Journal of Korean Medical Science
Vol 5, No. 3, 137-143, September 1990

Studies on Rat Liver Nuclear DNA Damaged by Chemical Carcinogen (3'-Me DAB) and AP DNA Endonuclease II. Kinetic Properties of AP DNA Endonucleases in Rat Liver Chromatin*

Yoon Soo Kim, M.D., Ph. D., Jong Wook Kim, M.D., Ph.D.,
Seo Eun Lee M.S., and Sang-Hwan Oh, Ph.D.

Department of Biochemistry, Yonsei University College of Medicine, Seoul, Korea

An experiment was designed to investigate the reaction mechanism of AP (apurinic or apyrimidinic) DNA endonucleases (APcI, APcII, APcIII) purified from rat liver chromation.

Sulfhydryl compounds (2-mercaptoethanol, dithiothreitol) brought about optimal activites of AP DNA endonucleases and N-ethylmaleimide or $HgCl_2$ inhibited the enzyme activities, indicating the presence of sulfhydryl group at or near the active sites of the enzymes. Mg^{2+} was essential and 4mM of Mg^{2+} was sufficient for the optimal activities of AP DNA endonucleases.

Km values of APcI, APcII and APcIII for the substrate (E.coli chromosomal AP DNA) were 0.53, 0.27 and 0.36μM AP sites, respectively. AMP was the most potent inhibitor among adenine nucleotides tested and the inhibition was uncompetitive with respective to the substrate. The Ki values of APcI, APcII and APcIII were 0.35, 0.54 and 0.41mM, respectively. The degree of nick translation of AP DNAs nicked by APcI, APcII and APcIII with Klenow fragment in the presence and absence of T_4 polynucleotide kinase or alkaline phosphatase were the same, suggesting that all 3 AP DNA endonucleases excise the phosphodiester bond of AP DNA strand to release 3-hydroxyl nucleotides and 5-phosphomonoester nucleotides.

Key Words: *AP DNA endonuclease, Kinetic properties, AMP, Strand excision*

INTRODUCTION

The mechanism on repair of AP (apurinic or apyrimidinic) sites in DNA has been known for years in *E.coli* (Ljungquist, 1977), however, the repair mechanism in eukaryotic cells is still uncertain (Gossard & Verly, 1978). It has been believed that the repair of AP sites begins with the incision of the DNA strand near the AP site in order to start the repair synthesis of the damaged DNA strand. AP DNA endonuclease of rat liver chromatin has been purified by Verly et al. (1981) who have shown that it hydrolyses the phosphodiester bond 5' to the AP site, leaving 3'-hydroxy and 5-phosphate ends. AP DNA endonucleases are widely distributed in prokaryotes and eukaryotes (Mosbaugh and Linn, 1980; Verly et al., 1981, Cesar and Verly, 1983), and their properties such as molecular weight, pH optimum, requirement for divalent cations and substrate specificities have been reported to be variable. (VanLanker and Tomura, 1974; Linsley et al., 1977; Shaper et al, 1982; Grafstrom et al., 1982; Goffin and Verly, 1984; Lee et al., 1986). AP DNA endonucleases in rat liver chromatin, nucleoplasm, cytoplasm, plasma membrane, nuclear membrane and mitochondria were found to have different properties (Verly and Thibodeau, 1979; Thibodeau and Ver-

Adress of correspondence: *Yoon Soo Kim, Department of Biochemistry, Yonsei University College of Medicine C.P.O. Box 8044, Seoul 120-749, Korea. Tel: (02) 392-0161 #3061*
**This study was supported by the research grant (1989) of Sam Mi Cultural Foundation on behalf of Korean Medical Association.*

ly, 1980; Goffin and Verly, 1984; Tomkinson et al., 1988) and some kinetic properties of AP DNA endonucleases purified from human fibroblasts have been reported (Mosbaugh and Linn, 1980). Recently, three kinds of AP DNA endonucleases (APcI, APcII & APcIII) in rat liver chromatin have been purified in our laboratory, and it has been shown that APcIII acted on DNA damaged by 3'-methyl-4-monomethylaminoazo benzene, an immediate metabolite of 3'-methyl-4-dimethylaminoazo benzene (3'-Me DAB) (Kim et al., 1990), but the kinetic properties including factors affecting on the enzyme activity have not yet been studied. This paper describes the kinetic characteristics of AP DNA endonucleases purified from rat liver chromatin.

