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Biochemistry

Isolation and characterization of monoclonal antibodies directed against the DNA repair enzyme uracil DNA glycosylase from human placenta

(gene regulation/immunoprecipitation/hybridomas/antigenic determinants/isoenzymes)

PABLO ARENAZ AND MICHAEL A. SIROVER

Fels Research Institute and the Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

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ABSTRACT **A series of monoclonal antibodies has been prepared against the base excision repair enzyme uracil DNA glycosylase isolated from human placenta. Spleen cells from BALB/c mice immunized with purified human placental uracil DNA glycosylase were fused with either P3X63 Ag8.653 or SP2/0 myeloma cells. Hybridomas producing antibodies directed against the placental glycosylase were identified in an enzyme-linked immunosorbent assay. Each positive hybridoma was cloned twice by limit dilution and tested for anti-glycosylase activity in an enzyme immunoprecipitation assay. Each of the four clones examined in detail precipitated enzyme activity in an immunoprecipitation reaction only in the presence of rabbit anti-mouse IgG as a second antibody. No anti-uracil DNA glycosylase activity was observed in a spontaneous hybridoma used as a control. Each monoclonal antibody immunoprecipitated uracil DNA glycosylases isolated from several human tissues. Partial crossreactivity was observed with rat liver glycosylase and with a hamster enzyme. In contrast, no crossreactivity was observed with yeast or *Escherichia coli* glycosylase. Glycerol gradient sedimentation analysis demonstrated that one of the antibodies bound to the glycosylase at a site that did not diminish its catalytic activity. A second monoclonal antibody bound at a determinant that affected catalytic activity. Analysis of antibody–glycosylase interactions suggests that human cells contain antigenically distinct glycosylase species that may be encoded by individual uracil DNA glycosylase genes. The potential use of these monoclonal antibodies in studies examining the regulation of glycosylase isoenzymes during cell proliferation in normal human cells and in cells from cancer-prone individuals is considered.**

Recent studies have indicated that there is a correlation between the proliferative state of a eukaryotic cell and the capacity of that cell to repair DNA after chemical or physical insult. Increases in the repair capacity of proliferating cells above basal levels present in quiescent cells have been observed for all DNA repair pathways that have been examined. These have included nucleotide excision repair after UV irradiation (1–6) or after exposure to *N*-acetoxyacetylaminofluorene (2, 6), base excision repair after exposure to methyl methanesulfonate (5, 6) or to sodium bisulfite (5), and repair after exposure to ionizing radiation (7). This enhancement of repair capacity has been observed with both asynchronous and synchronous cell populations, using various protocols to quantitate DNA repair. The *in vitro* quantitation of individual DNA repair enzyme activities during cell proliferation demonstrated that increases in the specific activities of the base excision repair enzymes uracil DNA glycosylase (8–13) and 3-methyladenine DNA glycosylase (12) as well as an increase in the specific activity of the O^6-methylguanine methyltransferase (14) were dependent on the proliferative state of the cell. These increases in enzyme activity, in the absence of cellular insult, indicated that the induction of repair enzymes was a normal regulatory event during cell proliferation (15, 16). Further analysis of DNA repair by using synchronized cells suggested that the repair pathways were regulated within a defined temporal sequence such that repair was enhanced prior to the induction of DNA replication (5, 6). The significance of these regulatory mechanisms of DNA repair during cell proliferation is suggested by recent observations that cells from cancer-prone individuals may be characterized by specific defects in the regulation of DNA repair during cell proliferation (17). Although the cellular deficiencies in these syndromes are well recognized (18–20), the molecular mechanisms that underlie these deficiencies remain unknown. In particular, none of the syndromes that have been examined appear to involve deficiency in the activity of individual DNA repair enzymes when assayed *in vitro* with exogeneous substrates (21–27).

