STIMULATION OF AMINO ACIDS INCORPORATION INTO RAT LIVER NONHISTONE CHROMATIN PROTEINS AFTER TREATMENT WITH DIETHYLNITROSAMINE

A. ALONSO and H. P. ARNOLD
Deutsches Krebsforschungszentrum, Institut für Experimentelle Pathologie, 69 Heidelberg, Germany

Received 24 February 1974

1. Introduction

The nonhistone proteins of the chromatin have been implicated in the regulation of transcription [1, 2]. The heterogeneity of these species- and organ-specific proteins, as against the more uniform pattern for histone, have supported this interpretation [3–5]. Some of these proteins are also able to bind to DNA [6]. In addition, their presence has been shown to enhance the rate of transcription of chromatin in vitro [7]. If the NHP play a role in the regulation of transcription, it is of interest to elucidate their behaviour during the early stages of carcinogenesis. Studies with alkylating carcinogens have revealed that these substances alkylate proteins and nucleic acids, decreasing their rates of synthesis [8, 9].

We have investigated the changes in precursor incorporation into the chromatin-bound nonhistone proteins and into rapidly labelled RNA after treatment with the potent liver carcinogen diäthylnitrosamine (DENA).

2. Material and methods

2.1. *Isolation procedures*

Sprague–Dawley rats were injected with 20 mg DENA/kg by.w. One hour before killing, they were injected i.p. with 50 μCi ^{14}C-protein hydrolysate or 2.5 mCi ^{3}H-leucine/kg by w. The animals were also injected with 200 μCi ^{3}H-uridine/kg by.w. 10 or 15 min before killing. The livers were perfused with 0.24 M surcrose–3 mM $CaCl_2$ and homogenised in the same medium. The sediment obtained after centrifugation at 2000 *g* for 10 min was washed twice and the crude nuclei were layered on to 2.2 M sucrose–3 mM $MgCl_2$ and centrifuged for 1 hr at 40 000 *g* (IEC B-60, Rotor SB-283). The isolated nuclei were extracted twice for 30 min with 0.14 M NaCl, and extract was centrifuged for 90 min at 105 000 *g* to separate membranes and ribosomes. The resulting supernatant is the 'globulin fraction'. The pellet remaining after the initial extraction with 0.14 M NaCl was further extracted with 0.2 N HCl to remove the histones. The remaining pellet was dissolved in phenol to isolate the NHP according to the method of Teng et al. [10]. After the last dialysis against 8.6 M urea, the proteins were dialysed against electrophoresis buffer and 1:4 E-buffer (E-buffer: 38 mM glycine, 5 mM Tris, 0.3% SDS and 1% 2-mercaptoethanol, pH 8.3). The RNA was extracted with hot phenol according to Scherrer and Darnell [11].

2.2. *Acrylamide electrophoresis*

The NHP were analysed using SDS-acrylamide electrophoresis according to the method of Weber and Osborn [12]. Slab gels were employed. Linear gradients were prepared with an acrylamide concentration from 7 to 12% and stabilised with sucrose. The gels were run at 20 mM for 7 hr and stained with Amido Black.

2.3. *Determination of radioactivity*

Aliquots of the proteins were solubilised with So-

luene and mixed with 10 ml dioxan-based scintillation fluid. The acrylamide gels were cut into 2 mm slices, solubilised with 0.2 ml H_2O_2 overnight at 65°C. and counted with 10 ml Triton-X 100 scintillation fluid.

2.4. *Analytical determinations*

The protein concentration was determined by the method of Lowry et al. [13] with a standard of bovine serum albumin. The RNA concentration was measured using the orcinol method [14].

3. Results and discussion

One hour after application of DENA, the rate of incorporation of ^{14}C-labelled amino acids into the NHP of the chromatin reached a value of 150% of the controls (table 1). Three hours later, the activity measured still corresponded to 130% of control values. This increase could by attributable to two different mechanisms: i) A higher rate of synthesis of this fraction; this seems improbable, however, since there is an overall inhibition of protein synthesis after DENA-treatment. We were not able to demonstrate an appreciable quantity of labelled phenol-soluble proteins in the cytoplasm, where the synthesis of these proteins probably takes place. It is nevertheless also possible that the newly synthesised proteins are so rapidly transported to the nucleus that no trace of them can be found in the cytoplasm after 1 hr; ii) DENA-treatment could produce an increase in the rate of exchange of bound and free nonhistone chromatin proteins. This view is supported by the finding that 1 hr after DENA-treatment, the specific activity of the nuclear globulins decreased, eventually returning to the normal

Table 1
Specific activities of the NHP, globulins and nRNA after DENA-treatment

	Control	1 hr	3 hr
NHP	195 (100)	294 (150)	259 (130)
0.14 M NaCl soluble proteins	616 (100)	412 (66)	510 (82)
nRNA	1347 (100)	3360 (281)	871 (67)

Values are presented as cpm/mg protein or RNA. The number in parentheses shows the percentage value related to the controls. Each value represents the mean value from three different animals. The experiment was repeated three times. The experiment was repeated using a dose of 5 mg DENA/Kg b.w. in which case a similar result was obtained. This indicates that the stimulation was not just a result of the high dose first employed.