MATERIALS AND METHODS

AMP, N-ethylmaleimide (NEM), ß-mercaptoethanol (2-ME), dithiothreitol (DTT), 2,5-diphenyloxazole (PPO), 1,4-bis-2 (5-pheyloxazolyl) benzene (POPOP), alkaline phosphatase, T_4 polynucleotide kinase were purchased from Sigma Chem. Co. and other reagents were obtained from local suppliers.

Preparation of enzyme

AP DNA endonucleases (APcI, APcII, APcIII) were purified from rat liver chromatin by the method described in our previous paper (Kim et al., 1990).

Enzyme assay

AP DNA endonuclease activity was measured by the method of Thibodeau et al. (1980). To 100ΣI of Tris-HCl buffer (pH8.0) containing 10mM $MgCl_2$, 10mM NaCl and ^{3}H-labeled *E.coli* chromosomal AP DNA (10,000 dpm/14μg DNA), 100μl of the enzyme solution was added and incubated at 37°C for 15min. To stop the reaction, the reaction mixture was transfered to an ice bath and subsequently 100μl of 200mg% bovine serum albumin and 60μl of 30% perchloric acid were added. After 15min, the contents were centrifuged at 10,000x*g* for 15min to sediment high molecular weight DNA. The radioactivities of the DNA fragments in the supernatants were counted, and the enzyme activity was calculated based on the relative counts obtained from the complete breakage of AP sites by alkali treatment)pH12.3) of the enzyme reaction mixture. The enzyme unit (EU) was defined as pmoles of substrate (PA site) excised/min.

The number of AP site was determined by the method of Zubroff and Sarma (1976).

Kinetic analysis of AP DNA endonucleases

Km value of AP DNA endonuclease for the AP DNA was determined from Lineweaver-Burk double reciprocal plot (Segel, 1975) on the concentration of substrate versus enzyme activities. Inhibition mode of AMP on AP DNA endonucleases was determined from the double reciprocal plot, and Ki value for AMP was determined from Dixon plot on the inhibitor concentration versus reaction velocity.

Influence of Mg^{2+} and sulfhydryl agents on AP DNA endonucleases

The effects of sulfhydryl compound on the AP DNA endonucleases were determined by measuring the enzyme activities in the presence or absence of 2-ME, DTT. NEM and $HgCl_2$ known as sulfhydryl inhibitor were tested for their effects by measuring the enzyme activity in the presence of either one. The effect of Mg^{2+} on the AP DNA endonucleases was also determined by the addition of various concentrations of $MgCl_2$ to the reaction mixture.

Determination of excision site of AP DNA strand by AP DNA endonucleases

To 100μl of Tris-HCl buffer (pH8.0) containing 10mM $MgCl_2$, 10mM NaCl and unlabeled AP DNA (31.7μg), each AP DNA endonuclease was added and incubated at 37°C for 15min, then devided into two groups. Alkaline phosphatase (2.0units, pH8.0) was added to one group and T_4 polynucleotide kinase (2units, pH6.0) to the other group. The reaction mixtures were incubated for 15 min at 37°C and then transferred to a 70°C water bath, being kept for 10min to inactivate the enzymes present.

The contents were cooled in ice, and dNTP (13nmol of DATP, dGTP, dCTP + 1μCi of 9pmol [^{3}H]dTTP), 2.5units of *E.coli* DNA polymerase I large fragment (Klenow fragment) were added, and nick translated for 40min at 15°C.