In order to examine the regulation of DNA repair in normal human cells and in cells from cancer-prone individuals at the molecular level, we have prepared a series of monoclonal antibodies to the base excision repair enzyme uracil DNA glycosylase isolated from human placenta. In this report, we describe the isolation and characterization of four such monoclonal antibodies, examine the crossreactivity of each monoclonal antibody to uracil DNA glycosylases from different human sources and glycosylases from other organisms, and determine the physical binding of the antibody to the glycosylase by differential sedimentation through glycerol gradients. The pattern of glycosylase–antibody interactions in sedimentation analysis suggested that human cells contain multiple glycosylase species that are antigenically distinct. Further, the increase in the uracil DNA glycosylase during cell growth suggests that the regulation of this enzyme during cell proliferation may be due to the selective expression of individual glycosylase genes.

MATERIALS AND METHODS

Purification of Human Placental Uracil DNA Glycosylase. Freshly obtained human placenta was washed in 0.15 M KCl and dissected free of its connective tissue. The remaining tissue was homogenized six times for 1 min each in a Waring blender at 4°C in buffer I (20 mM Tris·HCl, pH 7.9/1 mM dithiothreitol). The suspension was centrifuged at 20,000 × *g* for 20 min and the cell pellet was discarded. In this procedure, the mitochondria were pelleted and the mitochondrial glycosylase, which accounts for only 5–10% of the total glycosylase activity, was removed (28). The supernatant was adjusted to 20% (vol/

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Abbreviation: ELISA, enzyme-linked immunosorbent assay.

vol) glycerol and absorbed to a DEAE-cellulose column previously equilibrated with buffer II (20 mM Tris·HCl, pH 7.9/1 mM dithiothreitol/20% glycerol). The column was washed with 1 column volume of buffer II to elute the enzyme. Peak fractions of glycosylase activity were pooled and absorbed directly onto a phosphocellulose column. The column was washed with 1 vol of buffer II followed by a 0–1 M KCl gradient in buffer II. The peak fractions from the phosphocellulose column were pooled and dialyzed twice in 3.5 liters of buffer III (10 mM potassium phosphate, pH 6.5/2 mM K_2EDTA/0.5 mM dithiothreitol/20% glycerol). The dialysate was absorbed onto a hydroxylapatite column and eluted with a gradient of 10–300 mM potassium phosphate in buffer III.

Immunization and Preparation of Hybridomas. Uracil DNA glycosylase purified from hydroxylapatite column chromatography was dialyzed against 4 liters of phosphate-buffered saline. Three BALB/c mice, age 6–8 weeks, were injected intraperitoneally with the uracil glycosylase (75 μg per mouse) in phosphate-buffered saline mixed 1:1 with complete Freund's adjuvant. A booster of 15 μg per mouse, mixed 1:1 with incomplete Freund's adjuvant, was given intraperitoneally 3 weeks later. After an additional 3 weeks, a final booster of 11.5 μg per mouse, in phosphate-buffered saline, was given intravenously. The mice were sacrificed 3 days later and their spleens were removed.

Mouse myeloma lines SP2/0 and P3X63 Ag8.653 (V653) (GM3569 and GM3570, respectively, from the Human Genetic Cell Repository, Camden, NJ) were routinely maintained in midlogarithmic phase in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and gentamycin at 1 μg/ml. Fusion was accomplished by a modification of the procedure described by Kennett (29). The cells from each spleen were separated into two equal aliquots (5–6 × 10^7 cells per aliquot) and mixed with either SP2/0 or V653 (5–6 × 10^6 cells) in the presence of polyethylene glycol 1,000 for 1.5 min at 37°C. The cells were centrifuged at 250 × *g* for 6 min and suspended in selective medium containing hypoxanthine, thymidine, and aminopterin and cultured at 37°C in humidified 8% CO_2/92% air.

