Purification and Determination of the NH_2-Terminal Amino Acid Sequence of Uracil-DNA Glycosylase from Human Placenta†

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ABSTRACT: Uracil-DNA glycosylase has been purified approximately 130 000-fold from extracts of human placenta. Although all of the uracil-DNA glycosylase activity coeluted through six chromatographic steps, at least four distinct peaks of activity were resolved in the final purification on a Mono S column. Each of the peaks containing uracil-DNA glycosylase activity contained two peptides of M_r = 29 000 and M_r = 26 500, respectively, as analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Experimental evidence indicated that the M_r = 29 000 peptide was the uracil-DNA glycosylase enzyme. The amino-terminal sequence of each peptide was determined after blotting of the peptides from the gel onto Polybrene GF/C paper. The sequences were not related to each other, and neither was any significant homology to other proteins found. Uracil-DNA glycosylase had a molecular turnover number of approximately 600/min and an apparent K_m value of 2 μM. The enzyme is a basic protein and was stimulated about 10-fold by 60–70 mM NaCl whereas higher concentrations were inhibitory.

Uracil may be found in DNA either after incorporation of dUTP instead of dTTP (Tye et al., 1977; Wist et al., 1978; Brynolf et al., 1978) or as a result of deamination of cytosine in DNA (Lindahl & Nyberg, 1974; Shapiro, 1980). An enzyme activity that hydrolyzes the bond between uracil and deoxyribose was first detected in *Escherichia coli* and subsequently purified to homogeneity from the same source (Lindahl, 1974; Lindahl et al., 1977). An analogous enzyme activity has been found in a variety of prokaryotic and eukaryotic systems including mammalian cells (Caradonna & Cheng, 1980; Krokan & Wittwer, 1981; Talpaert-Borle et al., 1982; Arenaz & Sirover, 1983; Wittwer & Krokan, 1985). All human tissues examined express uracil-DNA glycosylase activity, but at varying degrees (Myrnes et al., 1983; Krokan et al., 1983). Increased activity is observed during the S-phase (Sirover, 1979). Furthermore, a specific association with replicating SV40 chromatin suggested that the activity might be physically complexed with the replication machinery (Krokan, 1981). Recently, a preliminary report suggested that uracil-DNA glycosylase is associated with the catalytic subunit of DNA polymerase α (Seal & Sirover, 1986).

This work describes the biochemical properties and the NH_2-terminal amino acid sequence of uracil-DNA glycosylase from human placenta.

Materials and Methods

Chemicals. DNA–agarose was prepared according to Bendich and Bolton (1968). Other chemicals and agents were obtained as described previously (Krokan & Wittwer, 1981; Vandekerckhove et al., 1985).

Assay of Uracil-DNA Glycosylase. Uracil-DNA glycosylase activity was determined according to the assay described previously (Krokan & Wittwer, 1981). One unit of uracil-DNA glycosylase is the amount of enzyme that releases 1 nmol of uracil/min at 30 °C.

Purification of Uracil-DNA Glycosylase. All operations were carried out at 0–4 °C if not otherwise stated. Placentae collected were stored at −20 °C until being used. To a total of 20 kg of tissue dissected free of connective tissue was added a total of 60 L of H_2O containing 0.01% NaN_3 and 0.5 mM PMSF,[1] and the tissue was homogenized in portions of 200 g in an Atomix homogenizer. The homogenate was squeezed by using a nylon bag (100-μm mesh) in a wine press (Desmond Walker, England) and the residue washed in another 30 L of the same solution. Then, 20 L of extraction buffer (100 mM Tris–acetate, pH 7, 50 mM NaCl, 0.01% NaN_3, and 0.5 mM PMSF) was added to the residue, which was extracted overnight under slow stirring. The extract was then squeezed out in the wine press as before and collected. This extraction procedure was repeated twice with the same residue. The combined extracts were then centrifuged (2500*g*, 40 min) to remove the precipitate and passed through a Buchner funnel (17 cm in diameter) containing 500 g of DEAE-cellulose (DE-52). The DE-52 was replaced after 30 L of extract were passed. This fraction is designated "crude extract".