To the reaction mixture, 1ml of 0.1M sodium pyrophosphate solution containing 5% trichloroacetic acid (NaPPT) was added to stop the reaction.

The nick translated DNA was precipitated and sedimented by centrifugation (10,000*g*, 10min). The pellet was washed 3 times with NaPPT and the radioactivities incorporated in nick translated DNA were counted by a liquid scintillation counter (Packard-Tricarb) to estimate the amount of free 3'-OH residues.

RESULTS

Effects of Mg^{2+} and sulfhydryl compounds on the activities of AP DNA endonucleases.

Mg^{2+} ion was essential for the activities of all 3 kinds of chromosomal AP DNA endonucleases, and about 4mM of Mg^{2+} was sufficient for the full activities of the enzymes (Fig. 1). EDTA (1mM), a chelating agent, alleviated the 62% of Mg^{2+} (5mM) effect.

However, addition of 0.1mM of 2-ME to the enzyme reaction mixture increased the activities of APcI, APcII and APcIII by 96%, 15% and 58%, respectively. Addition of 0.1mM DTT to the enzyme reaction mixture enhanced the activity of APcI by 37% and 1.0mM of DTT enhanced the activity by 85% (Fig. 2).

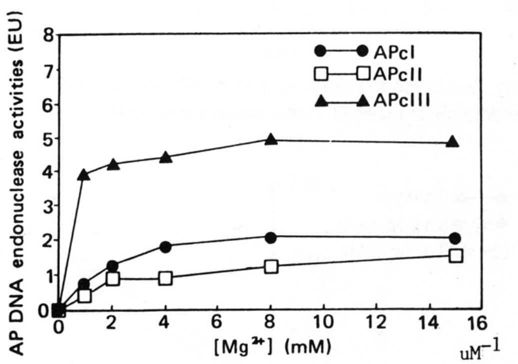

Fig. 1. Effect of Mg^{2+} on AP DNA endonuclease activities in the rat liver chromatin.

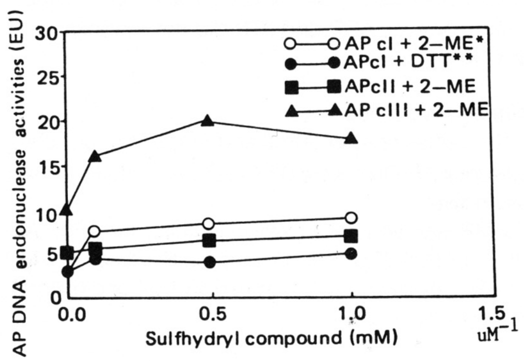

Fig. 2. Effect of sulfhydryl compounds on AP DNA endonuclease activities.
* 2-ME: ß-mercaptoethanol
** DTT: dithiothreitol

Inhibition by NEM, Hg^{2+} and AMP.

NEM (0.05mM) inhibited the activity of APcI by 46% and $HgCl_2$ (0.05mM) inhibited 65% of the activity (Fig. 3).

AMP inhibited all 3 chromosomal AP DNA endonucleases, and 0.1mM of AMP inhibited the activities of APcI, APcII, and APcIII in the presence of 1μM of AP site by 27, 11 and 13%, respectively, and 0.2mM

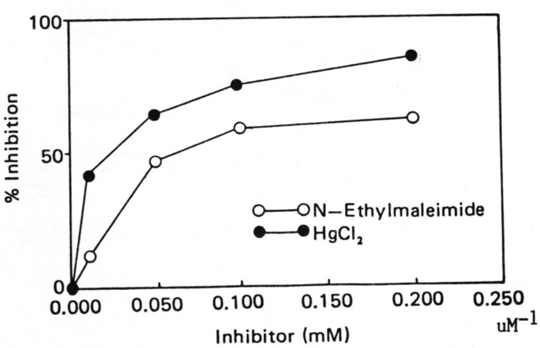

Fig. 3. Effect of N-ethylmaleimide and $HgCl_2$ on AP DNA endonuclease (APcI) activity.

