the case, chitobiose production should occur before the induced transcription of chitinase genes starts. To test this, samples were taken every hour from the inorganic salts medium culture supplemented with a 0.05% (w/v) colloidal chitin. After centrifugation, chitobiose concentration in the supernatant was determined, and the transcripts of the five cbi genes in *S. coelicolor A3(2)* were measured using the total RNAs from the mycelia harvested from the corresponding cultures.

HPLC-tandem mass spectrometry enabled us to measure chitobiose concentration with high sensitivity. The chitobiose concentration increased exponentially from 0.06 µM (zero time) to 8.4 µM (4 h later) and further increased to 13.9 µM at 5 h (Fig. 3b). The experiment was carried out twice and similar results were obtained. The chiC transcripts were detectable at 2 h after exposure to colloidal chitin, when the chitobiose concentration in the culture supernatant was 0.66 µM. The levels of the chiC transcripts increased exponentially until 4 h and reached maximum at 5 h (Fig. 3). The increase of the chiC transcripts from 2 to 4 h after the addition of colloidal chitin seemed to coincide with the increase of the chitobiose concentration in the culture supernatant. These results, together with the more immediate effect of chitobiose than colloidal chitin on the induction of chi genes transcription (Fig. 4), suggest that chitobiose produced from colloidal chitin could be involved in the induction of the expression of chitinase genes in *S. coelicolor A3(2).*

**Transcriptional induction of chitinase genes by α-chitin**

Chitinase production in *S. plicatus* was shown to be induced by insoluble chitin but not by chitobiose (Robbins et al., 1992). Crab shell chitin flakes retain the structure of α-chitin, unlike colloidal chitin, and are insoluble. To determine whether chiE, chiG and chiH are induced by chitin flakes, *S. coelicolor A3(2)* was grown in the presence of this substrate. Transcripts of the chiA gene were detected whereas those of chiE, chiG and chiH were not (data not shown).

**Determination of the transcription start sites of chiB, chiD and chiF**

In order to identify the promoter regions of the *S. coelicolor A3(2)* chitinase genes, the transcriptional initiation sites of the genes were deduced or determined. The promoter regions of the chiA and chiC genes of *S. coelicolor A3(2)* were deduced from the transcriptional initiation sites of the chiA and chiC genes of *S. lividans*.
Regulation of chitinase genes in *S. coelicolor*

(Fujii & Miyashita, 1993; Miyashita & Fujii, 1993), respectively, because the corresponding genes in the two species are almost identical in their upstream regions and coding regions (Saito *et al*., 1999). The deduced transcriptional initiation sites of *chiA* and *chiC* were the G and the ACT nucleotide residues at positions 51 and from 56 to 58 upstream of the putative translation initiation sites, respectively. To identify the promoter regions of *chiB*, *chiD* and *chiF*, their transcription initiation sites were determined by primer extension analysis using total RNAs prepared from cells grown for 2 h in an inorganic salts medium containing 250 µM chitobiose. For *chiB*, the extension product corresponded to the G residue 39 nt upstream of the putative translation initiation site (Fig. 5a). For *chiD* and *chiF*, the extension products matched with the ACCG and CC nucleotide residues at positions from 66 to 69 and 119 to 120 upstream of the putative translation initiation site, respectively (Fig. 5b, c). The possible RNA polymerase binding sites of *chiE*, *chiG* and *chiH*, whose transcripts were not detected by Northern-blot analysis, were searched using the SDC Genetix System (Software Kaihatsu Co.). A possible promoter sequence was found upstream of *chiE* (Fig. 6) whereas there was no obvious promoter sequence upstream of *chiG* and *chiH*.

Ni & Westpheling (1997) proposed that the direct repeat sequences in the *chi63* promoter region of *S. plicatus* direct its chitin-induced transcription. In *S. coelicolor* A3(2), sequences highly similar to the *S. plicatus* direct repeat are present in the upstream regions of *chiA*, *chiB*, *chiC*, *chiD*, *chiE*, *chiF* and *chiH* but not of *chiG*, while only one copy of the repeated sequence is present in the possible promoter region of *chiE* (Fig. 6).

Glucose repression of transcription of chitinase genes

To investigate the transcriptional repression of the five *chi* genes by glucose in *S. coelicolor* A3(2) and the involvement of *glkA* in this phenomenon, Northern-blot

---

**Fig. 5.** Primer extension analysis to determine the transcriptional start points of *chiB* (a), *chiD* (b) and *chiF* (c). Lanes 1 through 4 are A-, C-, G- and T-specific dideoxy sequencing reactions, respectively. Lane 5 is the primer extension product. The corresponding sequences of the coding strands are shown on the right. The nucleotides of transcriptional starts, and the −10 and −35 regions of the promoter consensus sequences are indicated.
In this study, the expression of eight chitinase genes in *S. coelicolor* A3(2) was analysed at the transcription level. Either colloidal chitin or chitobiose induced the transcription of *chiA*, *chiB*, *chiC*, *chiD* and *chiF* but not of *chiE*, *chiG* and *chiH*. The promoter activities of *chiA* and *chiC* were induced twice as much as those of *chiD* and *chiF*, while that of *chiB* was weak. The −35 hexamers of the promoter regions of the *chi* gene are more highly conserved (TTGACN) than the −10 hexamers [(T/c)AcNtT] (Fig. 6). No obvious −35 or −10 hexamers could be found upstream of *chiH* and *chiG*. The absence of a ‘good’ promoter region in *chiH* and *chiG* might be responsible for their lack of, or very low, expression.