(1) CM-Sephadex C-50 Chromatography. Enough of 2 M $MgCl_2$ was added to the crude extract to make a final concentration of 2 mM. Then 1 part of H_2O was added to 2 parts of extract. The diluted extract was applied to a CM-Sephadex C-50 column (14 × 9 cm) equilibrated in a buffer containing 65 mM Tris–acetate, pH 7, 35 mM NaCl, 1.5 mM $MgCl_2$, and 0.01% NaN_3. The column was washed with 10 L of the same buffer. The adsorbed proteins were eluted with a buffer containing 100 mM Tris–acetate, pH 7, 0.4 M NaCl, 2 mM $MgCl_2$, 0.01% NaN_3 and 10% glycerol. Fractions containing

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[1] Abbreviations: AP, apurinic/apyrimidinic; FLPC, fast protein liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

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Table I: Purification of Uracil-DNA Glycosylase

fraction	total activity (units)	total protein (mg)	specific activity (units/mg)	yield (%)	purification (x-fold)
crude extract	16560	255500	0.065	100	
CM-Sephadex C-50	16560	6250	2.65	100	41
DNA–agarose	11240	567	19.8	68	306
CM-Sephadex C-50	9605	337	28.5	58	440
Sephacryl S-200	7451	74.7	99.8	45	1540
Bio-Gel P-100	6810	30.2	225.5	41	3480
poly(U)–Sepharose 4B	4943	2.2	2257	30	34800
FPLC Mono S (all UDG act.)	3017	0.5	5693	18	87800
FPLC Mono S (UDG 4)	1485	0.27	5500		
FPLC Mono S (UDG 4 after rechromatography)	525	0.06	8473		131000

uracil-DNA glycosylase activity were pooled, dialyzed four times against 3 volumes of buffer A (50 mM Tris–acetate, pH 7, 2 mM $MgCl_2$, 0.01% NaN_3, and 10% glycerol), and centrifuged (15000*g* in 30 min) to remove a precipitate.

DEAE-cellulose (DE-52) and DNA–Agarose Chromatography. A DE-52 column (5 × 23 cm) and a single-stranded DNA–agarose column (2.6 × 32 cm) were coupled in series and equilibrated with buffer A. The CM-Sephadex C-50 fraction was applied to the columns, which were washed with 1.5 L of buffer A. Activity adsorbed to the DNA–agarose column was then eluted with a 925-mL linear gradient of NaCl (0–0.5 M) in buffer A. Fractions containing uracil-DNA glycosylase activity were pooled (DNA–agarose fraction) and dialyzed twice against 5 L of buffer A.

(2) CM-Sephadex C-50 Chromatography. A CM-Sephadex C-50 column (1.6 × 4.5 cm) was equilibrated with buffer A and the DNA–agarose fraction applied to the column, which was washed with 150 mL of the same buffer. The adsorbed proteins were eluted in a 300-mL linear gradient of NaCl (0–0.5 M) in buffer A. Fractions containing uracil-DNA glycosylase activity were pooled (CM-Sephadex C-50 fraction) and concentrated.

Gel Filtration on Sephacryl S-200. A Sephacryl S-200 column (2.6 × 89 cm) was equilibrated in buffer B (100 mM Tris–acetate, pH 7, 1 M NaCl, 2 mM $MgCl_2$, 0.02% NaN_3, and 10% glycerol). The CM-Sephadex C-50 fraction was applied to the column and eluted at a rate of 22 mL/h. Fractions containing uracil-DNA glycosylase activity were pooled and concentrated (Sephacryl S-200 fraction).

Gel Filtration on Bio-Gel P-100. A Bio-Gel P-100 column (1.6 × 65 cm) was equilibrated with buffer B and the Sephacryl S-200 fraction applied to the column, which was eluted at a rate of 6.4 mL/h. Fractions containing uracil-DNA glycosylase activity were pooled and dialyzed against buffer A (Bio-Gel P-100 fraction).

Poly(U)–Sepharose 4B Chromatography. A poly(U)–Sepharose 4B column (1.6 × 5 cm) was equilibrated with buffer A and the dialyzed Bio-Gel P-100 fraction applied to the column, which was washed with 300 mL of the same buffer. The adsorbed proteins were eluted in a 100-mL linear gradient of NaCl (0–0.5 M) in buffer A. The elution rate was 14 mL/h. Fractions of 2.8 mL were collected in siliconized glass tubes containing 2.8 μL of 100 mM spermidine to stabilize the enzyme. Fractions containing uracil-DNA glycosylase activity were pooled [poly(U)–Sepharose 4B] and concentrated by ultrafiltration (Amicon filter PM10).

