African Journal of Biotechnology Vol. 2 (7), pp. 185–188, July 2003
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2003 Academic Journals

Full Length Research Paper

Analysis of *AVR4* promoter by sequential response-element deletion

Olukosi, YA[1] and Iwalokun, BA[2]*

[1]Genetics Division, Nigerian Institute of Medical Research (NIMR), 6, Edmond Crescent, Yaba – Lagos, PMB 2013, Lagos - Nigeria.
[2]Biochemistry Dept, Lagos State University, PMB 1087, Apapa – Lagos, Nigeria.

Accepted 24 June2003

Several reports have associated the variability in physico-chemical properties of avidin protein to dynamism inherent in the consensus regulatory networks within the promoter region of avidin genes. An Avr4 promoter region ligated to chloramphenicol acetyltransferase plasmid vector (pBLCAT2) to produce recombinant plasmid Avr4pBLCAT2 was sequentially deleted to produce five distinct mutants: Avr4pBLCAT2$_{907-176}$, Avr4pBLCAT2$_{809-176}$, Avr4pBLCAT2$_{789-176}$, Avr4pBLCAT2$_{429-176}$ and Avr4pBLCAT2$_{302-176}$. The transformants elicited different chloramphenicol acetyl transferase (CAT) activities.

Key words: Avidin, *AVR4* promoter, chloramphenicol acetyl transferase.

INTRODUCTION

Avidin gene when expressed in oviductal tissues of oviparous animals and in response to inflammation results in the production of a tetrameric protein (Green, 1975; Zerega et al., 2001). The avidin protein is heterogeneous in function and shows variation in its several properties, including biotin binding, thermostability, antigenicity and immunomodulation (Bayer and Wilchek, 1990; Houen and Hansen, 1997; Chinol et al., 1998). These variations seem to provide indications that avidin gene display diversity in structure even within the 5' flanking promoter region containing a network of regulatory cis elements such as hormone response elements (HRE), heat shock response element (HSE) and acute phase protein (APRE) (Yamamoto, 1985; Bienz and Phelam, 1986). These consensus sequences have been found to be highly conserved in several organisms and provide domains for co-operativity via DNA-DNA interactions and serve as enhancer sequences for transcriptional factor mediation of avidin gene expression (Wallen et al., 1995; Ahlroth et al., 2001a). However, the patterns of interactions, number and type of response elements involved in the regulation of avidin gene expression are inadequately understood, and supportive experimental studies to improve understanding are few. Hybrid avidin mutants have been expressed in babiculovirus infected insects to enhance biotin binding for improved application as a detection tool in immunology, histopathology and molecular biology (Airenne et al., 1997). Furthermore, Marttila et al. (2000) engineered the biotin- binding pocket in avidin protein mutants to alter glycosylation and increase stability under high temperatures. By virtue of high conserved sequence and exons homology, avidin related genes (AVRs) have been found as perfect analogues of avidin gene for genetic studies (Laitinen et al., 2002). This is supported by the recovery of *AVR1*, *AVR2* and *AVR3* mRNAs from chicken during inflammatory conditions but no clues as to whether these transcripts express proteins were given (Kunnas et al., 1993). *AVR4* and *AVR5* are the other human avidin related genes that have been extensively studied (Keinanen et al., 1994).

Gene deletion experiments are exceedingly useful in the understanding of gene functions and regulation. In this study, the avidin *AVR4* promoter subcloned into

*Corresponding author: E-mail: bamwal@yahoo.com

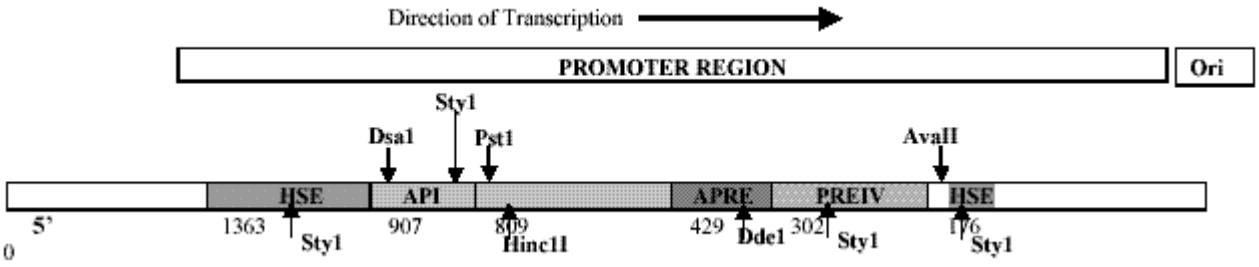

Figure 1. Avidin related gene 4 (*Avr4*) promoter region showing the map positions of consensus response elements from transcription start site and restriction enzyme cleavage sites.

pBLCAT2 expressing chloramphenicol acetyltransferase (CAT) was subjected to timed deletions. The influence of promoter activity was investigated by CAT activity.

