Vol. 209, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 6, 1995 Pages 126-130

Mutational Study at Ser300 Position of the Escherichia coli Lactose Repressor

S. Miura-Onai, M. Yabuta, and K. Ohsuye*

Suntory Institute for Medicinal Research & Development, 2716-1 Kurakake, Akaiwa, Chiyoda-machi, Ohra-gun, Gunma 370-05, Japan

Received February 21, 1995

SUMMARY : We have previously reported that a Ser300Asn mutant of the Escherichia coli lactose repressor protein produced a temperature-sensitive phenotype. In order to analyze the structure-function relationship of the lactose repressor protein, we conducted 18 amino acid substitutions at this Ser 300 site by using *in vitro* mutagenesis. The substitutions at this position that exhibited repressors with the wild-type phenotype *in vivo* were Gly, Ala, Ile, Thr, Met and Cys; the other 10 substitutions examined (Leu, Val, Tyr, Trp, Asp, Glu, Gln, His, Lys and Arg) resulted in the lacI$^-$ phenotype. In addition, the Ser300Phe mutation resulted in the lacI^{ts} phenotype, while the Ser300Pro mutation resulted in lacI^{ts,s}. It seems likely that the Ser300 position plays an important role in oligomer formation and inducer binding.

The lac repressor coded by the lacI gene is a tetrameric protein comprising four identical subunits, each consisting of 360 amino acids (1). This subunit consists of two functional domains, the N-terminal 59 amino acid residues of the protein that binds the operator DNA, and the remaining "core" region of the protein that is required for both oligomer formation and binding to an inducer such as IPTG (2, 3). Many studies on the structure-function relationship of the lac repressor have been carried out by suppressor mutation, and 13 amino acid replacements were produced by suppressing an amber codon (4). Over 1600 single amino acid substitutions of the lac repressor have been reported by using this method (4).

* To whom correspondence should be addressed: Fax: +81-276-86-5787.

Abbreviations: IPTG, isopropyl-β-D-thiogalactopyranoside; ONPG,ortho-nitro-phenyl-galactoside.

0006-291X/95 $5.00

In the previous study, we demonstrated that a Ser300Asn mutation in the C-terminal core region produced a temperature-sensitive phenotype (5). Ser300 is thought to be an important site for tetramer formation or inducer binding. In order to explore further the structure-function role of Ser300 in the lac repressor, site-directed mutagenesis was used to replace this residue with 18 amino acids. The mutants with altered repressors displayed a variety of phenotypes.

MATERIALS AND METHODS

E. coli MW3110, which is a lacI$^-$ lacZ$^+$ derivative of W3110, was used as the host strain and for expression studies (5). Synthetic oligonucleotides were prepared with a model 392 DNA synthesizer (Applied Biosystems, California, USA) and purified by reverse-phase HPLC. IPTG and ONPG were obtained from Boehringer Mannheim-Yamanouchi (Tokyo, Japan). Taq DNA polymerase and the restriction enzymes were used by following the manufacturer's instructions. Site-directed mutagenesis was performed with a PCR mixture containg 1 μg of plasmid DNA, each primer at 1 μmol, each dNTP at 0.2 mM, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, gelatin (0.01%) and 2.5 units of Taq DNA polymerase in a final volume was 50 μl. Denaturation was carried out at 94℃ for 1 min, annealing at 55℃ for 2 min, and elongation at 72℃ for 2 min, this cycle being repeated 30 times. A β-galactosidase assay was performed according to the method previously described (6).

RESULTS AND DISCUSSION

Construction of the expression vectors for the mutant lacI analogs

The lacI gene carried by plasmid pMC9 (7) was modified by site-directed mutagenesis with synthetic oligonucleotides to introduce a SalI cloning site, overlapping codons 301 and 302, without changing the coding amino acids. The SalI site is unique and was designed to flank closely the target Ser codon 300. The resulting plasmid was named pMC9-SalI and used as the starting plasmid.

Primer 1 (5'-GGGAATAAGGGCGACACGGA-3') is the 5' end forward sequences of nucleotides 4119-4138 of the pBR322 amp^r gene. The sequences of the mutagenic oligonucleotides are shown in Table 1. Primers 2-9 are the inverse sequences of 969-992 which contain the SalI site. PCR was carried out as described in Materials and Methods, the resulting DNA fragment being digested with BssHI and SalI, and the 0.15kb DNA fragment was isolated from agarose gel.