Spent culture fluid from growing cultures was assayed for anti-uracil-DNA glycosylase activity by enzyme-linked immunosorbent assay (ELISA) (30). Microtiter plates were coated with uracil DNA glycosylase by incubating each well with 100 μl of glycosylase (100 μg/ml) in phosphate-buffered saline at pH 7.0 for 2 hr at 37°C in a humidified atmosphere. The plates were washed twice with phosphate-buffered saline containing 1% bovine serum albumin, then incubated at 37°C for 20 min with 100 μl of phosphate-buffered saline/1% bovine serum albumin per well. The plates were washed twice with phosphate-buffered saline and 50 μl of spent culture fluid was added to each well (two wells per clone). The plates were incubated for 2 hr at 37°C in a humidified atmosphere. Each plate was washed twice with washing buffer containing 10 mM Tris·HCl, pH 7.4, and Tween 20. To each well was added 50 μl of a 1:250 dilution of alkaline phosphatase-conjugated $F(ab')_2$ fragments of sheep antiserum to mouse IgG (New England Nuclear) and incubated for 2 hr at 37°C. Plates were washed as above in washing buffer and washed twice in distilled water, and 50 μl of *p*-nitrophenyl phosphate was added to each well. After 14–16 hr at room temperature, plates were checked for positive wells. Supernatants from a previously isolated and spontaneous hybridoma clone were used as a negative control. Hybridomas scored as positive in the ELISA were cloned by limit dilution in DME medium with hypoxanthine and thymidine. Positive clones were recloned by limit dilution and tested again for anti-glycosylase activity by immunoprecipitation. Hybridomas that were positive in ELISA and that immunoprecipitated glycosylase activity were grown for collection of spent fluid and for ascites tumor production. To produce ascites tumors, 1–2 × 10^7 hybridoma cells were injected intraperitoneally into pristane-primed mice.

RESULTS

Characterization of Hybridomas Producing Antibodies to Uracil DNA Glycosylase. Two weeks after fusion, 46 out of 1,000 wells produced clones. Of these, 24 died in culture. Clones from the remaining 22 wells were subcultured by limit dilution. Clones from the limit dilution positive for anti-glycosylase activity were selected by ELISA; of 719 wells assayed, 157 were positive. The ELISA-positive clones were further screened by immunoprecipitation and 24 clones were selected on the basis of their ability to precipitate glycosylase activity. The cells from these clones were subjected to a second limit dilution and reassayed for anti-glycosylase activity by immunoprecipitation. There were 92 clones that exhibited exceptional anti-glycosylase activity out of 260 wells assayed. These 92 clones were further grown and retested for anti-glycosylase activity by immunoprecipitation. Of these, 4 clones, designated PM 16.11.08, PM 37.04.12, PM 40.10.09, and PM 42.08.07, were selected for further study. The antibodies from all the hybridomas were classified as IgG by Ouchterlony double-diffusion analysis and as IgG1 by ELISA.

To examine the anti-uracil DNA glycosylase activity of the monoclonal antibodies, immunoprecipitation reactions were performed with rabbit anti-mouse IgG as a second antibody. A spontaneous hybridoma (PM 1.05) that was ELISA negative and immunoprecipitation negative was used as a control. Enzyme immunoprecipitation assays demonstrated that the activities remaining in the supernatant after immunoprecipitation were 36%, 44%, 13%, and 41% of the PM 1.05 control for PM 16.11.08, PM 37.04.12, PM 40.10.09, and PM 42.08.07, respectively (Fig. 1). The precipitation was essentially complete with the addition of 0.05 mg of each antibody except for PM 40.10.09, which required 0.5 mg. The addition of antibody up to 2 mg per assay did not precipitate enzyme activity significantly beyond that observed at 0.5 mg. Further, there was no effect on the extent

