Proc. KSAM International Symposium
June 20-22, 2001, Chonan, Korea

Tk-PTP, Protein Tyrosine Phosphatase from Hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1 : Enzymatic Characteristics and Isolation of its Substrate Proteins

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Abstract

The *Tk-ptp* gene encoding a protein tyrosine phosphatase (PTPase) from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1 was cloned and sequenced. Sequence analysis indicated that *Tk*-ptp encoded a protein consisting 147 amino acid residues (16,953 Da). The wild type and the mutants were expressed in *Escherichia coli* cells as His-tagged fusion proteins and examined for enzyme characteristics. *Tk*-PTP possessed two unique features that were not found in eucaryal and bacterial counterparts. First, the recombinant *Tk*-PTP showed the phosphatase activity not only for the phosphotyrosine but also phosphoserine. Second, the conserved Asp (Asp-63), which was considered to be a critical residue, was not involved in catalysis. In order to know a specific substrate for *Tk*-PTP, C93S mutant was used to trap substrate protein. Proteins of 120, 60 and 53 kDa were isolated specifically from KOD1 cell lysates by affinity chromatography with *Tk*-PTP-C93S. It is suggested that these proteins are tyrosine-phosphorylated substrates of *Tk*-PTP.

Introduction

Protein tyrosine phosphorylation is evolutionarily conserved among eucarya, bacteria, and archaea (1). A large number of protein tyrosine phosphatases have been observed from eucarya and shown to play a key role in the control of various physiological processes, including growth, differentiation, cell cycle regulation, cytoskeletal function, as well as in the etiology and pathogenesis of certain diseases (2). In the present study, to determine the cellular function of the PTPase in archaea, we cloned the *Tk-ptp* gene, which encodes a new member of the PTPase family from *Thermococcus kodakaraensis* KOD1, and characterized the recombinant protein. Mutant *Tk*-PTP was constructed for substrate trapping experiments and specific cytoplasmic substrates were screened.

Materials and Methods

Site-directed mutagenesis: The mutant proteins of *Tk*-PTP were obtained through the use of site-directed mutagenesis using PCR (overlap extension method) as described previously (3).

Protein expression and purification: His-tagged *Tk*-PTP wild type and mutants were each expressed in *E.* coli BL21(DE3) cells and purification was carried out based on the produre of (4).

Phosphatase assay: All enzyme assays were performed as described using free O-phosphotyrosine as the substrate (5).

Preparation of trapping column: HiTrap NHS-activated sepharose column of 1-ml bed volume (Amersham Pharmacia Biotech) was used to immobilize 10 mg of purified recombinant *Tk*-PTP protein. The coupling procedure was performed as described by the manufactureís protocol.

Results and Discussion

The *Tk-ptp* gene encodes of protein of 147 amino acids with a predicted molecular mass of 16,953 Da and an estimated isoelectric point (pI) of 4.87. The deduced amino acid sequence of *Tk*-PTP is highly homologous to those of other archaea and possesses the PTPase signature motif, IHCMGGLGRSG, that uniquely defines this enzyme family. A series of site-directed mutants were generated by replacing Asp-63 with Ala (D63A), Cys-93 with Ser (C93S) or Ala (C93A), Arg-99 with Lys (R99K) or Met (R99M) in *Tk*-PTP. Recombinant *Tk*-PTP-His_6 showed the phosphatase activity for the phosphoserine as well as phosphotyrosine in vitro, which is a unique archaeal feature that was not found in other eucaryal and bacterial counterparts. Kinetic analysis of *Tk*-PTP-D63A mutant revealed that the conserved Asp-63 of *Tk*-PTP is not directly involved in catalytic activity. These data suggest that the *Tk*-PTP utilizes another negatively charged amino acid besides Asp-63 or catalytic mechanism is different from known system. The Cys-93 and Arg-99 mutants of *Tk*-PTP showed lower k_{cat} and K_m values for the phosphotyrosine, indicating that these cysteine and arginine residues played a critical role in substrate binding and catalysis. Among mutants, C93S possessed the highest affinity to phosphorylated Tyr. In order to know a specific substrate for *Tk*-PTP, C93S mutant was used to trap substrate protein. The experiment by substrate-trapping mutant has revealed that *Tk*-PTP has the substrate proteins of 190, 60 and 53 kDa in KOD1. However, the function of these proteins still remains unclear.

References

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