Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 409-414

# EFFECT OF ALPHA-1-ANTICHYMOTRYPSIN ON DNA SYNTHESIS IN PERMEABILIZED HUMAN CELLS

Michio Tsuda, Yuri Umezawa, Masashi Masuyama, Kurumi Yamaguchi, and Tsunehiko Katsunuma

Department of Biochemistry, School of Medicine, Tokai University, Isehara, Kanagawa, 259-11, Japan

Received March 5, 1987

SUMMARY: The effect of Alpha-1-antichymotrypsin(ACT) on DNA synthesis was studied using lysolecithine-permeabilized cultured human stomach carcinoma cells. ACT added in medium inhibited DNA synthesis and the degree of inhibition is incubation time dependent. It is proportional to ACT concentration and the concentration of ACT required for 50% inhibition was 0.8 mg/ml.


Alpha-1-antichymotrypsin(ACT) is an acute phase reactant protein and has been found to be elevated in the sera of the majority of individuals with malignant diseases, as well as in extensive inflammation and in tissue damage(1). Recently it has been found that ACT could be often detected by immunohistochemical technique in tumor cells or cell nuclei of human stomach, breast, and pancreas adenocarcinomas and hepatomas. Also, ACT present in carcinoma cell nuclei is incorporated from the blood circulation (2, 3). However, why ACT is present in cell nuclei of carcinoma cells is as yet known. In vitro assay system using activated calf thymus DNA as a template, ACT specifically inhibits DNA polymerase alpha but not polymerase beta(4).

This study reports the effect of ACT on DNA synthesis in lysolecithine-permeabilized cultured human cells, which exhibit acceptable preservation of gross structural integrity but are capable of incorporating large molecular components.

0006-291X/87 $1.50

## MATERIALS AND METHODS

Materials. [$^3$H]dTTP(20Ci/mmol), [$^3$H]thymidine(10Ci/mmol) and Protosol were obtained from New England Nuclear. Minimum Essential Medium(MEM) was purchased from Nissui Co.(Tokyo, Japan). Lysolecithine (type IV) and activated calf thymus DNA were from Sigma Chemical Company(St. Louis, Mo). Glass filter paper GA100 was from Tokyo Roshi Kaisya,Ltd.(Tokyo, Japan). All other chemicals were of analytical grade from Wako Pure Chemical Industries (Tokyo, Japan).

Cell culture. Cultured human stomach adenocarcinoma cell line, designated MK-1 cell, was established in our laboratory(3). The cells were grown in MEM with 10% foetal calf serum. DNA synthesis studies were performed with exponentially growing cells.

Cell permeabilization and assay of DNA synthesis. Permeabilizing cells with lysolecithine and measuring DNA synthesis was performed according to the method as described by Miller et al.(5). Briefly, MK-1 cells were collected by centrifugation (5 min, 500 x g), washed twice at 4°C in solution A (150 mM sucrose, 80 mM KCl, 5 mM $MgCl_2$, 35 mM Hepes, pH 7.4), and finally suspended in solution A at 4°C at 8 x $10^6$/ml. Lysolecithine was added to a final concentration of 0.5 mg/ml and incubated in ice water for 1 min. Permeabilization was monitored by trypan blue exclusion. Generally, 94 to 98% of the cells were permeabilized. For assay of DNA synthesis, permeabilized cells were diluted to 2 x $10^6$/ml in solution A (0.4 ml) containing 15 mM phosphoenolpyruvate, 4 mM ATP, 0.3 mM each CTP, GTP, and UTP, 0.75 mM each dATP, dCTP, and dGTP, 0.15 mM [$^3$H]dTTP (0.13 Ci/mmol)were preincubated at 4°C for 60 min in the presence or absence of ACT as indicated. The samples were then incubated at 37°C for 20 min or 40 min . The reaction of DNA synthesis was stopped by the addition of 4 ml of 5% TCA containing 2% sodium pyrophosphate. The mixture was applied onto a glass filter paper disc following washing with 15 ml of the same solution and 8 ml of ethanol. The disc was immersed in Protosol for 18 hr and its radioactivity was measured.

DNA synthesis of intact cultured cells. MK-1 cells were preincubated in MEM with 10% foetal calf serum (2 x $10^4$ cells/ml) with or without ACT for various periods (2 hr to 2 days), and were incubated with [$^3$H]thymidine (0.5 uCi/ml)at 37 C for 2hr. The cells were collected by centrifugation(5 min, 500 x g) and suspended in 5% TCA with 2% sodium pyrophosphate. The solution was applied onto a glass filter paper disc. The disc was washed and the radioactivity was counted as in permeabilized cells experiments.