MATERIALS AND METHODS

Recombinant plasmid and vectors

Chloramphenicol acetyltransferase plasmid vector (pBLCAT2) and *AVR4* promoter region ligated to pBLCAT2 to produce a recombinant plasmid Avr4pBLCAT2 was obtained from the Dept of Molecular Medicine University of Jyvaskyla, Finland. Stock culture of *E. coli* HB101 harbouring pBR322 plasmid was obtained from our laboratory.

The nucleotide sequence *AVR4* promoter region investigated was obtained from EMBL database search with accession number Z22883 and used to map the cleavage sites of the restriction endonucleases used (Figure 1).

Creation of 5' and 3' overhanged Avr4pBLCAT2 plasmids

A 5' overhang of Avr4pBLCAT2 susceptible to Exonuclease III deletion and 3' overhang resistant to the enzyme activity were obtained by SalI and SphI digestion, respectively. The linear plasmids were subjected to Exonuclease III digestion at 25^0C according to manufacturer's instructions. The assay mixtures were incubated at different time intervals to allow sequential deletion of response elements of Avr4 promoter within each recombinant plasmid in 5' - 3' direction. The incubation period ranged from 29 - 59 min and was terminated by the addition of 2μl 0.5M EDTA at 69^0C for 10min. This was followed by agarose gel electrophoresis and the desired bands were excised and eluted by using the QIAEX II (R) method (QIAGEN Inc, Canada), and re-ligated. *E. coli* DH5α cells were made competent prior to transformation with the plasmids according to the protocol of Hanahan et al. (1983).

Each AVR4pBLCAT2 mutant was digested with a pair of restriction enzymes to confirm the deletion of an upstream response element and the presence of the expected terminal response element as indicated in Figure 1. For instance a pair of DsaI and StyI was used to confirm the absence of HSE and the presence API as the terminal response element in AVR4CAT2 mutant 1 (API-API?-APRE-PREIV-HSE-pLCAT2).

Chloramphenicol acetyl transferase (CAT) assay

CAT activity *in vitro*, as a measure of chloramphenicol degradation in broth was determined by modifying the protocol of Charles and Alan (1999). Each *Avr4 E.coli* transformant (10^7 cells) was grown aerobically with shaking at 30^0C in LB medium containing 20 □g/ml chloramphenicol (Sigma, USA). At 2 hourly intervals cells were pelleted by centrifugation (3000 rpm for 5 min) and aliquots of supernatants were extracted with ethylacetate (equal volume). The resulting organic phase was dried under reduced pressure and heat and the extract reconstituted with 20 μl ethylacetate. The concentration of chloramphenicol in the reconstituted extract was determined by extrapolation from chloramphenicol standard curve as previously described (Shaw, 1975) CAT activity was measured as the micrograms of chloramphenicol degraded per hour under the assay conditions.

RESULTS AND DISCUSSION

In vitro Exonuclease III deletion of the 5' and 3' overhanged Avr4pBLCAT2 generated five distinct mutants at different incubation periods (Table 1). The CAT activities of the constructs are recorded in Figure 2.

The present study is aimed at understanding the importance of the upstream response elements in the regulation of avidin gene expression. The obliteration of DsaI cleavage site but the presence of StyI site confirmed API? as the 5'terminal response elements in mutant I. That mutant 2 has API as its 5'terminal cis element was established by the absence of PstI site within the API? locus of mutant I. Meanwhile, HincII cleavage within the API terminal of mutant 2 was the cause of the 786bp, 1521bp and 2811bp bands observed in this study. Similarly, DdeI produced different fragment length in mutants 3 and 4 to confirm APRE and PREIV as their terminal cis elements. Finally, the obliteration of AvaII site in mutant 5 confirmed PREIV deletion from the precursor mutant. While StyI established HSE as the terminal regulatory motif in this mutant. Similar sequential deletion studies have been conducted elsewhere (Ahlroth et al., 2001b).

The observed increased CAT activity among the transformants implies decreased promoter activity with decreased promoter length, indicating the importance of the different promoter elements.

In conclusion, the successful transformation of *E. coli* DH5α with all the generated mutants as observed in this study coupled with altered gene expression suggests a

Table 1. *AVR4* promoter constructs generated at different time intervals.

Mutant	Promoter element(s)	Generation time (min)
Avr4pBLCAT2$_{907-176}$	API-API?-APRE-PREIV-HSE	32
Avr4pBLCAT2$_{809-176}$	API?-APRE-PREIV-HSE	32
Avr4pBLCAT2$_{789-176}$	APRE-PREIV-HSE	36
Avr4pBLCAT2$_{429-176}$	PREIV-HSE	41
Avr4pBLCAT2$_{302-176}$	HSE	45