Table 1. Effect of Adenine nucleotides on rat liver chromosomal AP DNA endonucleases

AP DNA endonuclease	Inhibitor concentration (mM)	Enzyme activity (EU)*		
		AMP	ADP	ATP
APcI	None (control)	4.9	4.9	4.9
	0.1	3.6	4.8	5.1
	0.2	3.3	4.7	5.0
APcII	None (control)	8.7	8.7	8.7
	0.1	7.7	8.8	8.7
	0.2	6.6	8.5	8.6
APcIII	None (control)	8.3	8.3	8.3
	0.1	7.2	8.0	8.4
	0.2	5.9	8.0	8.0

*Enzyme activity was measured by the method described in the text. *E.coli* chromosomal AP DNA that had 1μM AP site was used as a substrate, and enzyme unit (EU) is defined as pmole of AP site excised per min.

of AMP inhibited them by 33, 24 and 29% respectively. However, ADP and ATP did not inhibit them at all (Table. 1).

Kinetic Properties.

Km values of APcI, APcII and APcIII for *E.coli* chromosomal AP DNA were 0.53, 0.27 and 0.36μM AP site, respectively.

AMP was an uncompetitive inhibitor for APcI, APcII and APcIII (Fig. 5, 6, 7), and the Ki values of AMP for APcI, APcII and APcIII were 0.35, 0.54 and 0.41mM, respectively (Fig. 8).

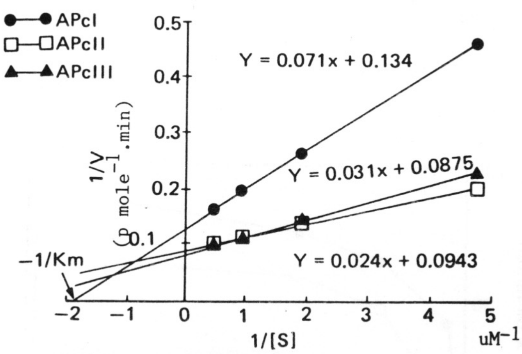

Fig. 4. Lineweaver-Burk reciprocal plot: 1/v versus 1/[S]

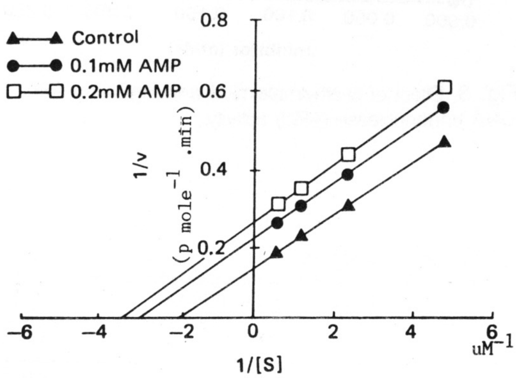

Fig. 5. Lineweaver-Burk reciprocal plot for AP DNA endonuclease (APcI) in the presence of AMP.

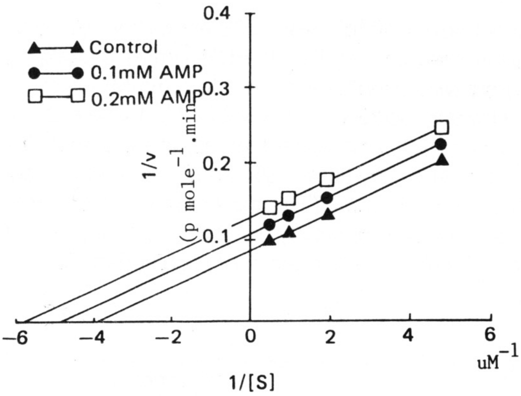

Fig. 6. Lineweaver-Burk reciprocal plot for AP DNA endonuclease (APcII) in the presence of AMP.

