Vol. 119, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1984 Pages 618-623

Rapid Inactivation of Ca^{2+},Mg^{2+}-dependent Endonuclease of Rat Liver Nuclei after Cycloheximide Treatment

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Received February 3, 1984

Ca^{2+},Mg^{2+}dependent endonuclease activity of isolated nuclei from rat liver disappeared completely within one to two hours after intraperitoneal administration of inhibitors of eukaryotic protein synthesis such as cycloheximide or puromycin. Actinomycin D, on the other hand, revealed no inhibition of the endonuclease activity, but even reversed the effect of cycloheximide by simultaneous addition.

Ca,Mg endonuclease was first shown in isolated rat liver nuclei to cleave chromatin DNA into a series of bands of limited length (1). Although the function of Ca,Mg endonuclease is not elucidated yet, involvement of the enzyme is suggested in several cellular metabolisms (1-8). The presence of Ca,Mg endonuclease-like activity has been well documented in many organs of a wide variety of creatures, possibly indicating an essential role in chromatin metabolism.

We have previously reported that cycloheximide (CH) treatment of cultured cells gave rise to relative enhancement of RNA polymerase II activity in isolated nuclei (9). During the course of further studies to investigate the relationship between protein synthesis and RNA synthesis by isolated nuclei, we have found that Ca,Mg endonuclease of rat liver nuclei is rapidly inactivated by pretreatment of the animal with CH.

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Abbreviations: Ca,Mg endonuclease, Ca^{2+},Mg^{2+}-dependent endonuclease; CH, cycloheximide

0006-291X/84 $1.50

Materials and Methods

Materials. Micrococcal nuclease and DNase I (DPFF grade) were purchased from Worthington; EGTA from Tokyo Kasei; Cycloheximide from Wako chemicals; Agarose from Dojin Chemicals; Puromycin from Boehringer; Actinomycin D from Merck, Sharp and Dohme.
Animals. The experiments were performed with male Donryu rats (200±20g). CH, puromycin or actinomycin D was administered into rats intraperitoneally at 3 mg, 6 mg or 0.2 mg per 100g body weight, respectively (10,11).
Isolation of rat liver nuclei and in situ digestion. Liver nuclei were prepared as described (12). The nuclear pellet was resuspended in 1mM Ca^{2+}-containing nuclei suspension buffer (13) and incubated at 25°C for in situ digestion by endogenous Ca,Mg endonuclease.

Results

Isolated nuclei from rat liver contained an endonuclease that cleaved chromatin DNA into a series of multiple bands, which are derived from nucleosomes (Fig.1a). Pretreatment of the animal with CH has, however, resulted in disappearance of such bands even after prolonged incubation of isolated nuclei

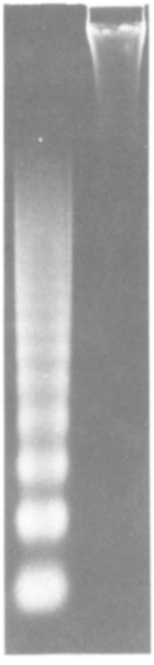

Fig.1 In situ digestion pattern of nuclear DNA. Isolated nuclei from untreated or CH-treated rat liver were incubated as described in Materials and Methods. DNA was extracted and fractionated according to size on an agarose gel. a, untreated nuclei; b, CH-treated nuclei for 4 hr.

(Fig.1b). CH, by itself, has exhibited no inhibitory effect on an endonuclease activity up to an extremely high concentration (such as 400µg/ml, unpublished results). The result shown in Fig.1 might raise the possibilities that CH treatment might change either the chromatin structure or the activity of endogenous Ca,Mg endonuclease. Susceptibility of chromatin DNA in nuclei from untreated and CH-treated rat to exogenous nucleases, including partially purified Ca,Mg endonuclease (14), micrococcal nuclease and DNaseI, appeared to be the same (unpublished data). This result eliminated the possibilities of a structural change in chromatin or the presence of inhibitor in CH-treated nuclei.

To study the kinetics of loss of enzyme activity, rats in groups of two were injected intraperitoneally with CH at variable times before being killed. At the time indicated, nuclei were isolated and the endogenous Ca,Mg endonuclease activity was assayed as in Fig.1. The typical oligonucleosomal DNA band pattern seen in control nuclei (Fig.2a) gradually disappeared as a period of treatment with CH prolonged. One to two hours treatment appeared to be sufficient for the sweep of DNA bands from oligonucleosome area (Fig.2A-c and -d). For confirmation, the Ca,Mg endonuclease activity of isolated nuclei after drug administration was quantitated. Fig.2B clearly depicts the decreasing activity of the enzyme as a function of time with an apparent half-life of about 30 to 40 min. Since the enzyme activity was detected neither in nuclear nor in cytoplasmic fraction in CH treated rat liver (unpublished data), the possibility of enzyme efflux from nucleus (15) was unlikely.