FPLC Chromatography on Mono S. An FPLC Mono S HR 5/5 column (1 mL) was equilibrated with buffer C (50 mM Hepes–NaOH, pH 8, and 10% glycerol). The poly(U)–Sepharose 4B fraction was adsorbed and protein eluted by gradually increasing the NaCl concentration. Fractions of 0.5 mL were collected in siliconized glass tubes which contained 10 mL of 1.6 M Tris–acetate, pH 7, 5 mM spermidine, 100 mM $MgCl_2$, and 0.5% NaN_3 to stabilize uracil-DNA glycosylase.

Transfer of Proteins from SDS–Polyacrylamide Gels to Polybrene-Coated Glass Fiber Sheets. Samples were separated in 12.5% SDS–polyacrylamide gels as described by Laemmli (1970). The preparation of Polybrene (PB) glass fiber sheets was done essentially as described by Vandekerckhove et al. (1985) as was the electrophoretic transfer from the SDS gels to two or three PB glass fiber sheets. The PB sheets were washed 4 × 10 min in 10 mM sodium borate, pH 8, and 25 mM NaCl, dried in the dark, and stained with a solution of fluorescamine in acetone (1 mg/300 mL to 1 mg/600 mL).

Acid Hydrolysis and Amino Acid Analysis. The part of the glass fiber sheet containing the protein was cut out and sonicated for 30 s in 10 mM sodium borate, pH 8, and 25 mM NaCl. It was then subjected to acid hydrolysis and amino acid analysis as described previously (Vandekerckhove et al., 1985). Amino acids were quantified after reaction with *o*-phthaldialdehyde essentially as described by Benson and Hare (1975).

Amino Acid Sequence Determination. The amino acid sequence of the protein immobilized on the glass fiber sheet was analyzed as described previously (Vandekerckhove et al., 1985), by using a gas-phase sequencer (Applied Biosystems Inc., Model 470A) directly linked to a phenylthiohydantoin analyzer (Applied Biosystems 120 A) with a gradient elution system described by Hunkapiller and Hood (1983).

Other Methods. SDS–polyacrylamide gel electrophoresis was done as described by Laemmli (1970) and silver staining according to Wray et al. (1981). Protein was determined according to Schaffner and Weissmann (1973) using bovine serum albumin as a standard.

Results

Purification of Uracil-DNA Glycosylase. Approximately 80% of the uracil-DNA glycosylase activity recovered after homogenization was in the residue, whereas about 70% of the total soluble protein was removed in the dilute homogenization buffer. After centrifugation and filtration through DEAE-cellulose, uracil-DNA glycosylase was subjected to CM-Sephadex C-50 chromatography, followed by DEAE-cellulose, DNA–agarose, and a second CM–Sephadex C-50 column as described under Materials and Methods (Table I).

The activity was further purified by gel filtration on Sephacryl S-200 followed by a Bio-Gel P-100 column (Figure 1). It was essential to include high salt concentrations in the gel filtration buffer (1 M NaCl); otherwise, the enzyme activity eluted as a very broad peak starting with the void volume. The activity was further purified by poly(U)–Sepharose chromatography where it eluted as a broad peak between 100 and 250 mM NaCl in the gradient. Finally, uracil-DNA glycosylase was purified by FPLC on a Mono S cation exchanger (Figure 2A). The activity eluted in an isocratic region at 100 mM NaCl as five different peaks (UDG 1–4 and UDGX). When

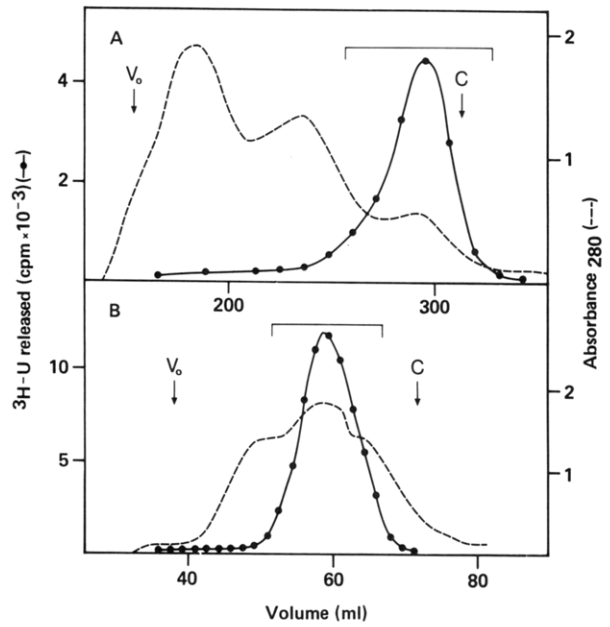

FIGURE 1: Purification of uracil-DNA glycosylase by gel filtration on (A) Sephacryl S-200 and (B) Bio-Gel P-100. V_0 represents the void volume and C the position of cytochrome *c* (in a separate experiment).