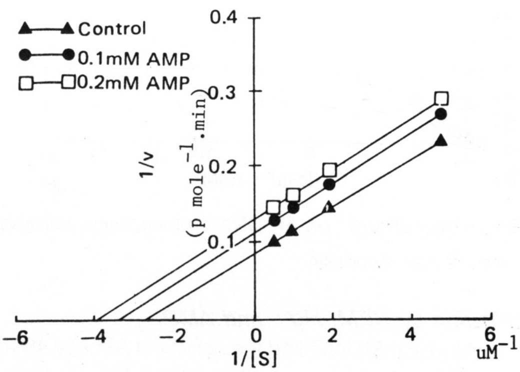

Fig. 7. Lineweaver-Burk reciprocal plot for AP DNA endonuclease (APcIII) in the presence of AMP.

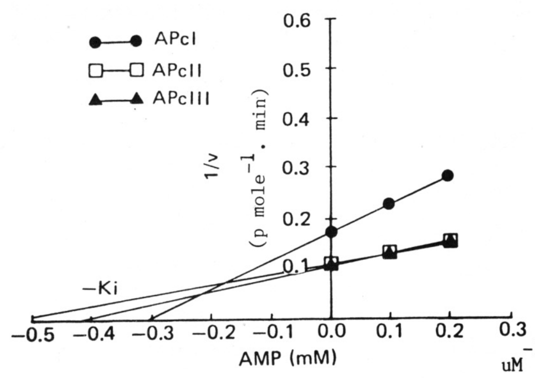

Fig. 8. Dix plot for AMP: 1/v versus [I] at a fixed concentration of substrate (2.06μM).

The excision sites of AP DNA strand

The degree of polymerization of AP DNA nicked by APcI, APcII and APcIII was elevated, but the treatment of nicked AP DNA with alkaline phosphatase or T_4 polynucleotide kinase could not bring about further elevation of the polymerization rate (Table 3 & Table 4). This result indicates the presence of 3'-hydroxy group at the nicked site of AP DNA generated by APcI, APcII and APcIII.

Table 2. Kinetic properties of AP DNA endonucleases

Enzyme	Km (μM)	Inhibition by AMP	
		Apparent Ki (mM)	Type
APcI	0.53	0.35	Uncompetitive
APcII	0.27	0.54	Uncompetitive
APcIII	0.36	0.41	Uncompetitive

Table 3. Translation of nicks produced by AP DNA endonucleases in the presence or absence of alkaline phosphatase

AP DNA endonuclease	Alkaline phosphatase	Total incorporation of (dpm)*	[^{3}H] dTTP (% Dev)	Net incorporation of [^{3}H] dTTP due to AP DNA endonuclease (dpm)*
—	–	830	—	0
APcI (2.12 EU)	–	37,909	27.9	37,079±10,596
APII (1.04 EU)	–	17,425	5.3	16,595±929
ApoII (1.04 EU)	–	45,544	14.3	44,714±6,415
—	+	7,045	—	0
APcI (2.12 EU)	+	41,665	3.0	34,620±1,252
APcII (1.04 EU)	+	23,002	11.0	15,957±2,533
APcIII (2.46 EU)	+	54,104	17.2	47,059±9,317

*AP DNA (31.7μg) was incubated for 45 min at 37°C, pH8.0 with enzyme (AP DNA endonucleases) followed by the incubation for 15 min with or without alkaline phosphatase (2 units, pH8.0), heated for 10 min at 70°C then cooled in ice water. To the reaction mixture, dNTPs (13 nmol of dATP dGTP and dCTP + 9pmol of [^{3}H]dTTP) were added with Klenow fragment of *E.coli* DNA polymerase I)1 unit), then incubated another 60 min at 15°C. The polymerization reaction was stopped by addition of 1ml of 5% TCA in 0.1M sodium pyrophosphate solution, followed by a centrifugation to precipitate DNA incorporated with radioactive nucleotides. The precipitate was washed 3 times with the same solution and then radioactivities in DAN were counted. [^{3}H]dTTP [^{3}H]dTTp due to

Table 4. Translation of nicks produced by AP DNA endonucleases in the presence or absence of T4 polynucleotide kinase

