ACTIVITY OF URACIL-DNA GLYCOSYLASE IN DIFFERENT RAT TISSUES AND IN REGENERATING RAT LIVER

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1. Introduction

DNA-glycosylases are the enzymes which release abnormal base residues from DNA [1]. Three types of DNA-glycosylases are known: 3-methyladenine–, hypoxanthine– and uracil–DNA-glycosylase [2–4].

It is still obscure how uracils are generated in DNA. Two ways are possible:

(i) The spontaneous (heat) deamination of DNA–cytosine [5–7];
(ii) The incorporation of deoxyuridylate from dUTP instead of thymidylate during DNA synthesis [8–10].

If uracil–DNA-glycosylase (UDG) is mainly released uracil incorporated during replication, then a correlation between activity of the enzyme and DNA synthesis should be expected, as observed for the replicative enzymes [11]. We have therefore tried to make a comparative study of the UDG activity and proliferation in different rat tissues as well as the UDG activity in regenerating rat liver after partial hepatectomy.

2. Materials and methods

2.1. *Preparation of [^{3}H]uracil-labeled DNA*

Substrates were prepared by copying the activated calf thymus DNA with purified *Micrococcus luteus* DNA-polymerase, using d[^{3}H]UTP (47 Ci/mmol) and 3 other normal deoxyribonucleoside triphosphates (dCTP, dGTP and dATP) as precursors [12]. Uracil-labeled DNA was isolated from the reaction mixture by chromatography through Sephadex G-200 column.

2.2. *Assay for uracil–DNA-glycosylase*

The activity of UDG was measured by following the release of acid-soluble radioactivity from [^{3}H]-uracil-labeled DNA template [13]. Thin layer chromatography shows that 90% of the acid-soluble radioactivity in this method is associated with uracil.

One unit of activity corresponds to the quantity of the enzyme which releases 0.015 pmol uracil (500 cpm) in 60 min at 37°C.

2.3. *Preparation of extracts*

White rat males (80–120 g) were used. All procedures were done at 0–4°C. Tissues were excised from the rat, cut with scissors and homogenized in a Dounce apparatus (1:10, w/v) in buffer A (10 mM Tris–HCl, pH 8.0; 10^{-3} M EDTA; 10^{-3} M DTT; 20% glycerol and 0.05 M KCl). Cell extracts were kept at 0°C for 30 min before centrifugation at 10 000 × *g* for 60 min. The supernatants were collected for the enzyme assay. Buffer B (2 ml) (buffer A + 1 M KCl) was added to the cell pellets and the suspensions were sonicated for 3 min and after 30 min at 0°C were centrifuged at 10 000 × *g* for 60 min. Supernatants were examined for the enzyme activity. The final sediments were dissolved in 2 ml buffer C (buffer A + 0.5% Triton X-100). After 30 min at 0°C the enzyme activity was determined without centrifugation.

2.4. *Isolation of erythrocytes*

Erythrocytes were isolated from rat spleen by sedimentation through Ficol-Hypaque gradients [14]. The erythrocytes were washed twice with phosphate-buffered saline and sonicated in buffer B.

The UDG activity in regenerating rat liver was studied within 21,24,32,36,40 and 44 h after a partial hepatectomy [15].

3. Results and discussion

The greatest part of the UDG activity was extracted

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Table 1
Uracil–DNA-glycosylase activity in rat tissues (units UDG/g tissue)

Tissue	0.05 M KCl	1 M KCl	0.5% Triton X-100	Total activity
Brain	460	575	36	1071
Heart	553	1900	92	2545
Liver	490	6697	224	7411
Kidney	672	7820	325	8817
Thymus	905	12 273	143	13 321

with buffers of low ionic strength, followed by those of high ionic strength and 0.5% Triton X-100. Since most of the activity was extracted with high salt concentrations (see table 1) we assume that UDG was associated with the chromatin fraction of the tissues. This observation is confirmed by our failure to find any UDG activity in spleen erythrocytes.

The proliferative activity of rat tissues (except thymus) is a well-established phenomenon [16]. Proliferation of thymus was measured by [^{3}H]thymidine incorporation into DNA per g of the tissue. UDG contents and the proliferative activities in tissues investigated are given in table 2. The corresponding values for liver are taken to be 1. Table 2 shows that there is a good correlation between the UDG activity and the proliferative capacity of a given tissue.

The activity of UDG have also been determined in spleen. While the proliferative activity of spleen is 5.5-times higher than that in liver, the activity of UDG varies from 0.76–1.3. The reason for this exception to the general rule is unknown.

To determine whether UDG is induced in regenerating rat liver we have compared the activity of the enzyme at different times after partial hepatectomy. To decrease individual differences between rats, the liver has been used as control for a particular rat. The

Table 2
Proliferative activity and uracil–DNA-glycosylase content in tissues[a]

Tissue	Proliferative activity [16]	UDG activity
Brain	0.21	0.14
Heart	0.42	0.34
Liver	1	1
Kidney	1.2	1.19
Thymus	1.85	1.80

[a] Values for the liver are taken to be 1

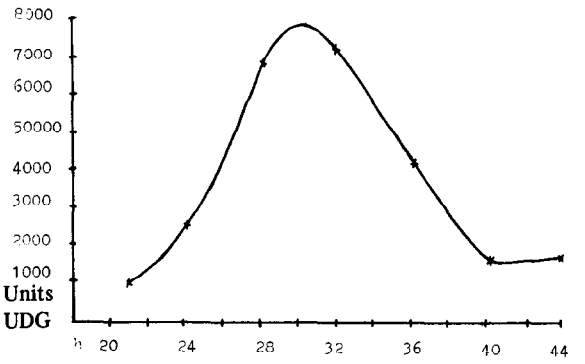

Fig.1. Uracil–DNA-glycosylase activity/g regenerating liver.

UDG activity was determined in extracts obtained with buffer B and sonication, since this procedure is most effective. During liver regeneration the UDG activity increases reaching a maximum 28–32 h after the operation (fig.1). α-DNA polymerase activity changes in a similar way [17].

The data obtained are in agreement with the hypothesis that the level of UDG reflects the proliferative capacity of a given cell population and that UDG is mainly used for excision of replicative uracil.

This agrees with the results of other laboratories demonstrating a 10-fold enhancement of UDG activity during lymphocyte stimulation by phytohemagglutinin [18].

Stimulation of UDG during the cell cycle of a synchronous population in human diploid fibroblasts reaches a maximum just prior to maximum DNA replication or the increase in DNA polymerase activity [19]. This has been interpreted to suggest that UDG is used to correct the pre-existing DNA modifications (template 'cleaning') before replication, that is mainly heat-induced uracil. From this point of view it is difficult, however, to explain why the cell in the non-dividing state tolerates heat-induced DNA uracil since this can lead to mutated proteins if the uracil is located in the transcribed DNA strand (C–U transition).

As to other repair enzymes it is known that phytohemagglutinin stimulation of lymphocytes increases repair synthesis 8-fold after UV-irradiation [20] and 20-fold after ionizing radiation [21]. However, this is not true for all the repair enzymes since the AP-endonuclease activity in dividing and undividing Chinese hamster cells remains unaltered [22]. Repair enzymes are probably either dependent or independent of DNA synthesis.

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