Correspondence

Adenine glycosylase activity in mammalian tissues: an equivalent of ribosome-inactivating proteins

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Several plant tissues contain enzymes, provisionally called ribosome-inactivating proteins (RIPs), which damage ribosomes in an irreversible manner (reviewed in [1–3]) by removing a specific adenine from the major rRNA. Subsequently it was observed that all RIPs remove adenine from DNA, and the denomination of polynucleotide:adenosine glycosidase was proposed for them [4], consistently with the official denomination of ricin (rRNA glycosidase, EC 3.2.2.22). In the present letter this denomination will be changed to adenine polynucleotide glycosylase (adenine glycosylase), by analogy with the denomination of DNA glycosylase used for similar enzymes.

The presence of RIPs in diverse organisms led to the notion that equivalent proteins could exist in the animal kingdom [5]. We report now that partially purified preparations of some animal tissues remove adenine from DNA, and that their activity increases in stressed and virally infected cells.

The crude extracts from several rat tissues did not exhibit any activity typical of RIPs; namely, they did not inhibit protein synthesis in a rabbit reticulocyte lysate nor caused any release of adenine from DNA. However, after the extracts were subjected to a chromatographic procedure on Procion-Red (Pharmacia) used to purify RIP, the extracts of rat spleens, lungs and brains exhibited a significant deadenylating activity (Table 1), still without any inhibitory activity on the reticulocyte lysate system at concentrations up to 100 μg/ml of protein (results not shown). The rat spleens extracts were active also, in decreasing order, on poly(A), on rRNA (rat liver 28S+5S) and on tRNA. Much less or no activity at all was found in similarly treated extracts of mice, ox, hog, rabbit and human spleens.

The activity was proportional to the concentration of extract present in the reaction mixture and had an optimum at pH 5.5–6.5 (optimum for plant RIPs acting on DNA is 4.0). When the semi-purified preparations were subjected to gel filtration on a TSK-3000 column (Toso-Haas), the enzyme activity appeared in the fractions of the effluent corresponding to a molecular mass between 20 000 and 40 000. Also, the activity was bound to cation-exchange chromatography media in conditions of binding basic proteins (results not shown), suggestive of a protein with a high p*I*.

Further purification of the enzyme activity from rat spleen extract was attempted on a micro-scale. After chromatography on Orange-matrix (Amicon), the activity was resolved in two peaks, although without a significant increase in specific activity of either peak. Upon chromatography on mono-*S* ion exchange column (Pharmacia), the activity appeared widely distributed during a gradient elution, with a low yield, but with an increase of approx. 10-fold of specific activity in selected fractions. These results indicate that these enzymes may be present in different isoforms, as it happens with plant RIPs. Fractions with higher specific activity were subjected to analysis of reverse-phase chromatography, still showing a substantial heterogeneity of protein species.

Rat spleen extracts enriched by Procion-Red chromatography had an activity on calf thymus DNA lower than on herring sperm-DNA, and had no activity at all on deoxy-adenosine or 3′dAMP and 5′dAMP (results not shown).

Table 1
Enzyme activity (pmol adenine released/mg protein/40 min)

Tissue	Substrate			
	Herring sperm DNA	tRNA	rRNA	Poly(A)
Rat spleens	257	37.5	56	179
Rat brains	9.5			
Rat lungs	332			
HELF fibroblasts				
Normal	5.1			
Stressed	15.8			
Poliovirus infected 1 h	18.3			
Poliovirus infected 6 h	45.4			
Poliovirus infected 18 h	2.3			
Poliovirus infected 30 h	7.7			

