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A DNA GLYCOSYLASE FOR OXIDIZED THYMINE RESIDUES IN *DROSOPHILA MELANOGASTER*

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A DNA glycosylase activity which excises fragmented thymine residues has been identified in cell extracts from *Drosophila melanogaster* embryos. The enzyme has an apparent M_r = 40,000, acts preferentially on double-stranded polydeoxyribonucleotide substrate and requires no co-factors. The DNA glycosylase presumably acts in excision-repair of pyrimidines damaged by hydroxyl radicals and other oxidizing species. This is the first identification of a DNA glycosylase in *Drosophila* cells.

Cellular DNA is constantly subject to premutagenic changes, which are efficiently corrected by various DNA repair enzymes [1]. One class of enzymes, the DNA glycosylases, initiates excision-repair by cleaving the base-sugar bond of altered bases. DNA glycosylases that liberate deaminated, methylated, oxidized and irradiated bases have been described in extracts from bacteria, fungi and mammalian cells [1,2]. In contrast, specific searches for uracil-DNA glycosylase and 3-methyladenine-DNA glycosylate in *Drosophila* cells have proved negative [3-5], and it has been suggested that Drosophila represents a class of organisms that does not rely on base excision repair as a mechanism for removing modified or non- conventional bases from DNA. Here I report the presence of a DNA glycosylase activity in *Drosophila* embryos.

EXPERIMENTAL PROCEDURES

Drosophila melanogaster (wild type, Oregon-R) early embryos (2-16 hr, average 9 hr) were a generous gift of Dr. David Glover, Imperial College, London. Cell extracts were prepared essentially as described by Banks et al. [6] and in Fig. 1. All

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operations were performed at 0-4°C. For gel filtration, 4 ml of the crude cell extract was supplemented with $(NH_4)_2SO_4$ to 80% saturation. The precipitated proteins were collected by centrifugation and redissolved in 2 ml of 0.5M NaCl/3mM EDTA/21 mM 2-mercaptoethanol/50 mM Tris-HCl, pH 7.5/5% glycerol, dialysed against the same buffer, and applied to a precalibrated Ultrogel AcA-54 column (2.6 x 150 cm, LKB Products). DNA glycosylase assays for the release of urea, thymine glycol, formamido-pyrimidine and uracil from damaged DNA were performed according to established procedures [7-11]. The substrate for oxidized pyrimidines was a poly(dA, [2-^{14}C]dT) copolymer containing 97% dAMP residues which had been treated either with $KMnO_4$ and NaOH to convert the thymine moieties into urea or with OsO_4 to yield thymine glycol [8]. To generate a double-stranded structure, it was annealed with an equimolar amount of poly(dT). To measure urea release the reaction mixture containing 11 pmol (1000 cpm) of substrate in a buffer consisting of 100 mM KCl/1 mM EDTA/1 mM dithiotreitol/70 mM Hepes-KOH (pH 7.8) was supplemented with 30-175µg protein and incubated for 20 min at 37°C. Protein and substrate were precipitated with ethanol at -20°C, centrifuged and the supernatant analysed by reverse phase HPLC or paper chromatography [7,8] (Fig. 1). For thymine glycol assay the procedure was scaled up five-fold. About 50% of the radioactive material can be released from these polymer substrates under conditions of enzyme excess [8]. AP endonuclease assays and urease digestion was performed as described previously [9].