It was of interest to see whether other kinds of eukaryotic protein synthesis inhibitor showed similar effects on Ca,Mg endonuclease. Puromycin and actinomycin D were tested for

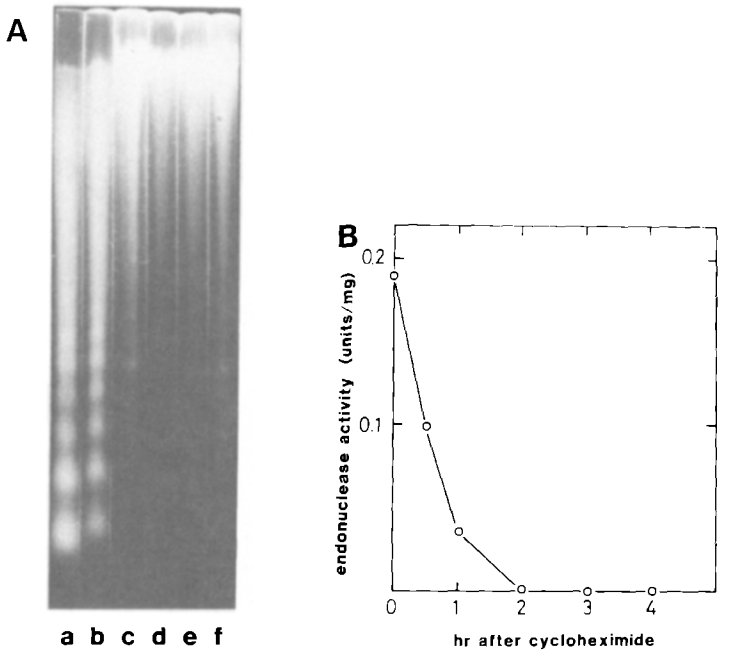

Fig.2 Kinetic studies of CH pretreatment on in situ digestion by isolated nuclei. CH was injected into rats intraperitoneally at times indicated before being killed. Nuclei were isolated and incubated for 60 min for in situ digestion as in fig.1. A, agarose gel electrophoretic pattern of extracted nuclear DNA. Period of CH pretreatment was for a,0; b,0.5; c,1; d,2; e,3; and f,4 hr.; B, Rate of decay of endogenous Ca,Mg endonuclease. Nuclei were incubated for 60 min either in the absence or presence of Ca^{2+}. Supernatant fraction was recovered by centrifugation and absorbance at 260 nm was monitored. The value assayed without Ca^{2+} was subtracted from that with Ca^{2+} and the difference was taken for the estimation of Ca,Mg endonuclease. The activity was expressed as commercial micrococcal nuclease-equivalent units.

this subject. As Fig.3b shows, puromycin inhibited the enzyme activity. From these experiments it was concluded that Ca,Mg endonuclease activity might be closely coupled with the translation process itself. On the other hand, actinomycin D revealed no inhibition of the endonuclease activity at all (fig.3c), and even reversed the inhibition of Ca,Mg endonuclease caused by CH when applied to the animal simultaneously (Fig.3d).

Discussion

The inactivation might be either due to a rapid turnover of the enzyme protein itself, the formation of an inactive complex of the enzyme molecule with some other nuclear components that

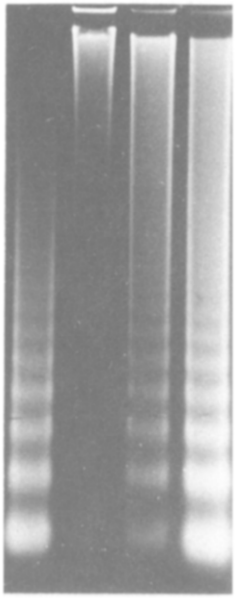

Fig.3 Effect of puromycin and actinomycin D. (b) Puromycin was injected intraperitoneally at 2hr and 1hr before the animal was killed (11), and (c) actinomycin D was administered as described (10). (d) CH and actinomycin D were injected simultaneously. Nuclei were isolated and in situ digestion was performed as in fig.1. After incubation, DNA was extracted and separated on an agarose gel. a, untreated control; b, puromycin-treated; c, actinomycin D-treated; d, CH and actinomycin D-treated.

may be activated by CH treatment, or the modification of the enzyme, such as phosphorylation, acetylation or ADP ribosylation etc. Among these, the first possibility seems less probable, since the enzyme in isolated nuclei was capable of digesting nuclear DNA for up to 2 hr in a linear fashion and solubilized crude enzyme was also fairly stable (unpublished results). For the second alternative, we have carried out "mixing experiments" in which untreated liver nuclei were incubated with either cytoplasm, nuclear sap or nuclei suspension from CH-treated liver. Since all these additives showed no inhibitory effect on in situ digestion process by normal nuclei (unpublished data), the release or the activation of Ca,Mg endonuclease-binding protein, if any, appeared also unlikely. With respect to enzyme modification, the in vitro inactivation of Ca,Mg endonuclease by

ADP ribosylation has been reported (16,17). Furthermore, since actinomycin D has been shown to strongly inhibit ADP ribosylation of Ca,Mg endonuclease in vitro (18), the result shown in Fig.3c appeared to further support the idea. There is a counter but related example of the direct inhibition of protein synthesis by diphtheria toxin through inactivation of the elongation factor-2 by ADP ribosylation (19). We are therefore tempted to favour the third possibility for the explanation of the phenomenon.

Acknowledgements

We are grateful to Mr.N.Kinoshita and Misses B.Noda and M.Honda for help. This work was supported in part by grants from Japanese Foundation for Multidisciplinary Treatment of Cancer, from Fukuoka Cancer Society and from the Japan Tobacco and Salt Public Corporation.

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