Table 1 Nucleotide sequence of the primers used for mutagenesis

Primer	Sequence	Amino acids changed
2	5'-AAGCGGTCGAC[AC(A, G, C)]GGTTGCCCC-3'	Cys, Arg, Gly
3	5'-AAGCGGTCGAC[CT(G, C, T)]GGTTGCCCC-3'	Glu, Gln, Lys
4	5'-AAGCGGTCGAC[CA(A, C, T)]GGTTGCCCC-3'	Met, Val, Leu
5	5'-AAGCGGTCGAC[AG(C, G, T)]GGTTGCCCC-3'	Thr, Pro, Ala
6	5'-AAGCGGTCGAC[GT(A, C, G)]GGTTGCCCC-3'	His, Asp, Tyr
7	5'-AAGCGGTCGAC[GAA]GGTTGCCCC -3'	Phe
8	5'-AAGCGGTCGAC[CCA]GGTTGCCCC-3'	Trp
9	5'-AAGCGGTCGAC[TAT]GGTTGCCCC-3'	Ile

[a] The position of the SalI site is underlined. [b] Boxes show the codons corresponding to the position of amino acid number 300.

This isolated fragment was ligated with SalI-BamHI (1.0kb) and BamHI-BssHI (4.9kb) of the pMC9-SalI plasmid. The ligated mixture was used to transform E. coli strain MW3110, and the transformants were incubated overnight on LB agar plates containing ampicillin (50 μg/ml) at 30℃. DNA sequencing by the dideoxy chain termination method was used to screen the mutants and to verify the entire sequence of the mutant DNA.

Phenotype analysis of the mutants

MW3110 strains carrying each plasmid were grown overnight in an LB medium containing 50 μg/ml of ampicillin at 30℃. An aliquot of the overnight culture was inoculated into the same medium and cultured at 30℃ and 37℃ in the presence or absence of the inducer IPTG. Cells were collected and subjected to the β-glactosidase assay with ONPG as a substrate, the results of this assay being shown in Table 2. The character of the side chain at this position could exert a significant influence on the surrounding structure that is required for oligomer formation and inducer binding. Substitutions at position 300 that produced repressors with the wild-type phenotype *in vivo* were Ser300Gly, Ser300Ala, Ser300Ile, Ser300Thr, Ser300Met and Ser300Cys; all the other substitutions examined resulted in the $lacI^-$, $lacI^{ts}$ or $lacI^{ts,s}$ phenotype. Approximately 30% (6/18) of the substitutions were tolerant to replacement, and about 50%

Table 2 Effect on phenotypic changes by amino acid substitution at the Ser300 position of the E. coli lac repressor

Phenotype	Amino acid	β-galactosidase activity (units / ml)			
		IPTG(-)		IPTG(+)[a]	
		30℃	37℃	30℃	37℃
$lacI^+$	Ser[b]	12	13	1977	3076
	Gly	16	62	2915	3416
	Ala	46	43	1834	3261
	Ile	13	44	2517	3458
	Thr	33	46	2363	3753
	Met	20	67	2405	2861
	Cys	15	19	2389	3708
$lacI^-$	Leu	3841	4648	4481	5201
	Val	3009	5208	3176	4805
	Tyr	2243	3583	3404	4218
	Trp	3455	3912	2943	4867
	Asp	3320	3465	3975	4214
	Glu	3554	3588	3568	4724
	Gln	1484	2654	2483	2824
	His	3656	2623	3606	3815
	Lys	3574	4241	4018	4368
	Arg	3650	4511	3288	4225
$lacI^{ts}$	Asn[c]	18	752	1894	3393
	Phe	33	870	2474	3706
$lacI^{ts, s}$	Pro	22	418	211	626

[a] A final concentration of 10 mM IPTG was used; [b]wild type; [c]Ser300Asn mutant.

(10/18) of the substitutions led to the $lacI^-$ phenotype. Replacement of Ser300 with bulky or charged amino acids resulted in a loss of the repressor activity and led to the $lacI^-$ phenotype (Ser300Leu, Ser300Val, S300Tyr, Ser300Trp, Ser300Asp, Ser300Glu, Ser300His, Ser300Lys, Ser300Arg and Ser300Gln). In a previous report, Ser300Arg resulted in the $lacI^-$ phenotype (8), the same result being obtained in our present work. It is interesting that Ser300Leu and Ser300Val also gave $lacI^-$, while, Ser300Ile resulted in the $lacI^+$ phenotype. New temperature-sensitive mutations, Ser300Phe and Ser300Pro, were identified. The Ser300Pro mutation also showed the $lacI^S$ phenotype; that is, induction with IPTG did not occur at 30 ℃ and 37℃. The region encompassing residues 226 to 337 has been predicted to consist of an extensive β-sheet formation punctuated by the β-turn (9). We speculate that this β-turn occurs at the Ser300 position , since both Ser and Pro residues exhibit a very high β-turn potential. Many $lacI^S$ sites

have been identified, one set of sites occurring at different positions (4). Miller *et al.* have speculated that the $lacI^S$ cluster might represent the end of a β-turn and the crevice into which the IPTG molecule fits is formed between these clusters on one side (4). Therefore, a conformational change surrounding the β-turn caused by the substitution of Ser300 to Pro300 might interrupt the fitting of the IPTG molecule to the crevice.

We have demonstrated in this study that the Ser300 position has an important function in the molecule. These results provide valuable information for structure-function studies of the lac repressor protein.

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