レンサ球菌のサイクリックADPリボース代謝酵素の一次構造の決定と活性部位の解析

1997 Fiscal Year Final Research Report Summary

The gene encoding streptococcal NAD^+-glycohydrolase : datermination of primary structure and analysis of enzymatic active sites

Research Project

Project/Area Number

08670303

Research Category

Grant-in-Aid for Scientific Research (C)

Allocation Type

Single-year Grants

Section

一般

Research Field

Bacteriology (including Mycology)

Research Institution

Kanazawa University

Principal Investigator

KARASAWA Tadahiro School of Medicine, Kanazawa University, Assistant Professor, 医学部, 講師 (90251917)

Project Period (FY)

1996 – 1997

Keywords

Streptococcus pyogenes / NAD / cyclic ADP-ribose / cloning

Research Abstract

1. Cloning of a DNA fragment corresponding to the N-terminus amino acid sequence of streptoccal NAD^+-glycohydrolase
An N-terminus amino acid sequence of streptococcal NAD^+-glycohydrolase was determined by using the purified enzyme. To amplify a DNA fragment which corresponded to the N-terminus amino acid sequence, PCR-primers were designed and PCR was done. A DNA fragment which had the expected size (120 bp) was amplified. The nucleotide and the deduced amino acid sequences of this fragment revealed that this fragment was a part of the gend

encoding NAD^+-glycohydrolase.
2. Primary structure of the gene encoding NAD^+-glycohydrolase and construction of its expression system
Southern blot analysis was done using the 120-bp DNA fragment. It was shown that the gene encoding NAD^+-glycohydrolase was contained as a single copy in chromosome. Plasmid libraries of chromosomal DNA from Streptococcus pyogenes C2556 were constructed. Single specific-PCR was preformed using primers designed from sequences of the plasmid and of the 120-bp DNA fragment. The nucleotide sequence of the amplicon was determined. The open reading frame was ca.1400 bp. Calculated molecular weight and pI were ca.50,000 and 8.5, respectively. These properties were consistent with those of the purified enzyme. Full length of the gene encoding NAD^+-glycohydrolase was cloned and expressed in Escherichia coli. Cell extracts showed not only NAD^+-glycohydrolase activity bu also cyclic ADP-ribose-synthesis and -hydrolysis activities. Enzymatic active portions are currently under investigation.

Research Products (8 results)

All | Other

All | Publications (8 results)

[Publications] 唐澤 忠宏: "Streptococcus pyogenesの産生するサイクリックADPリボース代謝酵素" 第43回毒素シンポジウム予稿集. 89-91 (1996)

[Publications] 唐澤 忠宏: "A群レンサ球菌の産生するNAD分解酵素" 長崎大学熱帯医学研究所共同研究報告集. 144-145 (1996)

[Publications] 唐澤 忠宏: "A群レンサ球菌cyclic ADP-ribose合成・分解酵素-遺伝子クローニングを中心として-" 長崎大学熱帯医学研究所共同研究報告集. (印刷中). (1997)

[Publications] Nata, K: "Human gene encoding CD38 (ADP-ribosyl cyclase/cyclic ADP-ribonse hydrolase) : organization,nucleotide sequence and alternative splicing." Gene. 186. 285-292 (1997)

[Publications] Karasawa, T.: "Cyclic ADP-ribose metabolizing enzyme produced by Streptococcus pyogenes (in Japanese)" Proceedings of the 43th Symposium on Toxins. 89-91 (1996)

[Publications] Karasawa, T.: "NAD^+-glycohydrolase produced by Streptococcus pyogenes (in Japanese)" Annual Reports of Institute of Tropical Medicine, Nagasaki University. 144-145 (1996)

[Publications] Karasawa, T.: "Streptococcal cyclic ADP-ribose forming/hydrolyzing enzyme : cloning and determination of nucleotide sequence (in Japanese)" Annual Reports of Institute of Tropical Medicine, Nagasaki University. (in press). (1997)

[Publications] Nata, K.: "Human gene encoding CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) : organization, nucleotide sequence and alternative splicing." Gene. 186. 285-292 (1997)

URL: https://kaken.nii.ac.jp/report/KAKENHI-PROJECT-08670303/086703031997kenkyu_seika_hokoku_

Published: 1999-03-15