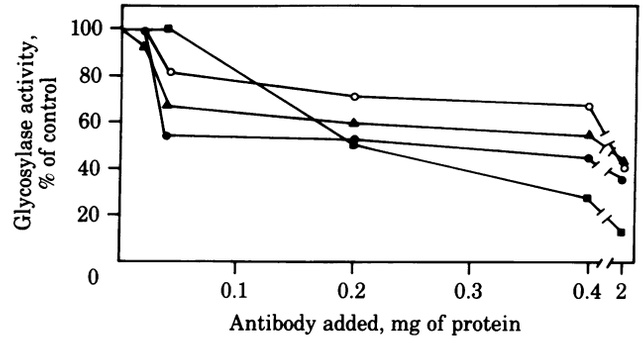

FIG. 1. Immunoprecipitation of uracil DNA glycosylase by monoclonal antibodies. Purified antibodies from each clone were incubated at increasing concentrations with 1.32 μg of purified uracil DNA glycosylase for 1 hr at 4°C. The glycosylase–antibody complexes were immunoprecipitated by the addition of 100 μl (3.8 mg) of rabbit anti-mouse IgG (Sigma) for 1 hr at 4°C. The immunoprecipitate was collected by centrifugation at 10,000 × *g* for 30 min at 4°C. Uracil DNA glycosylase activity in the supernatant was determined as described (5, 6, 8, 11, 17). The extent of inhibition was calculated as the percentage of glycosylase activity in supernatants from incubations with antibody from ELISA-positive clones as compared to that amount of enzyme activity in supernatants from incubations with antibody from the spontaneous hybridoma control which was ELISA negative. Hybridoma supernatants were used to quantitate anti-glycosylase activity of clones PM 16.11.08 (●), PM 40.10.09 (■), and PM 42.08.17 (○); ascites fluid was used as a source for PM 37.04.12 antibody (▲).

of enzyme inhibition by the addition of up to 4 times the standard quantity of second antibody used (data not shown). Thus, it appeared that none of the antibodies tested were able to totally precipitate glycosylase activity under conditions of both first and second antibody excess.

Specificity of Monoclonal Antibodies. To examine the antigenic specificity of the four monoclonal antibodies, the uracil DNA glycosylase was isolated from a variety of prokaryotes and eukaryotes for examination in the enzyme immunoprecipitation assay. As shown in Table 1, all four of the monoclonal antibodies crossreacted with uracil DNA glycosylase isolated from normal human skin fibroblasts as well as the glycosylase isolated from a human leukemia-lymphoma T-cell tumor cell (molt 4). The extent of enzyme inhibition by immunoprecipitation was comparable in each case to that observed for the glycosylase isolated from human placenta. The glycosylases from a hamster cell line (BHK-21) and rat liver were precipitated by the antibodies from hybridomas PM 37.04.12, PM 40.10.09, and PM 42.08.07, but no crossreactivity was observed with the antibody from hybridoma PM 16.11.08. No crossreactivity was observed with the uracil DNA glycosylase isolated from yeast or from *E. coli*, as noted by the lack of inhibition observed with any of the four antibodies examined. These results suggest that there may exist considerable antigenic diversity within uracil DNA glycosylase from different organisms. Further, the lack of crossreactivity between PM 16.11.08 and either the rat liver or the hamster enzyme suggests that the antibody is specific for an antigenic determinant on a human enzyme that is absent from both the hamster and rat liver enzymes.

Analysis of Enzyme–Antibody Complex. To examine the physical binding of the monoclonal antibody to the uracil DNA glycosylase, the enzyme–antibody complex was sedimented through a glycerol gradient. Placental glycosylase (100 μl; 13.2 μg) was incubated with 400 μl of bovine serum albumin at 1 mg/ml in water, with 400 μl (95 μg) of antibody from the control hybridoma (PM 1.05) or with 400 μl (101 μg) of antibody from PM 16.11.08 for 2 hr at 4°C. No second antibody was added. The antigen–antibody complex was then layered on a 10–35% glycerol gradient and centrifuged at 40,000 rpm for 16 hr at 4°C. Fractions were collected from the top and enzyme activity was determined. As shown in Fig. 2A, after incubation with bovine serum albumin, the glycosylase activity sedimented as a single peak near the top of the gradient. Mouse IgG, analyzed by itself, sedimented further into the gradient (Fig. 2A). After incubation with antibody from the spontaneous hybridoma control, the glycosylase sedimented as a single peak comparable to that observed for the glycosylase sedimented after incubation with bovine serum albumin (Fig. 2B). However, incubation of the glycosylase with antibody from PM 16.11.08 (Fig. 2B) demonstrated two distinct alterations in this sedimentation pattern: (*i*) there was a diminution in the extent of glycosylase activity sedimenting near the top of the gradient at a position similar to that observed with control antibody; and (*ii*) a second peak of glycosylase activity was observed that sedimented further into the gradient at a density characteristic of a higher molecular weight complex. The extent of activity in the second peak