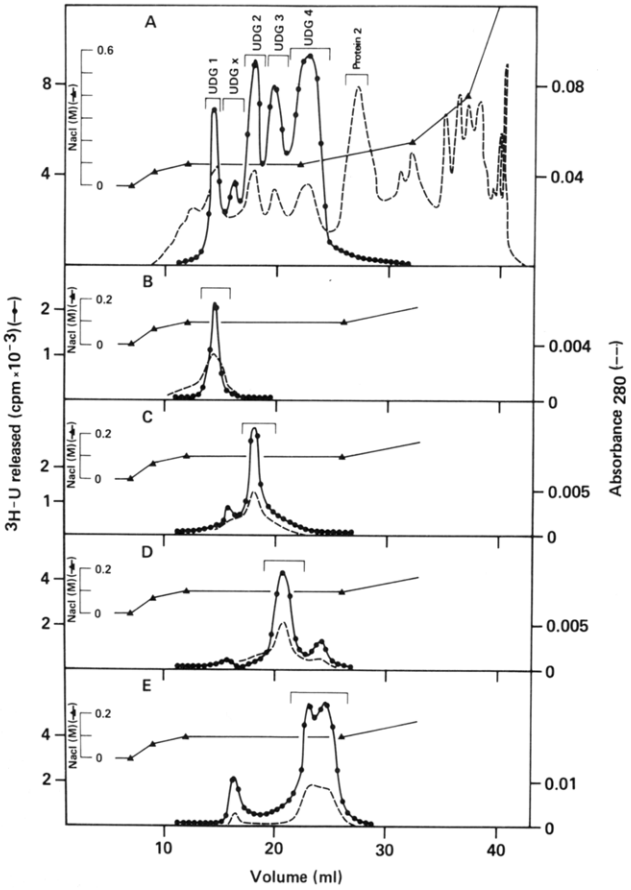

FIGURE 2: Purification of uracil-DNA glycosylase by FPLC on a Mono S cation exchanger (A). The following pooled fractions in (A) were rechromatographed on the same column: UDG 1 (B), UDG 2 (C), UDG 3 (D), and UDG 4 (E).

analyzed by SDS–polyacrylamide gel electrophoresis, two major bands were observed for each pooled peak of activity (Figure 3A–E). Their molecular weights were 26 500 and 29 000, respectively. A peak with very little uracil-DNA glycosylase activity was also pooled (protein 2 in Figure 2A) and analyzed by SDS-polyacrylamide gel electrophoresis,

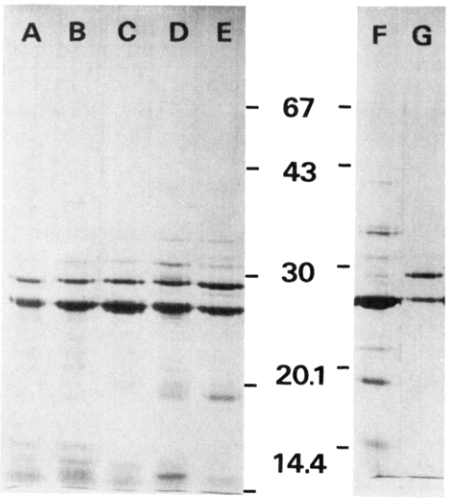

FIGURE 3: SDS–polyacrylamide slab gel electrophoresis. In the middle is marked the position of the size markers in kilodaltons: phosphorylase *b* (94), bovine serum albumin (67), carbonic anhydrase (30), soybean trypsin inhibitor (20.1), and α-lactalbumin (14.4). The following fractions from Figure 2 (0.2–0.5 μg) were applied to the gel: UDG 1 (A), UDG X (B), UDG 2 (C), UDG 3 (D), UDG 4 (E), protein 2 (F), and UDG 4 after rechromatography (G). The gel was silver stained.

where it appeared as a major band at M_r = 26 500 (Figure 3F). The major bands observed had the same R_f value with or without treatment with 2-mercaptoethanol, indicating that the proteins analyzed were single polypeptide chains.