Animals were from a breeding firm. Human spleens were from organ donors, and were kept for 24 h at room temperature in sterile saline before processing. The work was performed according to the guidelines of the University Ethics Committee. Human embryonic lung fibroblasts (HELF, from Istituto Zooprofilattico, Brescia, Italy) were grown in monolayers with Eagle's minimum essential medium supplemented with 10% fetal calf serum (FCS) and either infected or mock infected when they were 70–80% confluent with poliovirus type 1 isolated from the stool of a vaccinated child at a m.o.i. of 10 pfu/cell. Stressed cells consisted of 3.6×10^7 cells kept at 37°C in a CO_2 incubator for 5 days with 0.01% of FCS. Supernatant of tissue homogenates and cell lysates were chromatographed on Sephadex G-25 column and then on Procion-Red column. The column was eluted sequentially with phosphate-buffered saline, 5 mM phosphate buffer, pH 7.0, 10 mM Tris–HCl, pH 8.0, 0.25 M NaCl in 10 mM Tris–HCl, pH 8.0, and finally 3 M NaCl in 10 mM Tris–HCl, pH 8.0. The adenine glycosylase activity of protein eluted with the last eluent was measured from the amount of adenine released as described [4], except that adenine was measured by high-performance liquid chromatography with a XTerra MS C18 column (2.1×50 mm). Adenine was separated by a gradient of methanol in 10 mM ammonium acetate. The effluent was introduced into a Waters ZMD 4000 mass spectrometer detector and ions with mass 135.8 were measured.

As compared with plant RIPs, the activity of rat spleen extracts appeared to be more heat-labile, being destroyed by heating at 56°C for 30 min, was only partially resistant to proteolysis by trypsin (plant RIPs are quite resistant), and had an optimum of activity at a pH 5.5–6.5, higher than the optimum for plant RIPs acting on DNA (pH 4.0). Plant RIPs act on rRNA (rat liver 28S+5S) and some on poly(A), but the effect of most animal extracts on these substrates could not be evaluated due to the presence of high levels of RNase(s).

Assuming a specific activity in the same range of that of RIPs, the most active extracts should contain from 0.1 mg (referred to saporin-S6, the most active RIP) to 30 mg (referred to momordin I, the least active RIP) of enzyme protein/kg of tissue. It is possible that an even lower, and thus undetectable, amount of enzyme is present in the tissues in which no measurable activity was found with the method employed.

After chromatography on Procion-Red, the fractions active on DNA did not affect protein synthesis with a rabbit reticulocyte lysate (results not shown), indicating that the enzyme does not act, or acts only at a much higher concentration, on mammalian ribosomes. This is consistent with the known property of plant RIPs, which often act on plant ribosomes at concentrations much higher than those damaging animal ribosomes [1]. This suggests that autologous ribosomes are not the primary target of these glycosylases.

A higher activity than in normal cells was found in the extracts of stressed or virally infected animal cells (Table 1). The activity begins to increase at a very early stage after infection, reaches a peak at 6 h, and declines at subsequent times, when cells appear severely altered. This increase is consistent with the higher levels of RIPs observed in old, stressed or virally-infected plant tissues (review in [2]), suggesting that these proteins may have a role, both in plant and animal cells, in the pathogenesis of the apoptosis which occurs in these conditions, including poliovirus infection.

To our knowledge, the present results indicate for the first time the presence in animal tissues of an enzyme activity removing a normal purine base from normal DNA, but raise again the question of the function of RIPs, originally found in plants [1] and subsequently in fungi and at least one bacterium (*Shigella*). The role of RIPs in plants is still not definite, and the main hypotheses put forward were (i) a protective role, since they possess antiviral activity [1], and (ii) a role in the apoptotic process, since they are expressed at a higher level in senescent or stressed tissues. Furthermore, the notion that the damage to DNA by plant RIPs could contribute to the antiviral action of these proteins [2,3], could be envisaged for the animal enzyme. The presence of adenine glycosylase activity in quite different organisms and tissues and its increased expression in damaged tissues, i.e. when cells are bound to die, point towards a role of general significance, possibly in the mechanism leading to cell death and/or in randomly disrupting nucleic acid sequences which have to be inactivated, such as any naked DNA which may be present in the cells.

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