RESULTS AND DISCUSSION

Crude cell extracts of Drosophila melanogaster embryos contain readily detectable urea-DNA glycosylase activity (Fig.1), about 1 pmol min^{-1} mg^{-1} protein (1 unit mg^{-1}). This level is 15 times lower than that in E. coli, but similar to that in extracts of calf thymus and human fibroblasts [7-9]. The identity of the released material was further confirmed by its susceptibility to urease [9]. The enzyme acts on double-stranded but not single-stranded substrate, is fully active in the presence of 2 mM EDTA, is two-fold stimulated by KCl up to 100 mM, but inhibited at 200 mM, and is not product inhibited (<10%) by urea up to 2 mM. These properties are similar to those of the bacterial and mammalian enzymes [7-9]. On size- fractionation by gel filtration the enzyme eluted as a single, symmetrical peak consistent with a globular protein of M_r = 40,000±3,000. Thus, the apparent molecular weight is larger than that of the corresponding E. coli and calf thymus activities [7-9], but it is possible that the enzyme migrated as a dimer on AcA-54, although

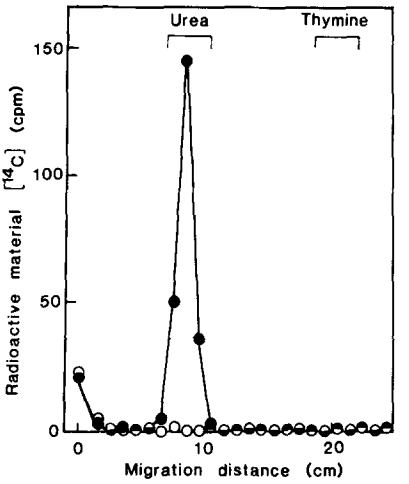

Fig.1. Enzymatic release of free urea from DNA containing fragmented thymine residues. Dechorionated Drosophila embryos (1g) were homogenized in 5 ml of a buffer containing 200 mM KCl/3 mM EDTA/5 mM DTT/1 mM phenylmethylsulfonylfluoride/10 mM sodium metabisulfite/50 mM Tris-HCl (pH8) by five strokes with a glass Teflon homogenizer, and the material was centrifuged at 30,000xg. Aliquots of the supernatant were stored frozen at -70°C. The ethanol soluble material released from the polydeoxyribonucleotide substrate containing urea residues by the crude cell extract of Drosophila embryos was analysed by chromatography on Whatman 3MM paper in ethylacetate/n-propanol/H_2O (4:1:2) [ref. 7]. The standard reaction mixture contained 11 pmol (1000 cpm) substrate. (•), 120µg extract added; (o), no extract. Reference compounds were chromatographed in separate lanes.

such behaviour has not been observed in the case of other DNA glycosylases.

The purified E.coli and calf thymus enzymes catalyze the release of urea (ring-fragmented thymine) as well as thymine glycol (ring-saturated thymine). Here, a small but significant amount of thymine glycol-DNA glycosylase activity was present in the most active AcA-54 fractions, with thymine glycol being released at 2-3% of the rate of urea. The lower rate of excision of thymine glycol compared to urea (as well as the lower overall activity) makes the Drosophila enzyme appear closer to the bovine and human activities than to that found in E. coli [7-9]. Though these activities have been discovered using model substrates, the E.coli enzyme has been shown to be fully active on DNA as well as the poly(dA.dT) polymers [12]. Homogenous preparations of the analogous E. coli enzyme cleave DNA at AP sites [8]. AP

endonuclease activity was not exclusively associated with the Drosophila DNA glycosylase on gel filtration. However, since a homogenous preparation is not available in this case, it is not clear whether the Drosophila DNA glycosylase also acts on AP sites. No DNA glycosylase activities excising uracil or formamidopyrimidine were detected in crude Drosophila cell extracts when assayed under standard conditions. The absence of uracil-DNA glycosylase is in agreement with previous work [3,4]. Formamidopyrimidine-DNA glycosylase is only present in low amounts in mammalian tissues and cells, and is not readily detected in crude extracts from such sources [9,13].

The main purpose of this work has been to establish the existence of a DNA glycosylase activity in Drosophila embryos. It is the first report of a DNA glycosylase in this species, and this observation does not support the proposal by Deutsch and co-workers [4,5] that the base excision-repair pathway is not employed for DNA repair in Drosophila cells.

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