UDG 1–4 were rechromatographed on the same Mono S column (Figure 2B–E). The major peaks of activity eluted at almost the same place as the original pooled activities (UDG 1–4) in Figure 2A. The rechromatographed UDG 4 (bracket in figure 2E) was analyzed by SDS–polyacrylamide gel electrophoresis (Figure 3G). Again two bands were seen at M_r = 26 500 and M_r = 29 000, respectively. The rechromatographed UDG 1–3 activities were also analyzed by SDS–polyacrylamide gel electrophoresis and showed a similar pattern (not shown). A summary of the purification of uracil-DNA glycosylase UDG 1–4 and X including the rechromatography of UDG 4 is given in Table I.

Biochemical Properties of Uracil-DNA Glycosylase. The most purified preparation of human placental uracil-DNA glycosylase (UDG 4 after rechromatography) had a specific activity of 8470 units/mg of protein. The apparent K_m value was determined by a double-reciprocal plot and was about 2 μM for UDG 4 in agreement with previous results (Wittwer & Krokan, 1985). UDG 4 was stimulated 10-fold by 70 mM NaCl whereas higher concentrations inhibited the activity (85% inhibition at 200 mM NaCl). Two experiments on a chromatofocusing column with a pH gradient from 6 to 9 (Pharmacia) showed that the bulk of uracil-DNA glycosylase activity eluted at a pH >8.7 which was the highest measurable value using this system.

A fraction of "protein 2" (Figure 2A) and one of rechromatographed UDG 3 (brackets in Figure 2D) were each treated with trypsin. The digests were analyzed by high-performance liquid chromatography using a reverse-phase column (data not shown). Apart from the peaks from trypsin autodigests, at least four of the main peaks showed identical retention times on both chromatograms. This indicates that the M_r = 26 500 protein in the protein 2 fraction and that in the fractions containing uracil-DNA glycosylase activity are identical or very similar.

Amino Acid Analysis of Uracil-DNA Glycosylase (UDG 4). About 3 μg of the UDG 4 preparation were subjected to

Table II: Amino Acid Compositions of the M_r = 29 000 Protein (Uracil-DNA Glycosylase Activity) and the M_r = 26 500 Protein[a]

amino acid	protein of M_r = 29 000	protein of M_r = 26 500
Asx	630 (17)	760 (20)
Thr	250 (7)	300 (8)
Ser	900 (24)	1200 (31)
Glx	830 (22)	1190 (32)
Gly	1410 (38)	1890 (49)
Ala	450 (12)	570 (15)
Val	470 (13)	140 (4)
Met	120 (3)	180 (5)
Ile	380 (10)	310 (8)
Leu	750 (20)	730 (19)
Tyr	1290 (35)	200 (5)
Phe	270 (7)	400 (10)
Lys	590 (16)	470 (12)
His	420 (11)	320 (8)
Arg	790 (21)	190 (5)

[a] The values are given in pmol. The estimated numbers of residues are shown in brackets. The calculations are based on the apparent molecular weights, and the values are given uncorrected for loss of proline, cysteine, and tryptophan. Inaccuracies because of unreliable values for glycine and methionine are neglected.

SDS–polyacrylamide gel electrophoresis and blotted onto a Polybrene glass fiber sheet. The visualized bands containing the M_r = 26 500 and M_r = 29 000 proteins were cut out separately, hydrolyzed, and subjected to amino acid analysis. The two proteins had very different amino acid compositions (Table II). By the method used, methionine is generally underestimated whereas glycine is generally overestimated (Vandekerckhove et al., 1985). The amino acid composition of the M_r = 29 000 protein (which we believe is uracil-DNA glycosylase, see Discussion) was significantly different from that of uracil-DNA glycosylase from *E. coli* (Lindahl et al., 1977). The content of basic amino acids was considerably higher for the human enzyme and may explain the higher apparent p*I* value compared to the *E. coli* enzyme.

