Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1681 (2005) 134–149

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

http://www.elsevier.com/locate/bba

Molecular characterization of NADase-streptolysin O operon of hemolytic streptococci☆

Hisashi Kimoto[a,*], Yutaka Fujii[b], Yoshifumi Yokota[a], Akira Taketo[c]

[a]Department of Molecular Genetics, Faculty of Medicine, Fukui University, 23-3 Shimoaizuki, Matsuoka, Fukui 910-1193, Japan
[b]Department of Molecular Biology and Chemistry, Faculty of Medicine, Fukui University, 23-3 Shimoaizuki, Matsuoka, Fukui 910-1193, Japan
[c]Department of Environmental and Biotechnological Frontier Engineering, Fukui University of Technology, 3-6-1 Gakuen, Fukui 910-8505, Japan

Received 22 July 2004; accepted 29 October 2004
Available online 11 November 2004

Abstract

Whether *slo*, the gene encoding streptolysin O (SLO), a streptococcal cytolysin, has its own promoter or not is unsettled as yet. Present analyses demonstrate that *slo* is a member of an operon covering the upper-stream *nusG* and *nga* (NADase) genes, from which transcription of *slo* proceeds polycistronically, and major transcript is produced by readthrough from *nga* promoter. Mutational conversion of the sixth nucleotide T at the putative −10 region of chromosomal *nga* gene into C caused a drastic decrease in both NADase and SLO activities and the disappearance of the two corresponding mRNA bands from the Northern blot profile. The initiation site of the transcription was determined at 56 bp upstream (NusG gene) and 25 bp upstream (NADase gene) of each initiation codon. Although the promoter region of *slo* gene is highly conserved between group A and C streptococci, the proper *slo* promoter is nonfunctional in group C strain H46A. Moreover, commonly conserved arrangement was limited to the *nusG-nga-orf1-slo* region. These results indicate an intimate relationship between NADase and SLO in the regulation of their biosynthesis. Additional results suggest that NADase, synthesized as precursor with feeble activity, is activated by removing the carboxyl terminal region during or after secretion into culture medium.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Streptococci; Gene targeting; Streptolysin; SLO; NADase; *nga*

1. Introduction

Streptolysin O (SLO), an oxygen-labile cholesterol-dependent cytolysin, is an extracellular protein produced by most strains of β-hemolytic streptococci belonging to Lancefield group A (*Streptococcus pyogenes*, GAS) and many strains of group C (GCS, including *Streptococcus dysgalactiae* subsp. *equisimilis*) and G (GGS) streptococci [1]. In addition to GAS, human isolates of GCS and GGS streptococci have been implicated as causative agents in outbreaks of purulent pharyngitis, wound infections and streptococcal toxic shock-like syndrome. SLO belongs to a single, highly homologous family of cytolysins found in species of five genera: *Streptococcus*, *Bacillus*, *Clostridium*, *Listeria*, and *Arcanobacterium* [2,3]. Upon binding to cholesterol-containing membrane as monomers, these proteins oligomerize to form large pores, comprising about 50 monomer subunits with internal diameter of up to 30 nm

Abbreviations: Ap, ampicillin; Em, erythromycin; Sp, spectinomycin; Tet, tetracycline; THY medium, Todd Hewitt broth supplemented with 1% yeast extract; LB, Luria-Bertani broth; BSA, bovine serum albumin; EDTA, ethylene diamine tetraacetic acid; TE, tris–EDTA buffer; SSC, standard saline citrate; PBST, phosphate-buffered saline containing 0.05% Tween 20; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis; CBB, Coomassie brilliant blue; PVDF, polyvinylidene difluoride; PCR, polymerase chain reaction; RT, reverse transcription; dGTP, deoxyguanosine 5′-triphosphate; TS, thermosensitive; *ori*, replication origin; ORF, open reading frame; HU, Hemolytic activity; SLO, streptolysin O; SLS, streptolysin S; ASLO, anti-streptolysin O serum; NADase, NAD-glycohydrolase; *nga*, NADase gene; *sag*, SLS-associated gene; CFU, colony-forming units; GAS, group A *Streptococcus*; GBS, group B *Streptococcus*; GCS, group C *Streptococcus*; GGS, group G *Streptococcus*; bp, base pair

☆ The nucleotide sequences reported in this paper have been submitted to DDBJ with accession numbers: AB128035, AB128036.

* Corresponding author. Tel.: +81 776 61 8314; fax: +81 776 61 8164.
E-mail address: hisashi@fmsrsa.fukui-med.ac.jp (H. Kimoto).

0167-4781/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbaexp.2004.10.011

[2,4]. These large pores permit permeation of not only ions and small metabolites but also macromolecules. Recently, Madden et al. [5] proposed for the first time from Gram-positive bacteria, a cytolysin-mediated translocation system which is a functional equivalent to the type III secretion system in Gram-negative bacteria [6]. Their data support a model in which the effector NADase (NAD-glycohydrolase), encoded by *nga* (*spn*), the gene located upstream of the *slo* gene, is transported through the SLO pore into the host cell [5]. Thus, SLO is of considerable biochemical and clinical interest and elucidation of transcriptional regulation of *slo* gene is important for understanding streptococcal pathogenesis as well. In contrast to extensive research on the structure and function of SLO protein, little information is available on the regulation of the *slo* gene [7–9].