Table 1. Immunoreactivity of the uracil DNA glycosylases from various sources

Glycosylase source	Immunoreactivity, % of PM 1.05 hybridoma control			
	PM 16.11.08	PM 37.04.12	PM 40.10.09	PM 42.08.07
Human				
Placenta	36	44	13	41
Skin fibroblasts	42	27	58	31
Human leukemia-lymphoma T-cell tumor line (Molt 4)	43	50	55	48
Rat liver	89	74	69	53
Hamster kidney (BHK-21)	93	55	52	62
Yeast	111	116	91	114
Escherichia coli	117	114	103	147

Enzyme inhibition assays with the indicated monoclonal antibodies were performed as described in the legend to Fig. 1 with rabbit anti-mouse IgG as a second antibody. Each experiment was performed a minimum of two times. The extent of inhibition was calculated by comparison to the extent of activity in incubations containing antibody from a spontaneous hybridoma control, which were performed in parallel.

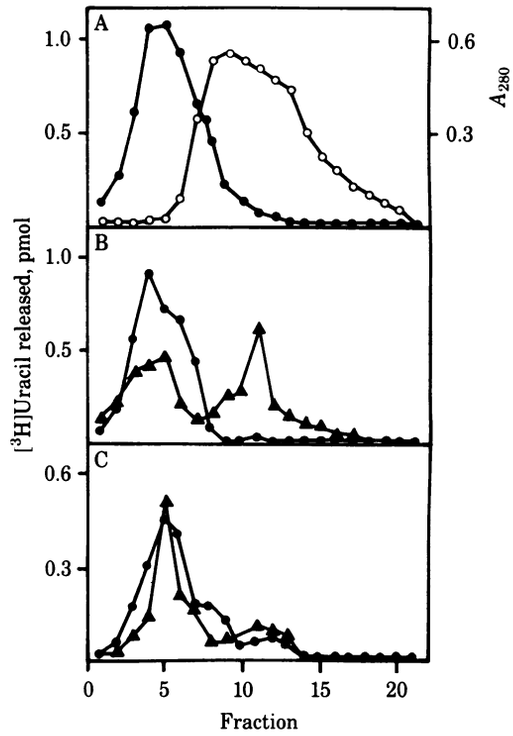

FIG. 2. Glycerol gradient analysis of glycosylase–antibody complexes. Human placental uracil DNA glycosylase (100 μl; 13.2 μg) was incubated with bovine serum albumin at 1 mg/ml and PM 1.05 antibody (400 μl; 95 μg) or PM 16.11.08 antibody (400 μl; 101 μg) for 2 hr at 4°C. BHK-21 glycosylase was treated in a similar manner. The mixture was layered on a 10–35% glycerol gradient containing 10 mM Tris·HCl at pH 7.8, 100 mM NaCl, and 1 mM K_2EDTA. The gradients were centrifuged at 40,000 rpm for 16 hr in a Beckman SW 50.1 rotor at 4°C. Fractions (300 μl) were collected from the top of the gradient. Uracil DNA glycosylase activity was measured in a reaction mixture (total volume 100 μl) which contained 100 mM Tris·HCl (pH 7.9), 1 mM dithiothreitol, 10 mM K_2EDTA (pH 7.0), and 1–5 mg of [^{3}H]uracil-labeled calf thymus DNA (3,500 dpm/pmol) or poly(dA)·poly([^{3}H]dU) (15,000 dpm/pmol). Reaction mixtures were incubated for 120 min at 37°C. The reaction was stopped by the sequential addition of 300 μl of ethanol, 100 μl of denatured calf thymus DNA (1 mg/ml), and 60 μl of 2 M NaCl. Reactions were assayed as described (6). Mouse IgG was determined by immunoprecipitation with rabbit anti-mouse IgG. Fifty microliters of each fraction was incubated with 100 μl (3.8 mg) of rabbit anti-mouse IgG for 60 min at 37°C and the immunoprecipitate was pelleted at 10,000 × *g* for 30 min. The pellets were resuspended in phosphate-buffered saline and washed twice by centrifugation at 10,000 × *g* for 10 min. The pellets were suspended in 0.1 M NaOH and absorbance was measured at 280 nm. (*A*) Human placental glycosylase incubated with bovine serum albumin at 1 mg/ml (●) or mouse IgG (○). (*B*) Human placental glycosylase incubated with control hybridoma (●) or with PM 16.11.08 antibody (▲). (*C*) Hamster glycosylase incubated with control hybridoma (●) or with PM 16.11.08 antibody (▲).