NH_2-Terminal Amino Acid Sequence Determination. Uracil-DNA glycosylase (UDG 4) (30 μg) was subjected to SDS–polyacrylamide gel electrophoresis to separate the M_r = 29 000 and M_r = 26 500 proteins, which were then transferred to three layers of Polybrene glass fiber sheets. The NH_2-terminal amino acid sequence was determined for both proteins. They were not related to each other (Figure 4). Since we only have indirect evidence that the M_r = 29 000 protein is uracil-DNA glycosylase, we prefer to present the amino-terminal sequence for both proteins. The SwissProt database was screened with UWGCG system Wordsearch. No homology to other proteins beyond five consecutive residues was found for either protein.

Discussion

Uracil-DNA glycosylase from human placenta was extensively purified to a specific activity of 8470 units/mg of protein and was judged to be approximately 50% pure from SDS gels. In comparison, an apparently homogeneous uracil-DNA glycosylase preparation from HeLa cells had a specific activity of 17 200 units/mg of protein and an M_r = 29 000 (Myrnes & Wittwer, 1988). From these results we calculated a molecular turnover number of about 600 uracil molecules released/min for uracil-DNA glycosylase from both human placenta and HeLa cells, a human cell line.

The following arguments indicate very strongly that uracil-DNA glycosylase from human placenta has a molecular weight of 29 000. First, when uracil-DNA glycosylase was rechromatographed on a Mono S cation-exchange column, the peak of enzyme activity corresponded nicely to absorbance at

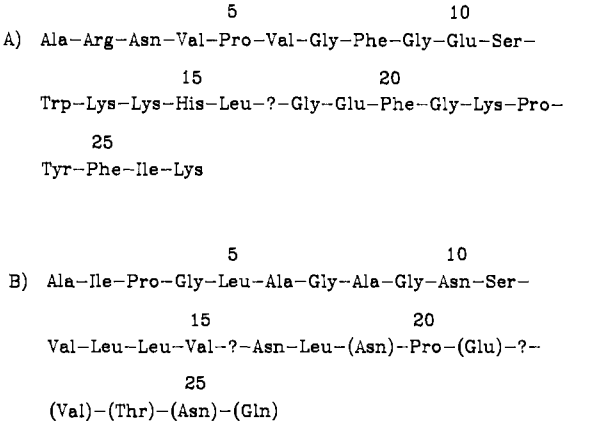

FIGURE 4: NH_2-terminal amino acid sequence of (A) the M_r = 29 000 protein (uracil-DNA glycosylase activity) and (B) the M_r = 26 500 protein.

280 nm. This rechromatographed fraction contained only two visible bands of M_r = 26 500 and M_r = 29 000, respectively, in silver-stained SDS gels (Figure 3, lane G). These two proteins were the only bands that increased in intensity during the purification (data not shown). Second, analysis of tryptic digests indicated that the M_r = 26 500 protein was identical, or at least very similar, in the rechromatographed fraction containing uracil-DNA glycosylase activity and in one fraction (protein 2, Figure 2A) that did not contain uracil-DNA glycosylase. Third, a homogeneous preparation of uracil-DNA glycosylase from HeLa cells (a human cell line) was recently found to have a molecular weight of 29 000 after SDS–polyacrylamide gel electrophoresis using conditions identical with those in the present paper (Myrnes & Wittwer, 1988).

Since these observations do not entirely exclude the possibility that the M_r = 26 500 protein may represent an inactive form of uracil-DNA glycosylase, we have chosen to present the amino-terminal sequence for both proteins (Figure 4).

In comparison, the apparent molecular weight of uracil-DNA glycosylase was reported to be 30 000 in blast cells of acute myelocytic leukemia patients (Caradonna & Cheng, 1980) and 28 000 in calf thymus (Talpaert-Borle et al., 1982). Both values were determined by SDS-polyacrylamide electrophoresis and are in good agreement with our results. We have previously reported a lower molecular weight for native HeLa cell uracil-DNA glycosylase after sedimentation in sucrose or glycerol (Wittver & Krokan, 1985). This indicates that either uracil-DNA glycosylase or the markers used sedimented anomalously, since we consider SDS–polyacrylamide gel electrophoresis to be a more reliable method. In previous experiments the apparent molecular weight found after gel filtration was approximately 50 000 if we did not have a high salt concentration in the buffers. This is most likely due to a specific or unspecific association with other proteins. Similar findings have also been observed by others (Guyer et al., 1986; Seal & Sirover, 1986).