The *slo* gene was first cloned and sequenced by Kehoe and Timmis [7] and Kehoe et al. [10] from *S. pyogenes* strain Richards. Their transposon insertion and truncation experiments of the *slo* region using *E. coli* system suggested the existence of two strong and weak *slo* promoters upstream from the open reading frame [7]. Savic et al. [8] also reported that *slo* possesses its own autonomous promoter, which is of low strength and is located 155 bp upstream of the *slo* gene. On the other hand, Madden et al. [5] reported that insertional inactivation of *nga* (*spn*) gene in *S. pyogenes* strain JRS4 abolished expression of SLO, and a nonpolar in-frame deletion mutant of *nga* (*spn*) gene did not affect expression of SLO. This mutational experiment suggests that *nga* (*spn*) gene and *slo* gene are cotranscribed from a promoter located upstream of *nga* (*spn*) gene (*nga-slo* operon).

Recently, we cloned the *slo* genes of *S. pyogenes* strain Sa and *S. dysgalactiae* subsp. *equisimilis* strain H46A [11,12]. The cloned *slo* genes from Sa and H46A were, however, inactive in the production of each hemolysin protein in *E. coli*, as examined by blood agar plate assay and cell lysate analysis. This finding is consistent with the report of Madden et al. [5] on knockout mutation of *S. pyogenes* strain JRS4 *nga* (*spn*) gene. Together, these results suggest that transcriptional regulation of *slo* gene might vary with streptococcal species and strain. Here, we present a more detailed study of the transcriptional mechanism of *nga-slo* operon.

2. Materials and methods

2.1. Materials

Spectinomycin dihydrochloride, mutanolysin, and lysozyme were purchased from Sigma Chemical Co. (St. Louis, MO, USA); ampicillin sodium, erythromycin and tetracycline hydrochloride were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DNA modification and restriction enzymes were products from Takara Shuzo Co., Ltd. (Kyoto, Japan), Toyobo Co., Ltd. (Osaka, Japan) or Nippon gene (Toyama, Japan). Marker DNA (lambda DNA digested with *Sty*I) was obtained from Nippon gene. Yeast extract and tryptone were purchased from Difco Laboratories (Detroit, MI, USA). All other chemicals were reagent grade or molecular biological grade.

2.2. Bacterial strains and culture conditions

The bacterial strains used in this study are listed in Table 1. Streptococci were grown in Todd Hewitt broth (Becton Dickinson, Cockeysville, MD, USA) supplemented with 1% yeast extract (THY medium) at 30 °C or 37 °C. *Escherichia coli* XL1-Blue (Stratagene Inc., La Jolla, CA, USA) was used as the cloning host for recombinant plasmids and cultured in Luria-Bertani broth (LB) at 37 °C. When necessary, antibiotics were added to the medium at the following concentrations: erythromycin (Em), 200 μg/ml for *E. coli* and 10 μg/ml for streptococci; spectinomycin (Sp), 100 μg/ml for *E. coli* and 30 μg/ml for streptococci; tetracycline (Tet), 20 μg/ml for *E. coli* and 2 μg/ml for streptococci; ampicillin (Ap), 100 μg/ml for *E. coli*. Blood agar plates were made by the addition of 10% (vol/vol) defibrinated sheep blood to THY agar medium (for streptococci) or LB agar medium (for *E. coli*).

2.3. DNA manipulations

Plasmid pUC19 was used as the cloning vector [13]. Electroporation was used for introduction of plasmid DNA

Table 1
Bacterial strains used in this study

Bacterial strain	Genotype or phenotype[a]	Reference or source
S. dysgalactiae subsp. *equisimilis* strains		
H46A (ATCC 12449)	Wild type, group C *Streptococcus*	[7]
KT1	Isogenic mutant, *slo::Em^r*, Em^r, SLO$^-$	[19]
KT2	Isogenic mutant, *sagB::Sp^r*, Sp^r, SLS$^-$	[19]
KT3	Isogenic mutant, *slo::Em^r*, *sagB::Sp^r*, Em^r, Sp^r, SLO$^-$, SLS$^-$	[19]
KT4	KT2 carrying pHY300PLK, Sp^r, Tet^r	This study
KT5	KT3 carrying pSLO1, Em^r, Sp^r, Tet^r	This study
KT6	KT3 carrying pSLO2, Em^r, Sp^r, Tet^r	This study
KT7	KT3 carrying pSLO3, Em^r, Sp^r, Tet^r	This study
KT8	Isogenic mutant, *nusG::*pCONS.h8, Em^r, Sp^r, SLS$^-$	This study
KT9	Isogenic mutant, *nusG::*pCONS.d, Em^r, Sp^r, SLS$^-$, NADase$^-$, SLO$^-$	This study
KT10	KT9 carrying pNGA1, Em^r, Sp^r, Tet^r, NADase$^+$, SLO$^+$	This study
S. pyogenes strain		
Sa	Wild type, group A *Streptococcus*	[42]
E. coli strain		
XL1-Blue	*recA1, endA1, gyrA96, thi-1 hsdR17, supE44, relA1, lac/F'* [*proAB lacI^q lacZ M15::Tn10* (*Tet^r*)], Tet^r	Stratagene

[a] Em^r, erythromycin resistance; Sp^r, spectinomycin resistance; Tet^r, tetracycline resistance; Ap^r, ampicillin resistance.

Download English Version:

https://daneshyari.com/en/article/9885599

Download Persian Version:

https://daneshyari.com/article/9885599

Daneshyari.com