Assay for DNA polymerase. Exponential MK-1 cells were collected by centrifugation(5 min, 500 x g), washed twice in 0.9% NaCl and suspended in 50 mM potassium phosphate buffer, pH 7.5, containing 0.1 mM EDTA, 1mM $MgCl_2$, 25% glycerol, 1mM dithiothreitol and 0.5 M NaCl. The solution was sonicated and used as a crude polymerase preparation. DNA polymerase activity was measured as described earlier(4).

Preparation of ACT. The purification of ACT from human pooled serum was performed according to the method described previously (6, 7). The homogeneity of the purified ACT preparation was confirmed by slab polyacryramide gel electrophoresis(8) and

sodium dodecylsulfate polyacrylamide gel electrophoresis(9).

Immunohistochemical analysis of ACT. Immunohistochemical staining was performed as described previously(2).

## RESULTS AND DISCUSSION

It has been reported that ACT could be often detected in tumor cell nuclei (2) and that ACT specifically inhibits the activity of DNA polymerase alpha purified from tumor cells using activated calf thymus DNA as a template(4). We wished to examine whether ACT inhibits DNA synthesis of cultured cells as well as in purified enzyme system to elucidate the biological role of ACT present in cell nuclei. However no ACT was detected by immunohistochemical analysis in the nuclei of intact cultured cells, which had been grown with ACT (1 to 3 mg/ml)for various periods(2 hr to 2 days), (not shown), although MK-1 cells transplanted into nude mice could incorporate ACT from the blood circulation as reported earlier(3). The reason why intact cultured cells cannot incorporate ACT is not known. In this intact cultured cell system, ACT did not have any effect on its DNA synthesis, which was measured by the incorporation of [$^3$H]thymidine into the acid insoluble cell fraction(Table 1).

Table 1. Effect of ACT on DNA synthesis in intact cultured human cells (MK-1 cells)

| Addition | | DNA Synthesis Incorporation of [$^3$H]thymidine |
|---|---|---|
| | mg/ml | cpm/1x10$^5$ cells/2 hr |
| None | | 35,176 ± 1,081 |
| ACT | 1.0 | 34,831 ± 748 |
| | 3.0 | 36,231 ± 1,124 |
| Bovine serum albumin | 3.0 | 34,972 ± 1,123 |

Exponentially growing MK-1 cells were preincubated with ACT or bovine serum albumin for 24 hr and then incubated with [$^3$H]thymidine at 37°C for 2 hr for assay of DNA synthesis as described in "Materials and Methods".

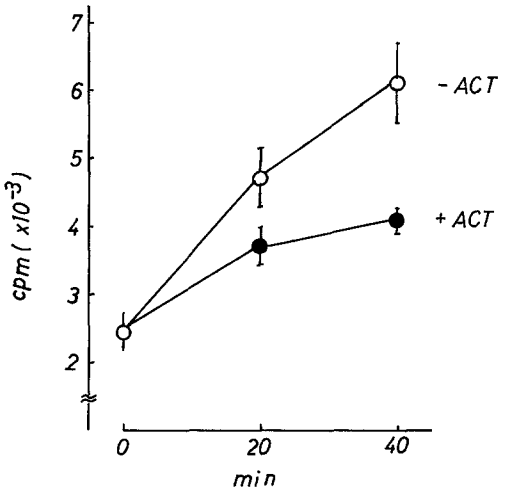


Fig 1. Effect of ACT on DNA synthesis in permeabilized cells. Exponentially growing MK-1 cells were permeabilized with lysolecithin(0.5 mg/ml),preincubated with or without ACT(1.7 mg/ml) and incubated with [$^3$H]dTTP. The radioactivity in acid insoluble fraction of cells was counted as described in "Materials and Methods".

Therefore a permeabilized human cell system reported by Miller et al., which had been capable of incorporating large molecular components, was used as a model of cells containing ACT. Fig 1 shows the effect of ACT on DNA synthesis in permeabilized MK-1 cells. ACT inhibited the DNA synthesis during the first 20 min of incubation, and much more for a further 20 min. It takes at least 20 min for ACT to be incorporated into cell nuclei and shows maximum inhibition of DNA synthesis. So we calculated the inhibition rate as follows.

$$\text{Inhibition rate (\%)} = \frac{A40 - A20}{C40 - C20} \times 100$$

A40 : cpm at 40 min with ACT. A20 : cpm at 20 min with ACT.
C40 : cpm at 40 min without ACT. C20 : cpm at 20 min without ACT