AP DNA endonuclease	T4 polynucleotide kinase	Total incorporation of (dpm)*	[^{3}H]dTTP (% Dev)	Net incorporation of [^{3}H]dTTP due to AP DNA endonuclease (dpm)*
—	–	1,538		0
APcI (2.12 EU)	–	31,800	8.9	30,262±2,693
APcII (1.04 EU)	–	18,377	12.1	16,839±2,037
APcIII (2.46 EU)	–	46,514	12.1	44,976±5,442
—	+	1,573		0
APcI (2.12 EU)	+	30,430	12.0	28,857±3,462
APcII (1.04 EU)	+	20,649	10.7	19,857±2,041
APcIII (2.46 EU)	+	45,492	11.9	43,919±5,226

*AP DNA)31.7μg) was incubated for 45 min at 37&C with each AP DNA endonuclease followed by the incubation for 15 min with or without T4 polynucleotide kinase (2 units, pH6.0), heated for 10 min at 70°C, then coooled in ice water. To the reaction mixture, dNTPs (13nmol of dATP, dGTP and dCTP + 9pmol of [^{3}H]TTP) were added with 1 unit of Klenow fragment of *E.coli* DNA polymerase I, then incubated another 60 min at 15°C. The polymerization reaction was stopped by addition of 1 ml of 5% TCA in 0.1M sodium pyrophosphate solution, followed by a centrifugation to precipitate the incorporated radioactive nucleotides. The precipitate was washed 3 times with the same solution and then radioactivities were counted.

DISCUSSION

A mammalian cell may lose up to about 10,000 purines and 200 pyrimidines from its genome spontaneously in each generation (Lindahl and Nyberg, 1972). The loss of bases from the genomic DNA (AP DNA) may be caused by environmental harmful agents such as heat, acid and ultraviolet light (Lindahl , 1979; Povirk and Goldberg, 1985).

AP DNA endonuclease, responsible for the excision repair of AP DNA is widely distributed in prokaryotes and eukaryotes. (Brent, 1976; Hanawalt et al, 1989). There were at least 3 kinds of AP DNA endonuclease in rat liver (Verly and Thibodeau, 1979), and the enzymes localized in plasma membrane and nucleoplasm were thought to be the precursors of the one in the chromatin, which is responsible for the repair of chromosomal AP DNA (Thibodeau and Verly, 1980; Goffin and Verly, 1984). However, no evidence is presented yet, that the precursor of the chromatin AP DNA endonucleases are localized at the plasma membrane. Three types of AP DNA endonucleases (APcI, APcII and APcIII) were purified from rat liver chromatin in out laboratory, and the enzyme activities were elevated by the addition of sulfhydryl compounds (2-ME, DTT) with optimal activities at about 0.5mM.

NEM and $HgCl_2$, the typical sulfhydryl group inhibitors inhibited the AP DNA endonucleases, suggesting that the enzymes have sulfhydryl groups at or near active sites. These results agree well with those of Kane and Linn (1981) who reported that the activity of AP DNA endonuclease isolated from HeLa cell was increased by sulfhydryl compounds. But the results are not compatible with the results of Kuhnlein and his associates (1976) who reported that AP DNA endonuclease isolated from human fibroblasts was not influenced by sulfhydryl compounds. The discrepancy between these results is still in controversal.

