TEMPLATE ACTIVITY IN LIVER DNA FROM RATS FED WITH MALONDIALDEHYDE

O.L.KLAMERTH *
Institut für Strahlentechnologie, Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe, Germany

H.LEVINSKY
Tierphysiologische Abteilung, Institut für Biologie am Reaktorzentrum, Seibersdorf, Austria

Received 31 March 1969

It is now generally accepted that the integrity and biological function of DNA is reflected in its capacity to act as a template in the DNA-dependent RNA polymerase (E.C.2.7.7.6) system [1, 2]. Using this system, Ono et al. [1] investigated the effects of ultraviolet light, and Phillips et al. [3] those of hydroxylamine on the template properties of polycytidine. As previously reported [4, 5], the bifunctional aldehydes glyoxal and malondialdehyde (MDA), both formed during the radiolysis of aqueous sugar solutions and glutamic acid [6, 7], strongly inhibit the growth of cells in culture by blocking DNA replication in particular. Moreover, mutagenic effects of irradiated sucrose solutions were observed in cells by Shaw and Hayes [8], presumably attributable to the dialdehydes formed. It has been further demonstrated that MDA interacts with the guanine and cytidine bases of the DNA in vitro, preventing total DNA degradation by DNase due to incomplete strand separation of the DNA helix [9]. As the RNA polymerase seems to read the template DNA accurately and sequentially [10], it could be assumed that DNA, after treatment with MDA, would show altered template activity in this system.

Therefore, experiments were performed with rats to investigate the template activity of liver DNA after incubation *in vitro* with various amounts of aqueous MDA, as well as of liver DNA from animals fed with MDA.

Groups of 3 to 4 young rats (Sprague Dawley, strain CFE, white, weighing from 60 to 70 g unless otherwise stated) were fed normally, with the addition of daily doses of 100 μg MDA/g body weight; in some cases the MDA was applied intreperitoneally. The MDA was freshly prepared as previously described [9] and given in saline, pH 6.7, each morning by means of a tube inserted into the esophagus. Simultaneously mock-treated animals from the same litter served as controls. After the intervals indicated, the animals were sacrificed by decapitation, the livers perfused *in situ* with ice-cold 0.25M sucrose/0.005M Tris, quickly removed, and the nuclei isolated according to Blobel and Potter [11]. The isolated nuclei were suspended in saline/EDTA and disrupted by stirring with SDS (end concentration 2%). The viscous solution was brought to 1 M NaCl and extracted at 45–50° with 90% phenol, pH 8.5; this procedure was repeated once more at 20°. The nucleic acid obtained from the aqueous supernatant by precipitation with alcohol in the usual manner was freed from RNA by a 30-min treatment with RNase (20 μg/ml, 37°). RNase was digested by pronase (50 μg, preincubated for one hour at 37°) and the solution, after deproteinization with chloroform-amylalcohol (24 : 1 v/v), precipitated again. The DNA obtained

* Present address: Institute for Human Genetics, University of Heidelberg, Mönchhofstrasse 15A, Heidelberg, Germany.

was free from RNA and protein. Determination of the phosphorus content of the DNA was in agreement with a value of 50 μg DNA/$A_{260m\mu}$.

For the *in vitro* experiments, liver DNA from control animals was incubated at 37° in 0.1 M Tris, pH 4.6, with various concentrations of MDA according to Brooks and Klamerth [9]. The rate of interaction with the dialdehyde was calculated by measuring the amount of acid-soluble products formed after 5 and 10 minutes by enzymic degradation with DNase (2.5 μg/ml), as described previously [9].

Nucleotide triphosphates were purchased from Cal Bio-Chem, RNase and pronase from Serva (Heidelberg), and ^{3}H-UTP from New England Nuclear. RNA polymerase (highly purified from micrococcus lysodeikticus) was a product of the Miles Laboratories. The radioactivity was measured in a liquid scintillation spectrometer (Packard, Model 3380). Protein was determined according to Lowry et al. [12], and phosphorus according to Chen et al. [13].

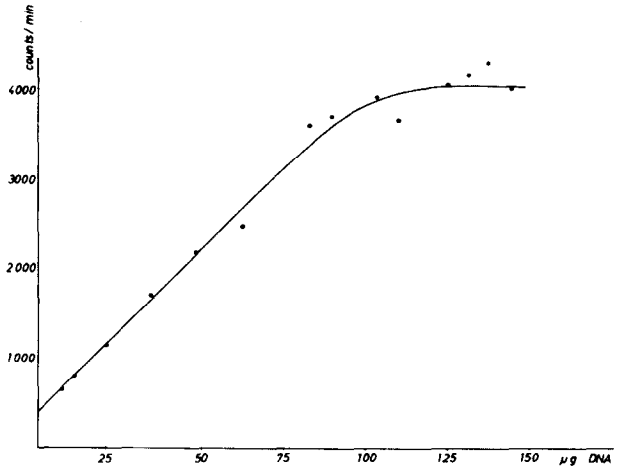

Fig. 1. DNA dependence of ^{3}H-UTP incorporation into the acid-insoluble fraction. Various amounts of DNA (as indicated in the graph) were added to the standard reaction mixture at zero time, and the assay performed as described.