A different molecular weight for human placental uracil-DNA glycosylase was reported by Vollberg et al. (1987). They studied the in vitro synthesis of uracil-DNA glycosylase. After SDS–polyacrylamide gel electrophoresis, immunoblot analysis with anti-human uracil-DNA glycosylase mouse monoclonal antibody revealed a band of M_r = 37 000. In vivo translated uracil-DNA glycosylase from human placenta and fibroblasts also showed a molecular weight of M_r = 37 000 (Vollberg et al., 1987; Seal et al., 1987). This significant difference in molecular weight might be due to specific proteolysis in intact cells. Alternately and possibly more likely, the anti-human

uracil-DNA glycosylase monoclonal antibody may recognize a protein firmly associated with uracil-DNA glycosylase, as the antibody was produced from a uracil-DNA glycosylase preparation of low purity (Arenaz & Sirover, 1983).

Four chromatographically slightly different peaks of uracil-DNA glycosylase were observed after purification on a Mono S column. These appeared to have identical molecular weights. However, we did not attempt any further characterization of each peak. It is likely that the slightly different chromatographic properties may be due to microheterogeneity of some sort. However, we do not have any information that could give a clue to what the differences are. Based upon the information about the amino-terminal sequence, it may now be possible to clone the gene for human uracil-DNA glycosylase. This may require the use of probes derived from both sequences determined.

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REFERENCES

Arenaz, P., & Sirover, M. A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5822–5826.

Bendich, A. J., & Bolton, E. T. (1968) *Methods Enzymol. 128*, 635–639.

Benson, J. R., & Hare, P. E. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 619–622.

Brynolf, K., Eliasson, R., & Reichard, P. (1978) *Cell (Cambridge, Mass.) 13*, 573–580.

Caradonna, S. I., & Cheng, Y. C. (1980) *J. Biol. Chem. 255*, 2293–2300.

Guyer, R. B., Nonnemaker, J. M., & Deering, R. A. (1986) *Biochim. Biophys. Acta 868*, 262–264.

Hunkapiller, M. W., & Hood, L. E. (1983) *Methods Enzymol. 91*, 486–493.

Krokan, H. (1981) *FEBS Lett. 133*, 89–91.

Krokan, H., & Wittwer, C. U. (1981) *Nucleic Acids Res. 9*, 2599–2613.

Krokan, H., Haugen, Aa., Myrnes, B., & Guddal, P. H. (1983) *Carcinogenesis 4*, 1559–1564.

Laemmli, U. K. (1970) *Nature (London) 277*, 680–681.

Lindahl, T. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3649–3653.

Lindahl, T., & Nyberg, B. (1974) *Biochemistry 13*, 3405–3410.

Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., & Sperens, B., (1977) *J. Biol. Chem. 252*, 3286–3294.

Myrnes, B. & Wittwer, C. U. (1988) *Eur. J. Biochem. 173*, 383–387.

Myrnes, B., Giercksky, K.-E., & Krokan, H. (1983) *Carcinogenesis 4*, 1565–1568.

Schaffner, W., & Weissmann, C. (1973) *Anal. Biochem. 56*, 502–514.

Seal, G., Arenaz, P., & Sirover, M. A. (1987) *Biochim. Biophys. Acta 925*, 226–233.

Seal, G., & Sirover, A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7608–7612.

Shapiro, R. (1980) in *Chromosome Damage and Repair* (Seeberg, E., & Kleppe, K., Eds.) pp 3–18, Plenum, New York.

Sirover, M. A. (1979) *Cancer Res. 39*, 2090–2095.

Talpaert-Borle, M., Campagnari, F., & Creissen, D. M. (1982) *J. Biol. Chem. 257*, 1208–1214.

Tye, B. K., Nyman, P. O., Lehman, I. R., Hochhauser, S., & Weiss, B. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 154–157.

Vandekerckhove, J., Bauw, G., Puype, M., Van Damme, J., & Van Montagu, M. (1985) *Eur. J. Biochem. 152*, 9–19.

Vollberg, T. M., Cool, B. L., & Sirover, M. A. (1987) *Cancer Res. 47*, 123–128.

Wist, E., Unhjem, O., & Krokan, H. (1978) *Biochim. Biophys. Acta 520*, 253–270.

Wittwer, C. U., & Krokan, H. (1985) *Biochim. Biophys. Acta 832*, 308–318.

Wray, W., Boulikas, T., Wray, V. P., & and Hancock, R. (1981) *Anal. Biochem. 118*, 197–203.