It is assumed that addition of 0.5mM sulfhydryl agents to the enzyme reaction mixture is required to get the optimal activity. Mg^{2+} was an essential cofactor for AP DNA endonucleases, and 4mM of Mg^{2+}, a sufficient amount for the optimal activities is a little lower than the value of 10mM reported by Kuhnlein et al (1976). The difference between this study and the previous study might be arised due to the difference in the substrates (*E.coli* chromosomal AP DNA vs PM-2 phage AP DNA) used. APcI, APcII and APcIII differed in their Km values, indicating that they have different molecular structure and different affinity for the *E.coli* chromosomal AP DNA. However, these values (0.53μM for APcI, 0.27μM for APcII and 0.36μM for APcIII) were much lower than the value (1.3mM) of AP DNA endonuclease from HeLa cells reported by Kuhnlein and his associates (1976). The difference in the values is assumed to be due to the difference in the substrates and the sources of the enzyme. Adenine, hypoxanthine, adenosine, AMP, ADP-ribose and NAD^+ inhibited AP DNA endonuclease isolated from HeLa cells but DAP,ATP,NADH and pyrimidine could not inhibit the enzyme activity (Kane and Linn, 1981). Tomkinson et al. (1988) reported that AP DNA endonuclease isolated from mouse cell mitochondria was not influenced by adenine and NAD^+. In the present study, AMP inhibited 3 AP DNA endonucleases in rat liver chromatin uncompetitively (Table 1: Fig. 5,6,7) indicating that AMP binds only to the enzyme-substrate complex. The relative Ki values of APcI, APcII and APcIII for AMP (0.35mM for APcI, 0.54mM for APcII and 0.41mM for APcIII) were inversely to the respective Km values in the present study, suggesting that the affinity of AP DNA endonuclease for substrate (AP DNA) is a contrast to the affinity of the AMP for enzyme substrate complex. T_4 polynucleotide kinase has 3'-phophatase activity in addition to 5'-phosphate kinase activity and alkaline phosphatase hydrolyzes orthophosphate residues (Haukanes et al, 1988; Haukanes et al, 1989). So, the degree of polymerization of nicked DNA strand with 3'-phosphate group by Klenow fragment (*E.coli* DNA polymerase I large fragment) is increased after the treatment with either enzyme.

However, the treatment of T_4 polynucleotide kinase or alkaline phosphatase on the nicked DNA generated by AP DNA endonucleases did not influence on the degree of polymerization (Table 3, 4). These results indicated that AP DNA endonucleases (APcI, APcII, APcIII) excise AP DNAs to generate the nicked strand with 3'-hydroxyl residues which may be an immediate substrate for DNA polymerase.

The present results agree with that of Verly et al. (1981) and that of Goffin and Verly (1984). The results also agree with that isolated from mouse cell mitochondria (Tomkinson et al., 1988).

Recently, it has been demonstrated that AP DNA endonuclease purified from human placenta excises bebween 3'-side of AP site and t'-side of phosphate group and produces 3'-deoxyribose and t'-phosphomonoester nucleotides (Haukanes, 1989). In this case, 3'-deoxyribose would be removed by proof reading of Klenow fragment so that nick translation rate would be same as in the case of the nicked strand with e'-hydroxyl residues. But it is still unknown whether e'-side or t'-side of AP site of AP DNA is cleaved by APcI, APcII AND APcII.

REFERENCES

Brent TP: *Purification and characterization of human endonuclease specific for damaged DNA. Biochem Biophys Acta 454:172-183, 1976.*

Cesar R, Verly WG: *The apurinic/apyrimidinic endodeoxyribonuclease of rat liver chromatin. Eur J Biochem*

129:509-516, 1983.

Friedberg EC: *Excision repair I. DNA glycosylases and AP endonucleases. In EC Friedberg ed. DNA repair, New York, W.H. Freeman and Co. 1984, pp141.*

Goffin C, Verly WG: *Repair of depurinated DNA with enzymes from rat liver chromatin. Biochem J 220:133-137, 1984.*

Goldmark PJ, Linn S: *An endonuclease activity from Escherichia coli absent from certain rec$^-$ strains. Proc Natl Acad Sci USA 67:434-441, 1970.*

Gossard F, Verly WG: *Properties of the main endonuclease specific for apurinic sites of Eschericia coli (Endonuclease VI). Eur J Biochem 82:231-332, 1978.*

Grafstrom RH, Shaper NL, Grossman L: *Human placental apurinic/apyrimidinic endonuclease. Mechanism of action. J Biol Chem 257:13459-13464, 1982.*