The inhibion of DNA synthesis in the permeabilized cells by ACT was concentration dependent as shown in Fig 2. The concentration of ACT required for 50 % inhibition was 0.8 mg/ml. In some cases, the level of ACT in sera from patients with malignant tumor was elevated up to 1.8 mg/ml, so that ACT level used in these

Table 2. Polymerase activity in cultured human cells(MK-1 cells)

| Addition | Polymerase activities dTTP/$10^6$ cells/30 min |
|---|---|
| | pmoles |
| None | 50.0 |
| Aphidicolin (30 ug/ml) | 4.5 |

Exponentially growing MK-1 cells were collected by centrifugation(5 min, 500 x g), washed twice with 0.9% NaCl, and suspended in 50 mM potassium phosphate buffer, pH 7.5, containing 0.1 mM EDTA, 1 mM $MgCl_2$, 25% glycerol and 1 mM dithiothreitol. The solution were sonicated and used as a crude polymerase preparation. DNA polymerase activity was measured as described in "Materials and Methods".

experiments was of physiological concentration.

The finding that 91% of total DNA polymerase activity in MK-1 cells is aphidicolin sensitive, polymerase alpha(Table 2), high concentration of ACT inhibited up to 90% of DNA synthesis in permeabilized cells(Fig. 2), and the degree of the inhibition of DNA synthesis by ACT in permeabilized cells was similar to that in purified DNA polymerase alpha(ID50 = 1.0 mg/ml)(4) suggested that ACT inhibited DNA synthesis via the inhibition of DNA polymerase alpha, a putative replicative polymerase(10, 11).

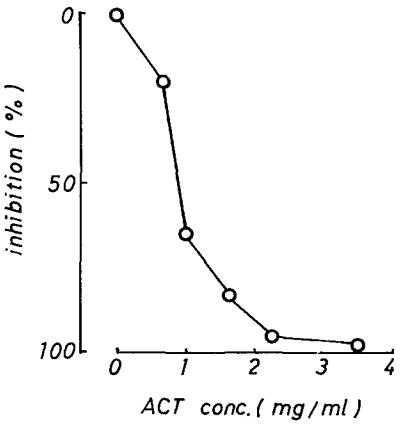


Fig 2. Effect of ACT on DNA synthesis in permeabilized cells. The inhibition of DNA synthesis at various concentration of ACT was measured as in Fig 1. The inhibition rate was calculated as described in Text.

Therefore ACT present in carcinoma cell nuclei may play a significant role in host defense mechanism against tumor growth via the inhibition of DNA synthesis, although the mechanism of the incorporation of ACT by carcinoma cells still remains to be solved.

ACKNOWLEDGMENTS

We are grateful to Nadia El Borai for correcting the manuscript. This work was supported in part by Research promotion grant,The Tokai University General Research and a grant from Yamanouchi Foundation for Research on Metabolic Disorders.

REFERENCES

1. Kelly,V.L.,Cooper,E.H.,Alexander,C.,and Stone,J. (1978) Biomedicine, 28, 209-215.
2. Takada,S.,Tsuda,M.,Mitomi,T.,Nakasaki,H.,Noto,T.,Ogoshi,K., and Katsunuma,T. (1982) Gann, 73, 742-747.
3. Takada,S.,Tsuda,M.,Fujinami,S.,Yamamura,M.,Mitomi,T.,and Katsunuma,T. (1986) Cancer Res., 46, 3688-3691.
4. Tsuda,M.,Masuyama,M.,and Katsunuma,T. (1986) Cancer Res., 46, 6139-6142.
5. Miller,M.R.,Castellot,J.J.,Jr.,and Pardee,A.B. (1978) Biochemistry, 17, 1073-1080.
6. Katsunuma,T.,Tsuda,M.,Kusumi,T.,Ohkubo,T.,Mitomi,T., Nakasaki,H.,Tajima,T.,Yokoyama,S.,Kamiguchi,H.,Kobayashi,K., Shinoda,H. (1980) Biochem.Biophys.Res.Commun., 93, 552-557.
7. Tsuda,M.,Ohkubo,T.,Kamiguchi,H.,Suzuki,K.,Nakasaki,H., Mitomi,T.,and Katsunuma,T. (1982) Tokai J.Exp.Clin.Med., 7, 201-211.
8. Davis,D.J. (1964) Ann.NY Acad.Sci., 121, 404-427.
9. Laemmli,U.K. (1970) Nature(Lond.), 227, 680-685.
10. Weissbach,A. (1977) Annu.Rev.Biochem. 46, 25-47.
11. Miller,M.R.,Ulrich,R.G.,Shu-Fong Wang,T.,and Korn,D. (1985) J.Biol.Chem., 134-138.