Polymerase assay: The reaction mixture contained in 0.8 ml 60 μmoles Tris/HCl (pH 8.4), 5 μmoles $MgCl_2$, 1 μmole $MnCl_2$, 60 μmoles KCl, 20 μmoles β-mercaptoethanol, 3 μmoles ATP, CTP and GTP each, 0.2 μmole ^{12}C-UTP, 2.5 μC ^{3}H-UTP (specific activity 1.31 C/mmole), 3.3 to 125 μg DNA (as specified), and 7 units polymerase. The reaction mixture was kept at 37° for 20 min and the reaction stopped by the addition of 0.2 ml of an aqueous solution containing 500 μg high-molecular yeast RNA and 0.5 μmole inactive UTP. The sample was quickly cooled in ice and precipitated with 3.0 ml ice-cold 0.6 N-perchloric acid/0.05 M sodium pyrophosphate; the precipitate was washed three times with 0.5 N-perchloric acid/0.05 M sodium pyrophosphate, and finally dissolved in 1 ml formic acid. 0.1 ml aliquots were applied to glass fiber disks (Whatman), dried in vacuum at room temperature, and the radioactivity counted.

The use of the incorporation rate of a nucleotide triphosphate into RNA as a parameter for the functional activity of DNA in the biological sense is feasible only under certain experimental conditions. As already observed by Fox et al. [2], "For a given amount of enzyme a certain quantity of DNA is required to bind the enzyme fully and allow for maximal rates of RNA synthesis". The correlation between the rate of incorporation of various concentrations of DNA at a constant enzyme level is demonstrated in fig. 1, which also shows the saturation point of the system.

Experiments *in vitro* with DNA which had interacted with MDA after previous incubation with the dialdehyde in various concentrations revealed an almost complete loss of template capacity (table 1).

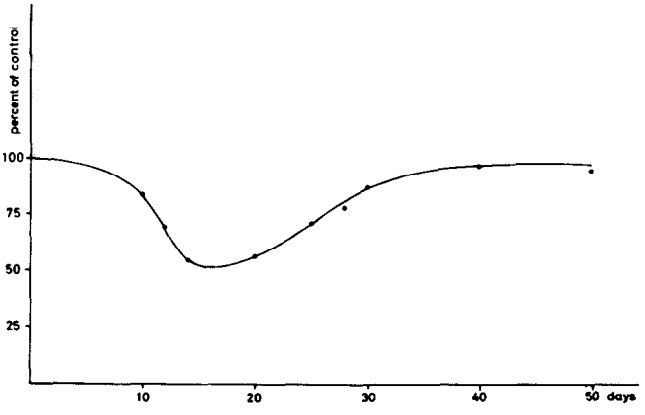

Fig. 2. Template activity of liver DNA from animals after *in vivo* treatment with MDA, measured in the standard *in vitro* system described by the incorporation of ^{3}H-UTP into the acid-insoluble sediment. Each dot represents the average of three independent experiments with 100 μg nuclear DNA from three to four pooled rat livers.

Table 1
Inhibition of the incorporation of ^{3}H-UTP into the acid-insoluble sediment after treatment with MDA *in vitro*

	"Cross-linking" (arbitrary units)	Counts per minute
Standard assay mixture		
+ 20 μg DNA (untreated)	–	1133
+ 20 μg DNA (treated)		
MDA molar ratio 1:1	20	190
MDA molar ratio 10:1	40	15
MDA molar ratio 100:1	70	0

Although it is not proved that such a reactive substance as MDA can reach the liver unchanged, the experiments with MDA-fed rats indicated a diminished template activity in the liver DNA, the decrease being dependent upon the age and weight of the animals and the duration of the MDA application. The curve in fig. 2 demonstrates that liver DNA from young animals treated for approximately 14 days shows the greatest decrease in activity, whereas longer treatment results in partial recovery. A smaller effect was observed in rats whose weight exceeded 150 g at the time of treatment. The lowest rate of activity was observed after intraperitoneal application of MDA in young rats (60 g body weight), but the experimental material was too limited to permit a final conclusion. It seems plausible that the older animals may have developed enzymes which degrade the dialdehyde before it reaches the liver. The effect in young animals can also be explained by the fact that in growing animals the liver cells are still multiplying, and the S-phase is presumed to be the most vulnerable point of attack.

This study was carried out within the framework of the International Programme on the Irradiation of Fruit and Fruit Juices (Seibersdorf Project) at the Institute of Biology, SGAE, Reactor Centre, Seibersdorf, Austria. The authors are indebted to Mr. F.J.Kocsis for his excellent technical assistance.

References

[1] J.Ono, R.G.Wilson and L.Grossman, J. Mol. Biol. 11 (1965) 600.
[2] C.F.Fox, R.I.Gumport and S.B.Weiss, J. Biol. Chem. 240 (1965) 2101.
[3] J.H.Phillips, D.M.Brown, R.Adman and L.Grossman, J. Mol. Biol. 12 (1965) 816.
[4] O.L.Klamerth, Biochim. Biophys. Acta 155 (1968) 271.
[5] O.L.Klamerth, Proc. Symposium on Quantitative Biology of Metabolism, ed. A. Locker (Springer-Verlag, 1968) p. 177.
[6] K.Ambe and A.Tappel, J. Food Science 26 (1961) 448.
[7] J.Morre and S.Morrazatti-Pelletier, Compt. Rend. Acad. Sci. Paris 262 (1966) 1729.
[8] M.W.Sahw and E.Hayes, Nature 211 (1966) 1254.
[9] B.R.Brooks and O.L.Klamerth, European J. Biochem. 5 (1968) 178.
[10] W.S.Robinson, W.T.Hsu, C.F.Fox and S.B.Weiss, J. Biol. Chem. 239 (1964) 2944.
[11] G.Blobel and V.R.Potter, Science 154 (1966) 1662.
[12] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J.Biol. Chem. 193 (1951) 265.
[13] P.S.Chen, T.Y.Toribara and H.Warner, Anal. Chem. 28 (1956) 1756.