Recently, Nguyen (1999) reported that Reg1, a regulatory protein for the amylase gene of *S. lividans* (Nguyen *et al*., 1997), binds to the promoter regions of several genes, including the *chiA* gene of *S. lividans*, that are subject to glucose repression. It was suggested that common sequences in the promoter regions, which are different from the direct repeat mentioned above, are involved in the DNA–protein interactions. Although sequences highly similar to the common ones were not observed in all the promoter regions of the chitinase genes of *S. coelicolor* A3(2), the binding ability of Reg1 to the promoters and the involvement of the protein in the regulation of the chitinase genes need to be elucidated.

**Discussion**

In this study, the expression of eight chitinase genes in *S. coelicolor* A3(2) was analysed at the transcription level. Either colloidal chitin or chitobiose induced the transcription of *chiA*, *chiB*, *chiC*, *chiD* and *chiF* but not of *chiE*, *chiG* and *chiH*. The promoter activities of *chiA* and *chiC* were induced twice as much as those of *chiD* and *chiF*, while that of *chiB* was weak. The −35 hexamers of the promoter regions of the *chi* gene are more highly conserved (TTGACN) than the −10 hexamers [(T/c)AcNtT] (Fig. 6). No obvious −35 or −10 hexamers could be found upstream of *chiH* and *chiG*. The absence of a ‘good’ promoter region in *chiH* and *chiG* might be responsible for their lack of, or very low, expression.

The 12 bp direct repeat in the *chi63* promoter region of *S. plicatus* directs both induction and repression of the *chi63* promoter (Delić *et al*., 1992; Ni & Westpheling, 1997). Two copies of the 12 bp putative operator sequence were found in the promoter regions of most of the *S. coelicolor* *chi* genes (Fig. 6). The proposed consensus for this putative operator is TGGTC(C/T)-(A/G)GACC(T/A). One of the repeats is between the −10 and −35 region and the other is located (from 1 to 7 bp) upstream of the −35 hexamer. The distance between the two repeats greatly varies from gene to gene: 4/5 bp in *chiH* and *chiF*, 9/10 bp in *chiC* and *chiD*, 19/20 bp for *chiA* and *chiB*. It is noteworthy that the more upstream repeat is absent from the *chiE* promoter region whereas the two repeats are very close to each other (4 bp) in the *chiH* promoter region. All the actively transcribed chitinase genes *chiA*, *B*, *C*, *D* and *F* share a similar organization with the two repeats on both sides (but at various distances) of the −35 hexamer. The sequence of the more upstream repeat of the *chiB* gene is the most divergent from the proposed consensus. This might account for the very poor expression of *chiB*.
glkA, a gene encoding an ATP-dependent glucose kinase (Angell et al., 1992), was shown to be involved in the glucose repression of the expression of the glycerol kinase, agarase and α-amylase genes in Streptomyces (Seno & Chater, 1983; Virolle & Bibb, 1988; Angell et al., 1992). The glkA gene also appeared to be involved in the glucose repression of chitinase production in S. lividans (Saito et al., 1998). In the current study, it appears that glkA is involved in the glucose repression of transcription of the five chitinase genes (chiA, chiB, chiC, chiD and chiF) in S. coelicolor A3(2) strain M145. On the other hand, it is reported that a mutation in the glkA gene had no effect on glucose repression of the chi63 gene in an S. coelicolor ccrA1 genetic background (Ingram & Westpheling, 1995). The ccrA1 mutation might suppress the glkA mutation directly or indirectly.

When S. coelicolor A3(2) was exposed to colloidal chitin, the chitobiase concentration in the culture supernatant increased during the first hour from 0.06 to 0.22 µM, although no chi gene transcript was detected during that period. We assume that low levels of chitinases constitutively produced initially digested colloidal chitin into chitobiase, which, upon accumulation, triggered the induction of chi gene transcription. Upon reaching the peak at 5 h of exposure to colloidal chitin, the transcripts of the five chitinase genes started to decrease (Fig. 2). Similar phenomena have been observed in various other systems (Baumann et al., 1996; Schwartz et al., 1998; Leveau et al., 1999; Iyer et al., 1999). For instance, in 2,4-dichlorophenoxyacetate (2,4-D)-utilizingRalstoniaeutrophaJM134(pJP4), the 2,4-D-induced transcription from TfdR/S-regulated promoters decreased soon after the initial increase (Leveau et al., 1999). In this system, 2,4-dichloromuconate, a pathway intermediate in 2,4-D utilization, is the signal for TfdR/S-mediated expression. It was assumed that the induction of intracellular enzymes that degrade the signal molecule caused the decrease in the tfd gene expression (Leveau et al., 1999). Although the signal molecule(s) for the induction of the chitinase genes in S. coelicolor A3(2) is still not identified, the other enzymes of the metabolic pathway for chitin or chitobiase utilization might degrade the signal molecule(s) or its precursor(s), resulting in the decrease of chitinase genes transcripts.

The five chitinase genes (chiA, B, C, D and F) were expressed simultaneously in the presence of colloidal chitin, although the induction levels of chiB were by far the lowest. This suggests that the five different chitinases work synergistically to degrade chitin, which is a heterogeneous polysaccharide. The importance of the multiplicity of the S. coelicolor A3(2) chitinases in chitin degradation could be elucidated by the enzymic characterization of the gene products.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid Bio Design Program from the Ministry of Agriculture, Forestry, and Fisheries of Japan (BDP-00-VI-2-4).

REFERENCES


Received 3 May 2000; revised 17 August 2000; accepted 21 August 2000.