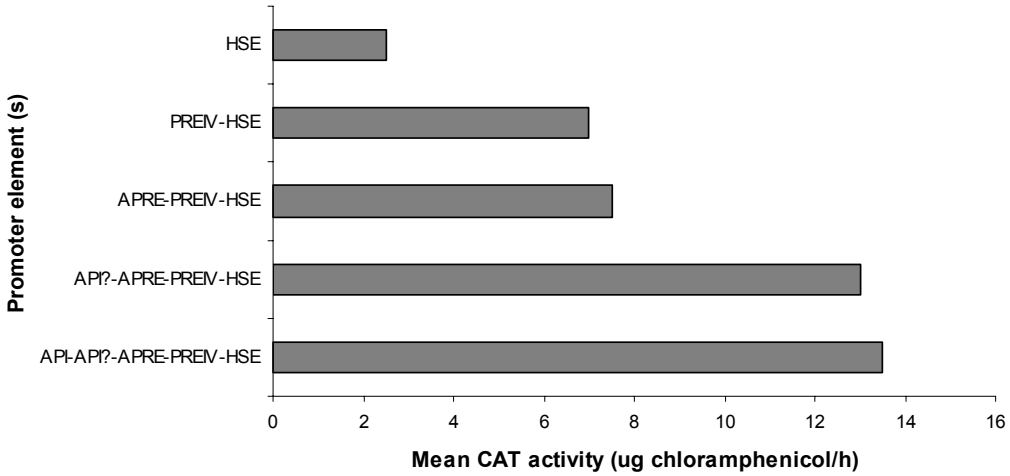

Figure 2. Mean CAT activity of the *Avr4* promoter deletion constructs.

possible means of studying avidin gene expression. There is also the need to ascertain the stability of these mutants for long-term storage and use towards elucidating the mechanisms of avidin gene expression as well as applicability in future avidin-biotin technological challenges.

REFERENCES

Ahlroth MK, Alhroth P, Kulomaa MS (2001b). Copy - number flunctuation by unequal crossing - over in chicken avidin gene family. Biochem Biophys Res Commun. 26: 400 – 406.

Ahlroth MK, Grapputo A, Laitinen OH, Kulomaa MS (2001a). Sequence features and evolutionary mechanisms in the chicken avidin gene family. Biochem. Biophys. Res. Commun. 285: 734-741.

Airenne KJ, Oker-Blom C, Majormaki VS, Bayer EA, Wilchek M, Kulomaa MS (1997). Production of biologically active recombinant avidin in baculovirus -infected insect cells. Protein Expr. Purif. 9: 100-108.

Bayer EA, Wilchek M (1990). Application of avidin-biotin technology to affinity -based separations. J. Chromatogr. 510: 3-11.

Bienz M, Pelham RB (1986). Heat shock regulatory elements function as an inducible enhancer when linked to a heterologous promoter. Cell 45: 753-760.

Chinol M, Casalini P, Maggiolo M, Canevari S, Omodeo ES, Caliceti P, Veronese FM, Cremonesi M, Chiolderio F, Nardone E (1998). Biochemical modifications of avidin improve pharmacokinetics and biodistribution and reduced immunogenicity. Br. J. Cancer 78: 189-197.

Green NM (1975). Avidin. Adv. Prot. Chem. 29: 85–133.

Hanahan D (1983). Studies on transformation of *E. coli* with plasmids. J. Mol. Biol. 166: 557-580.

Houen G, Hansen K (1997). Interference of sugars with the binding of biotin to streptavidin and avidin. J. Immunol. Methods 210: 115-123.

Keinanen RA, Wallen MJ, Kristo PA, Laukkanen MO, Toimela TA, Helenius M, Kulomaa MS (1994). Molecular cloning and nucleotide sequence of chicken avidin-related genes 1-5. Eur. J. Biochem. 220: 615–621.

Kunnas TA, Wallen MJ, Kulomaa MS (1993). Induction of chicken avidin and related mRNAs after bacterial infection. Biochim. Biophys. Acta 1216: 441- 445.

Laitinen OH, Hytopnen VP, Ahlroth MK, Pentikainen OT, Gallacher C, Norlund HR, Ovd V, Martilla AT, Porkka E, Heino S, Johnson MS, Airenne KJ, Kulomaa MS (2002). Chicken avidin-related proteins show altered biotin-binding and physico-chemical properties as compared with avidin. Biochem. J. 363: 609–617.

Marttila AT, Laitinen OH, Airenne KJ, Kulik T, Bayer EA, Wilchek M, Kulomaa MS (2000). Recombinant NeutraLite avidin: a non-glycosylated, acidic mutant of chicken avidin that exhibits high affinity for biotin and low non-specific binding properties. FEBS Lett. 467: 31-36.

Shaw WV (1975). Chloramphenicol acetyltransferase from chloramphenicol resistance bacteria. Methods Enzymol. 43: 737-755.
Sohaskey CD, Barbour AG (1999). Esterases in serum-containing growth media counteract chloramphenicol acetyltransferase activity *in vitro*. Antimicrob. Agent Chemother. 43: 655-660.
Wallen MJ, Laukkanen MO, Kulomaa MS (1995). Cloning and sequencing of the chicken egg-white avidin-encoding gene and its relationship with the avidin-related genes (Avr1-Avr5). Gene 61: 205-209.
Yamamoto KR (1985). Steroid receptor regulated transcription of specific genes and gene networks. Annu. Rev. Genet. 19: 209 - 252.
Zerega B, Camardella L, Cermelli S, Sala R, Candedda R, Descalzi-Candedda F (2001). Avidin expression during chick chondrocyte and myoblast development *in vitro* and *in vivo*: regulation of cell proliferation. J. Cell Sci. 114: 1473-1482.