was approximately equivalent to the diminution of activity observed in the first peak and is comparable to that observed in the immunoprecipitation reaction. The glycerol gradient was performed with an antibody-to-enzyme ratio of 7:1. Thus under condition of first antibody excess, only 50% of the activity was complexed to the monoclonal antibody. In contrast, incubation of hamster glycosylase with PM 16.11.08 antibody did not affect the extent of enzyme activity nor did it affect the sedimentation pattern of the enzyme on the gradient (Fig. 2C). These results correlate with the observed ability of the PM 16.11.08 antibody to selectively inhibit human placental glycosylase but not the hamster glycosylase as noted in the immunoprecipitation reaction. Furthermore, these data show that the ability of the monoclonal antibodies to inhibit glycosylase activity in the immunoprecipitation reaction is specifically related to the capacity of the antibody to bind directly to the glycosylase. However, although the PM 16.11.08 monoclonal antibody binds directly to the uracil DNA glycosylase, it appears to bind at a site that does not affect catalytic activity.

To determine the specificity of the physical binding of another antibody to the placental uracil glycosylase, a similar experiment was performed using the monoclonal antibody from PM 37.04.12. Glycosylase activity after incubation with control antibody sedimented as a single peak (Fig. 3A) similar to that observed above. When the placental glycosylase was incubated with PM 37.04.12 at an antibody-to-enzyme ratio of 40:1, there appeared to be a diminution of activity of the glycosylase (approximately 50%) at a position similar to that observed with the control antibody. This diminution of enzyme activity is comparable to the 44% diminution of enzyme activity as quantitated in the immunoprecipitation reaction. In contrast to PM 16.11.08 antibody, no second peak of glycosylase activity was observed. This same pattern of diminution of activity was also observed for hamster glycosylase incubated with 37.04.12 antibody (Fig. 3B) and corresponds with the ability of this antibody to cross-react with the hamster enzyme. The extent of diminution of enzyme activity is comparable to the partial crossreactivity with hamster glycosylase as measured by immunoprecipitation.

To determine that the extent of immunoprecipitation did not simply reflect low antibody affinity for the glycosylase, reprecipitation experiments were performed. Placental glycosylase (13.2 μg) was incubated with either a fraction (400 μg) from ascites produced by the parental clone SP2/0 (GM 3569) or ascites purified antibody from PM 16.11.08 (400 μg) and sedimented through a glycerol gradient. In the PM 16.11.08 gradient, two peaks of activity were observed. The first peak of glycosylase activity (that which contained no IgG) was pooled and 100 μl was reincubated with either GM 3569 antibody or PM 16.11.08 antibody and resedimented through a second glycerol gradient. When the glycosylase from the first peak of the PM 16.11.08 gradient was reincubated with GM 3569, there appeared a single peak (Fig. 4) similar to that observed previously (Fig. 2). This was also observed when the glycosylase from this gradient was reincubated with PM 16.11.08 antibody (Fig. 4). It should be noted that the height and amplitude of both the control peak and the peak reincubated with PM 16.11.08 antibody were virtually identical. Furthermore, there was no second peak of glycosylase activity observed in the gradient in contrast to that noted in Fig. 2. Similar results were observed in reprecipitation experiments using PM 37.04.12 antibody. Therefore, these results demonstrate that low binding affinity