Hanawalt PC, Cooper PK, Ganesan AK, Smith CA: *DNA repair in bacteria and mammalian cells. Ann Rev Biochem 48:783-836, 1979.*

Haukanes BI, Helland DE, Klepper L: *Analysis of cleavage products of DNA repair enzymes and other nucleases. Characterization of an apurinic/apyrimidinic specific en donucleases from mouse plasmacytoma cells. Nucleic Acid Rec 16:6871-6882, 1988.*

Haukanes BI, Wittwer CU, Helland DE: *Mechanism of incision by an apurinic/apyrimidinic endonuclease present in human placenta, Nucleic Acid Res 17:5529-5535, 1989.*

Kane CM, Linn S: *Purification and characterization of an apurinic/apyrimidinic endonuclease from HeLa cells. J Biol Chem 256:3405-3414, 1981.*

Kim YS, Woo EK, Koo W, Oh SH: *Studies on rat liver nuclear DNA damaged by chemical carcinogen (3'-Me DAB) and AP DNA endonuclease. I. Purification and some propeties of AP DNA endonucleases in rat liver chromatin (submitted for publication to this Journal), 1990.*

Kuhnlein U, Denhoet EE, Linn S: *An altered apurinic DNA endonuclease activity in group A and group D xeroderma pigmentosum fibroblasts. Proc Natl Acad Sci USA 73:1169-1173, 1976.*

Lindahl T: *DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision-repair. Prog Nucl Acids Res Mol Biol 22:135-192, 1979.*

Lindahl T, Nyberg B: *Rate of depurination of native deoxyribonucleic acid. Biochemistry 11:3610-3618, 1972.*

Linsley WS, Penhoet EF, Linn S: *Human endonuclease specific for apurinic/apyrimidinic sites in DNA. Partial purification and characterization of multiple forms from placenta. J Biol chem 252:1235-1242, 1977.*

Ljungquist S: *A new endonuclease from Escherichia coli acting at apurinic sites in DNA. J Biol chem 252:2808-2814. 1977.*

Mosbaugh DW, Linn S: *Further characterization of human fibroblast apurinic/apyrimidinic DNA endonucleases. J Biol Chem 255:11743-11752, 1980.*

Porvirk LF, Goldberg IR: *Endonuclease resistant apyrimidinic sites formed by neocarcinostatin at cysteine residues in DNA: Evidence for a possible role in mutagenesis. Proc Natl Acad Sci USA 82:3182-3186, 1985.*

Segel IH: *"Enzyme Kinetics". Behavior and analysis of rapid equilbrium and steady-state enzyme systems. Wiley-Interscience, 1975, p214.*

Shaper NL, Grafstrom RH, Grossman L: *Human placental apurinic/apyrimidinic endonuclease. Its isolation and characterization. J Biol Chem 257:13455-13458, 1982.*

Thibodeau L, Verly WG: *Cellular localization of the apurinic/apyrimidinic endonuclease in rat liver. Eur J Biochem 107:555-563, 1980.*

Tomkinson AE, Bonk RT, Linn S: *Mitochondrial endonuclease activities specific for apurinic/apyrimidinic sites in DNA from mouse cells. J Biol Chem 263:12532-12537, 1988.*

Van Lancker JL, Tomura T: *Purification and some properties of a mammalian repair endonuclease. Biochim Biophys Acta 353:99-114, 1974.*

Verly WG, Colson P, Zocchi G, Goffin C, Liuzzi M, Buchsenschmidt G, Muller M: *Localization of the phosphodiester bond hydrolyzed by the major apurinic/apyrimidinic endonuciease from rat liver chromatin. Eur J Biochem 118:195-201, 1981.*

Verly WG, Thibodeau L: *In Nicolini C. A. ed, Chromosome structure and function, part A, New York, Plenum Press, 1979, pp803-826.*

Zurbroff J. Sarma DSR: *A nonradioactive method for measuring DNA damage and its repair in nonproliferating tissues. Anal Biochem 70:387-396, 1976.*