Expression of the Taka-amylase A gene (amyB) of Aspergillus oryzae is induced by starch or maltose. The A. oryzae amyB gene promoter contains three highly conserved sequences, designated Regions I, II, and III, compared with promoter regions of the A. oryzae glaA encoding glucoamylase and the agdA encoding α-glucosidase. To identify the function of these sequences within the amyB promoter, various fragments containing conserved sequences in the amyB promoter were introduced into the upstream region of the heterologous A. nidulans amdS gene (encoding acetamidase) fused to the Escherichia coli lacZ gene as a reporter. Introduction of the sequence between -290 to -233 (the number indicates the distance in base pairs from the translation initiation point (+1)) containing Region III significantly increased the expression of the lacZ reporter gene in the presence of maltose. The sequence between -377 to -290 containing Region I also increased the lacZ activity, but its maltose inducibility was less than that of Region III. The sequence between -233 to -181 containing Region II had no effect on the expression. These results indicated that Region III is most likely involved in the maltose induction of the amyB gene expression.

Key words: promoter; Aspergillus oryzae; Taka-amylase A; gene regulation

Aspergillus oryzae secretes a large amount of enzymes involved in the degradation of starch. These include α-amylase (Taka-amylase A), glucoamylase, and α-glucosidase, which are produced in the presence of starch or malt-o-oligosaccharides but not of glucose. We have reported the cloning and sequencing of genes of Taka-amylase A (amyB),1) glucoamylase (glaA),2,3) and α-glucosidase (agdA)5) from A. oryzae. By comparing with these promoter sequences, two regions, designated Region I and II were found to be highly conserved,5) and more recently, an additional conserved sequence, Region III, was identified.5) To discover the function of these homologous regions in starch induction, fusion genes of the amyB, glaA, or agdA promoter and the Escherichia coli uidA, encoding β-glucoronidase (GUS) were expressed in A. oryzae and deletion analyses were done;5-8) these sequences, in particular Region I and III, were found to be important in starch induction. More detailed analysis of the conserved sequences is required for understanding of the regulatory circuit for amylase gene expression. In this study, DNA fragments encompassing the consensus sequences of the amyB promoter were introduced into the heterologous A. nidulans amdS gene promoter and were expressed in A. nidulans.

Figure 1 shows the nucleotide sequence of the promoter region of the amyB gene. Within the promoter region there are three highly conserved sequences, indicated by bold characters in Fig. 1 and designated Region I, II, and III, common to the A. oryzae amyB, glaA, and agdA genes. To analyze the function of these conserved sequences in starch or maltose induction, various DNA fragments containing the sequences were synthesized by PCR and introduced into the heterologous A. nidulans amdS gene promoter. A fragment from -315 to -181 counting from the start codon, contains three homologous regions, I + III + II. Fragments from -315 to -233 and -290 to -181 contain Regions I + III and III + II, respectively. Fragments from -377 to -290, -290 to -233 and -233 to -181 independently contain Regions I, III, and II, respectively. A fragment from -377 to -181 contains two putative CAAT boxes at -376 and -192. To generate BgII and BamHI sites at both ends of amplified DNA fragments, additional sequences, (5'-CCGAGATCT) and (5'-GCGGTATCC), were added to the 5'-end of the upstream and downstream primers, respectively. These fragments were introduced into pAN49-1,9) which carries the fusion gene of the upstream region of the amdS gene and E. coli lacZ. Each fragment was inserted in both orientations at the BamHI site -81 from the major transcription start point (identified by Corrick et al.,)10) within the amdS promoter of pAN49-1 (Fig. 2). The resultant plasmids

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Fig. 1. The Nucleotide Sequence of the 5′-Upstream Region of the amyB Gene.

Numbering is from the start codon (+1) of translation. Two putative CAAT sequences (−376 and −192) and TATA box are underlined. Region I (from −306 to −299), Region II (from −200 to −193), and Region III (from −263 to −248) are indicated by bold characters.

were introduced in A. nidulans WG355 (biaA1, argB2, bga0)\textsuperscript{10} which is an arginine auxotroph and produces no significant amount of endogenous β-galactosidase activity, to analyze the effects of inserted fragments on the promoter activity.

The constructed plasmids were introduced in A. nidulans WG355, and argB\textsuperscript{+} transformants were selected on minimal medium. Six transformants with each fusion promoter plasmid were analyzed by Southern blot hybridization, and then the transformants containing a single copy of the plasmid integrated at the resident argB locus were selected for promoter activity assay. As shown in Fig. 3, β-gal activities were measured in mycelial extracts of selected transformants grown on minimal medium containing maltose as an inducing carbon source or glucose as a non-inducing one. It is noted that β-gal activities of the transformants grown on glucose were almost the same irrespective of the constructed plasmids and the control, pAN49-1.