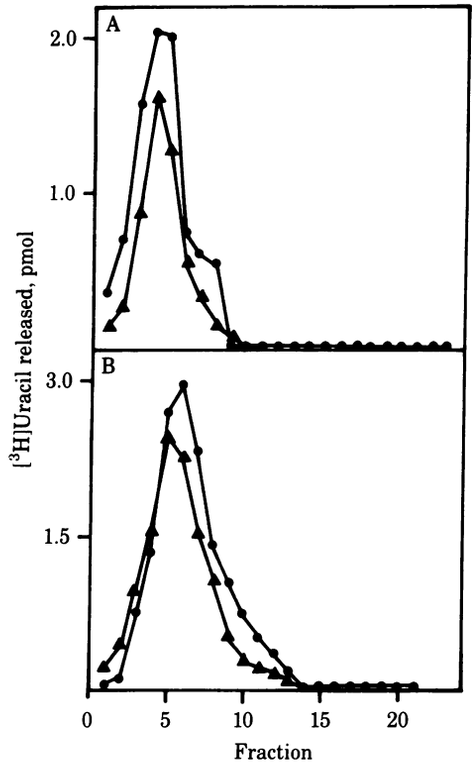

FIG. 3. Glycerol gradient determination of antibody specificity. Human placental uracil DNA glycosylase (100 μl; 13.2 μg) was incubated with PM 1.05 antibody (400 μl; 95 μg) or PM 37.04.12 antibody (400 μl; 450 μg) for 2 hr at 4°C. BHK-21 glycosylase was treated in a similar manner. The mixture was layered onto a 10–35% glycerol gradient containing 10 mM Tris·HCl (pH 7.8), 100 mM NaCl, and 1 mM K_2EDTA. The gradients were centrifuged at 40,000 rpm for 16 hr in a Beckman SW 50.1 rotor. Fractions were collected and uracil glycosylase activity was measured as described for Fig. 2. (A) Human placental glycosylase incubated with control antibody (●) or with PM 37.04.12 antibody (▲). (B) Hamster glycosylase incubated with control antibody (●) or with PM 37.04.12 antibody (▲).

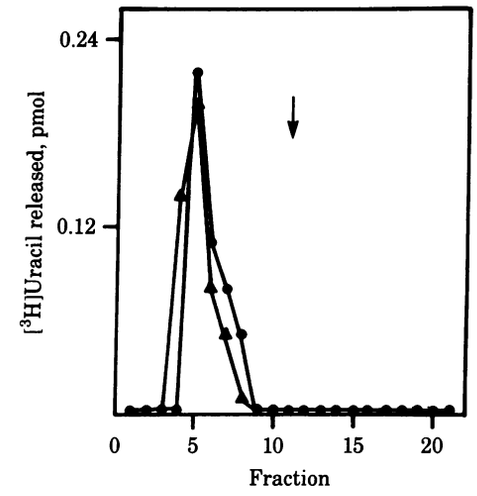

FIG. 4. Effect of reprecipitation of uracil DNA glycosylase. Human placental uracil DNA glycosylase (100 μl; 13.2 μg) was incubated with PM 16.11.08 antibody (400 μg; 400 μl) for 2 hr at 4°C. The mixture was layered onto a 10–35% glycerol gradient as described for Fig. 2. Fractions were collected and uracil glycosylase activity was measured as described for Fig. 2. The solutions containing the first peak of glycosylase activity were pooled and incubated (100 μl) with GM 3569 antibody (400 μg; 400 μl) or PM 16.11.08 antibody (400 μg; 400 μl) for 2 hr at 4°C. The mixture was layered onto a 10–35% glycerol gradient and all procedures were performed as described for Fig. 2. Human placental glycosylase was reincubated with GM 3569 antibody (●) or with PM 16.11.08 antibody (▲). The arrow indicates the position of the second peak from the first gradient.

cannot account for the inability of these monoclonal antibodies to immunoprecipitate more than 50–60% of the uracil DNA glycosylase activity.

