Identification and characterization of mammalian 5-formyluracil-DNA glycosylase

Mayumi Matsubara¹, Aya Masaoka², Tamon Tanaka¹, Hiroaki Terato¹, Yoshihiko Ohyama¹ and Hiroshi Ide¹
¹Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hirosima 739-8526, Japan and ²Department of Veterinary Science, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Sakai 599-8531, Japan

ABSTRACT
5-Formyluracil is a major oxidative thymine lesion with mutagenic and cytotoxic properties. In this study, we have partially purified and characterized a mammalian 5-formyluracil-DNA glycosylase (FDG) from rat liver. FDG was a monofunctional DNA glycosylase and removed 5-formyluracil, uracil, 5-hydroxyuracil, 5-hydroxymethyluracil in single-stranded and double-stranded DNA. Several lines of evidence indicate that FDG is a rat SMUG1 homologue. Human SMUG1 also exhibited similar enzymatic properties.

INTRODUCTION
In aerobic organisms, reactive oxygen species are produced by normal metabolism and generate oxidative damage to DNA. 5-formyluracil (fU) is a major lesion produced by oxidation of the methyl group of thymine (Fig. 1). fU is mutagenic since the ionized form of fU directs misincorporation of dGMP during DNA replication (1). In addition, fU is toxic for bacterial and mammalian cells (2, 3). In general, oxidative base lesions are repaired by the base excision repair pathway. We have previously shown that Escherichia coli 3-methyladenine DNA glycosylase II (Alk A) efficiently removes fU from DNA (4, 5). However, hMPG, a human functional homologue of Alk A, did not recognize fU. Thus, the mammalian DNA glycosylase for fU has not been identified to date. In the present study, we have partially purified and characterized 5-formyluracil-DNA glycosylase (FDG) from rat liver (6, 7).

RESULTS AND DISCUSSION
A 25-mer oligonucleotide containing site-specific fU was chemically synthesized and purified by HPLC as reported previously (1). The FDG activity was purified by 160-fold from rat liver using several column chromatographic steps. Analysis of FDG by gel filtration chromatography revealed that the molecular mass of FDG was around 35 kDa. When incubated with a duplex oligonucleotide substrate containing fU, FDG excised from DNA fU but did not incise the resulting abasic site. Accordingly, FDG is a monofunctional DNA glycosylase with an N-glycosylase activity alone (Fig. 2). FDG recognized fU, 5-hydroxymethyluracil (hmU), 5-hydroxyuracil (hoU), and uracil (U) in single-stranded (ss) and double-stranded (ds) DNA. Known repair enzymes for oxidative damage such as NTH1, OGG1, NEIL1 and NEIL2 are bifunctional DNA glycosylases with an N-glycosylase activity alone (Fig. 2). FDG recognized fU, 5-hydroxymethyluracil (hmU), 5-hydroxyuracil (hoU), and uracil (U) in single-stranded (ss) and double-stranded (ds) DNA. Known repair enzymes for oxidative damage such as NTH1, OGG1, NEIL1 and NEIL2 are bifunctional DNA glycosylases with an N-glycosylase activity alone. Thus, the enzymatic properties of FDG are distinct from those of other known DNA glycosylases for oxidative damage. In human, uracil-DNA glycosylase (UDG) and single-strand selective...
monofunctional uracil-DNA glycosylase (SMUG1) are known to excise U from ssDNA as well as dsDNA. However, FDG was not inhibited by a UDG inhibitor (UGI), showing that FDG was not UDG. Therefore, FDG is likely a rat homologue of SMUG1. To clarify whether FDG was a rat SMUG1 homologue (rSMUG1), we cloned and expressed rSMUG1. Purified rSMUG1 exhibited a damage specificity and a salt concentration dependence similar to those of FDG. Human SMUG1 (hSMUG1) also showed similar enzymatic properties with respect to the damage specificity and the salt concentration dependence. The activities of FDG and the cell free extract of rat liver for fU and hmU were both inhibited by polyclonal antibodies for hSMUG1. On the basis of the above data, we have concluded that FDG is rSMUG1. So far, SMUG1 is considered as a back-up enzyme of UDG in mammalian cells. However, the present results indicate that SMUG1 is involved in repair not only of uracil resulting from deamination of cytosine but also of oxidative pyrimidine lesions such as fU, hmU and hoU.

ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Scientific Research from JSPS (H.I.).

REFERENCES