Introduction of the fragments from −377 to −290 containing Region I (pAN49-1-I) resulted in 7-(forward) and 5-(reverse) fold increases in the maltose/glucose ratio (ratio of β-gal activities grown on maltose against those on glucose, referred to as “induction efficiency”), indicating that the region containing Region I is capable of activating the gene expression in the presence of maltose. We have previously shown that the induction efficiency was not completely lost by deleting up to −290 but was abolished by deleting up to −233.\textsuperscript{7,8} Since the fragment from −290 to −233 contains Region III, this similar sequence could also be required for maltose induction. Introduction of this fragment in both orientations causes higher efficiency of maltose induction (10–11 fold) than did that of Region I, thus suggesting that Region III is more important for induction than Region I. It is known that glucose has a repressive effect
Fig. 2. Construction of Fusion Promoter in Plasmid, pAN49-1.

Fragments containing Region I, II, or III in the amyB promoter were inserted into the BamHI site (at −81 nt from the major transcription start point) in the amdS promoter. Bg, BglII; E, EcoRI; H, HindIII; S, SauI; X, XbaI: argB⁺, mutated argB allele; PamdsS, promoter region of the amdS gene; TamdsS, terminator region of the amdS gene; amdS::lacZ, fusion gene of the amdS and lacZ coding region, connected with first 33 amino acids of the amdS and 16th amino acid of the lacZ.

Fig. 3. β-Galactosidase Activities of the Transformants Carrying Various Fragments Containing Conserved Sequences in the amdS Promoter.

Approximately 10⁶ conidia of each transformant were inoculated into 10 ml of minimal medium containing 1% glucose or maltose as a carbon source, incubated at 37°C for 18 h with shaking, and then were harvested. Preparation of cell-free extracts of transformants were done as described by Tada et al. β-Galactosidase activity was measured as described by Miller. The left panel shows the amyB promoter region including two putative CAAT boxes, (−376, −192) and Regions I, II, and III. Various fragments introduced into the A. nidulans amdS promoter are shown with arrows. The rightward arrows indicate the forward orientation (plasmid marked with F) and the leftward arrows indicate the reverse orientation (plasmid marked with R). β-Galactosidase activity of each transformant is given in the right panel.

on amylose gene expression. It could, therefore, be possible that the fragment containing Region III confers efficient expression ability on the amdS promoter in the absence of glucose. However, a high expression level of β-gal was not observed in the transformant grown in the medium containing fructose as a carbon source (data not shown), suggesting that the fragment is responsible for maltose induction.

On the other hand, introduction of Region II caused the same induction level as that of the control and it seems that this sequence alone is not substantially responsible for maltose induction. Moreover, induction efficiencies with Region I + III and Region III + II are same as that of Region III alone. Recently, we have shown that the gene expression decreased greatly and maltose induction was abolished by internal deletion of Region III from the agdA promoter, but was not affected by that of Region II. Internal deletion of Region I resulted in a significant reduction of gene expression but did not affect maltose induction. Taken together, Region III alone is sufficient to confer maltose induction on a heterologous promoter. However, in this study, it is suggested that Region I is also implicated in maltose induction. Since the fragment from −377 to −290 contains a long flanking sequence other than Region I, a cryptic element responsible for maltose induction might be in the flanking sequence.

Although induction efficiency was increased by insertion of Region III to a level similar to that of amyB::lacZ, in which 617 bp of the whole amyB promoter was fused to lacZ, high-level expression of lacZ was not reached. This suggests that other sequences
or regions are required for a full high level of gene expression. One of the candidates is the putative CAAT element at −376, which was shown to be a binding site of the nuclear protein AnCP1,12,13 and to be concerned with efficient expression. Since this CAAT element is contained in the region from −377 to −290 but located at the 5′-terminus, a sequence flanking the element may be required for efficient expression. Alternatively, other elements such as transcription initiation sequences may be involved. An experiment introducing upstream sequences of the abaA gene (A. nidulans conidiation regulatory gene) into the trpC promoter showed that the expression level was influenced by the presence of trpC transcription initiation sequences.14 This direct or indirect communication between different elements was also suggested in the experiment where the gpd box was introduced into the amds upstream region.9

In this study, we show that the conserved sequence, Region III, is mainly involved in maltose induction for the amyB gene. However, the contribution of its flanking sequences in the fragments used to gene expression cannot be excluded. More precisely, insertion analysis of the conserved sequences alone must be done. Furthermore, identification of the putative trans-acting factor(s) which may interact with Region III is underway.

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