DISCUSSION

Recent results from this laboratory have suggested that human cells actively regulate excision repair genes during cell proliferation (5, 6, 8, 11, 12, 17). Specifically, we have demonstrated that human cells increase their capacity for nucleotide excision repair after ultraviolet irradiation and for base excision repair after exposure to methyl methanesulfonate (5, 6, 17). Further, an increase in the activity of the uracil DNA glycosylase was parallel with an enhanced capacity for base excision repair (5, 6). In human lymphocytes, we demonstrated that quiescent cells possess two major glycosylase species in approximately a 1:1 ratio. Induction of cell proliferation by phytohemagglutinin increased only one of the glycosylase species (8). Because phytohemagglutinin specifically induces T-cell proliferation (31), our initial supposition was that the noninduced species was a B-cell enzyme. The glycosylase–antibody interactions described in this report suggest an alternative explanation. With the exception of PM 40.10.09, all the antibodies were capable of precipitating only 56–66% of the glycosylase activity. These results were obtained when using either enzyme immunoprecipitation reactions or glycerol gradient analysis to quantitate enzyme inhibition. This extent of inhibition was constant in the immunoprecipitation reaction even when there was a 4-fold higher than normal quantity of second antibody. Further, glycerol gradient sedimentation analysis demonstrated that only 50% of the enzyme was complexed to PM 16.11.08 antibody at an antibody-to-enzyme ratio of 7:1 or to PM 37.04.12 antibody at a 40:1 ratio. No second antibody was present in these experiments. Similar results were noted in an immunoprecipitation reaction using uracil DNA glycosylase isolated from relatively homogeneous human cells. Thus, it seems unlikely that distinct immunoreactive glycosylases that retain glycosylase activity could result from proteolysis during enzyme purification from placenta (32, 33).

It may be argued that these results suggest incomplete immunoprecipitation of the uracil glycosylase activity due to low antibody affinity. However: (*i*) In the reprecipitation experiment in which the first peak of glycosylase activity from the PM 16.11.08 glycerol gradient was reincubated with PM 16.11.08 and sedimented through a second gradient, the peak of uracil glycosylase activity was equivalent to that observed for the control. Further, there was no second peak of glycosylase observed in the second gradient. Similar reprecipitation experiments using PM 37.04.12 antibody from its first gradient produced similar results. (*ii*) If the antibodies were of low affinity, then one would expect to see a smearing of glycosylase activity along the gradient. This was never observed. (*iii*) The antibody-to-antigen ratios in both the immunoprecipitation reactions (up to 2,000:1) and the glycerol gradient experiments (up to 400:1) were such that they would compensate for any effect of low antibody affinity.

These cumulative data suggest that human cells may contain two uracil DNA glycosylases that are antigenically distinct. Because the mitochondrial glycosylase was removed during the enzyme purification (28), these two enzymes are presumably major species within human cells. It would appear that each antibody may recognize one or the other isoenzyme with the possible exception of PM 40.10.09, which may recognize an epitope common to both isoenzymes. Each isoenzyme may be encoded by a separate structural gene. Recent evidence suggests that some isoenzymes in mammalian cells are encoded by multiple gene families, which may be differentially regulated (34). Thus, one glycosylase species may be constitutively expressed in human cells independent of the proliferative state of the cell. The second isoenzyme would be differentially regulated during the defined, temporal program of gene regulation observed during cell proliferation (15, 16). In particular, its expression would be selectively increased as a function of cell growth. The isolation and characterization of such sets of uracil DNA glycosylase genes is now required to examine the relationship between such regulation and the repair capacity of human cells.

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