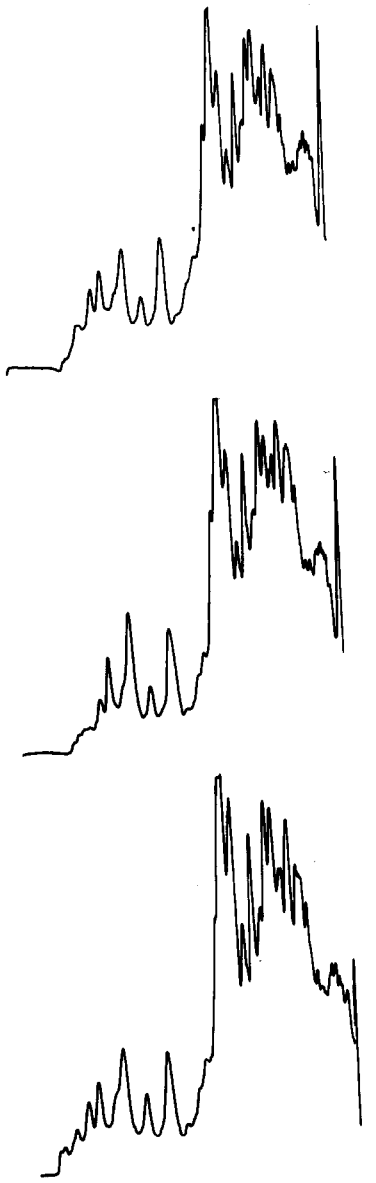

Fig. 1. Densitometric pattern of the NHP after separation with SDS-acrylamide electrophoresis. Upper curve: control, middle: 1 hr DENA-treatment, lower: 3 hr DENA-treatment The separation of the proteins was carried out on slab gels, with an acrylamide gradient from 7 to 10% or from 7 to 12%. Direction of separation from right to left.

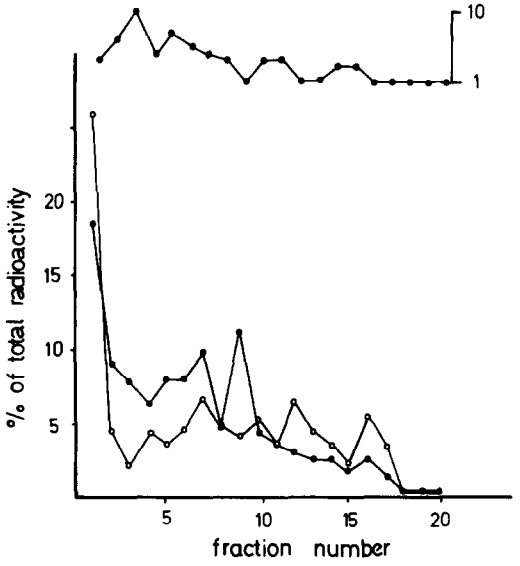

Fig. 2. Radioactivity values of the gel electrophoresis as a percentage of the total radioactivity. (o——o) Controls, (•——•) 1 hr treated animals. Upper figure: relationship between the absolute values in cpm from 1 hr treated animals to the controls.

value 3 hr later (table 1). In this fraction, Stein and Baserga [15] have demonstrated in HeLa cells the existence of a pool of free NHP.

Analysis of the proteins by acrylamide gel electrophoresis showed that at all the times investigated, the pattern as revealed by densitometry was the same (fig. 1). In the gradients, there is a concentration of bands corresponding to polypeptides of molecular weight larger than 100 000. In the lower part of the gels there are two bands which could correspond to the histones F2b and F2al. Measurement of the radioactivity of the gels from DENA-treated rats (fig. 2) showed an increase of up to 10-fold in the region of the gels where the molecular weight was greater than 100 000. No increase was observed in the other fractions, and in some cases a decrease in radioactivity with respect to the controls could be seen. Gronow [16] also did not find differences in the gel electrophoretic pattern between the chromatin-bound NHP of controls and of DENA-treated animals after chronic treatment; the specific activity of the proteins when labelled with ^{14}C-N-ethylmaleimide, however, was increased.

Pulse labelling with ^{3}H-uridine for 10 or 15 min demonstrated an increase in the incorporation into the rapidly labelled nuclear RNA to 280% with respect to control values 1 hr after treatment. In addition, the labelling was reduced three hours later (table 1). This agrees with the results of Villa Trevino [17] who has demonstrated an increase in the synthesis of this nuclear RNA 1 hr after treatment with the related carcinogen dimethylnitrosamine. This enhanced incorporation parallels the stimulation of the incorporation of amino acids into the NHP; such a correlation has been interpreted by other authors as representing a type of control of mRNA synthesis by the NHP [2].

From our results it can be concluded that diethylnitrosamine on the one hand has an inhibitory effect on the incorporation of amino acids into the soluble proteins of the cytoplasm (results not shown). On the other hand, incorporation was stimulated into the acidic fraction of the chromatin proteins. It is not known at present whether this stimulation plays a role in the early stages of DENA carcinogenesis.

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