Acta Chemica Scandinavica B 33 (1979) 171—174

Effects of Soluble Fractions from Untreated and SiO_2-treated Subcellular Particles of Macrophages on Nucleic Acid Metabolism in Isolated Nuclei of Experimental Granulation Tissue

PIRJO LEHTINEN

Department of Medical Chemistry, University of Turku, SF-20520 Turku 52, Finland

Nuclei isolated from proliferating granulation tissue were incubated with 20 000 *g* supernatants from untreated and SiO_2-treated subcellular particles of rat peritoneal macrophages in the presence of radioactive nucleic acid precursors. The supernatant from SiO_2-treated subcellular particles increased the incorporation of [^3H]CTP into nuclear RNA maximally by 26 % at 5 min, and that of [*methyl*-^3H] dTTP into DNA by 16 % at 20 min. The release of radioactivity from labeled DNA was suppressed simultaneously. An RNase preparation from rat peritoneal macrophages enhanced the release of radioactivity from labeled DNA similarly as the soluble fraction from untreated subcellular particles of macrophages. The results suggest that the effects of the soluble fractions upon DNA metabolism of granuloma cells are at least partly independent of the effects on RNA metabolism and that the soluble fraction from SiO_2-treated subcellular particles of macrophages stabilizes DNA through inhibition of nuclease activity.

Several reports have accumulated on the presence of fibrogenic factors in the supernatants of macrophages.[1–4] The 20 000 *g* supernatant from SiO_2-treated subcellular particles of macrophages [4] has been shown to stimulate the synthesis of collagen and to increase the amount of RNA and phospholipids in the rough endoplasmic reticulum of experimental granulation tissue.[5,6] It also maintains the stability of polysomes and enhances the cell-free synthesis of proteins by polysomes from experimental granuloma.[7] Specific macrophage ribonuclease (RNase) is suggested to regulate the RNA turnover and, hence, the protein synthesis in the adjacent fibroblasts.[8,9] The incorporation of radioactive precursors into DNA in proliferating granulation tissue slices (during a 3 h incubation) and in isolated nuclei (during a 30 min incubation) was also observed to be increased.[5,6] The purpose of the present work was to explore further how the soluble fractions from untreated and SiO_2-treated subcellular particles of macrophages influence the nucleic acid metabolism in nuclei isolated from experimental granulation tissue.

EXPERIMENTAL

Production of macrophage preparations. Peritoneal macrophages were obtained from adult male rats of Wistar strain, 15 at a time.[1] The production of preparations from untreated and SiO_2-treated subcellular particles of macrophages has been described earlier.[4] In short, macrophages were homogenized by repeated freezing and thawing, and the 7000/500 *g* sediment (containing plasma membranes, endoplasmic membranes, mitochondria and lysosomes but not nuclei and cytosol) was incubated overnight at 37 °C with SiO_2 particles in 0.1 M Tris-HCl buffer, pH 7.8. Penicillin (100 U/ml) and streptomycin (50 μg/ml) (Orion, Espoo, Finland) were always added. The concentration of SiO_2 was 3 mg/ml (Dörentrup Quartz, DQ 12, < 5 μm). The control supernatant was prepared similarly but no SiO_2 particles were added. An aliquot (25 μl) of the 20 000 *g* supernatant of the mixture, corresponding to 2×10^7 macrophages/ml, was added to the incubations with isolated nuclei.

Production of experimental granulation tissue. Female albino rats of Wistar strain (2 – 3 months old, weight 130 – 200 g) were kept on standard laboratory diet (from Hankkija, Helsinki, Finland) supplemented with vegetables. Growth of granulation tissue was induced by pieces of viscose cellulose sponge (dry size 4 mm × 14 mm × 14 mm; dry weight 60 – 70 mg,

0302-4369/79/030171-04$02.50

made by Säteri Oy, Valkeakoski, Finland).[10] The granulomas were collected in the proliferative phase, 7 days after implantation.[11] The surrounding capsules were removed, and the granulomas used immediately.

Isolation of nuclei from experimental granulation tissue. The granulation tissue was homogenized in TEMM buffer (10 mM Tris-HCl, 1 mM EDTA, 6 mM β-mercaptoethanol, 4 mM $MgCl_2$, pH 7.8) with an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen i. Breisgau, Germany), using 1 g of tissue per 5 ml of buffer. The homogenate was filtered through a nylon cloth and the nuclei recovered by centrifugation at 800 *g* for 5 min. The pellet was washed four times with TEM buffer (10 mM Tris-HCl, 1 mM EDTA, 6 mM β-mercaptoethanol, pH 8.0).[12]

Nucleic acid metabolism in isolated nuclei. The incorporation of radioactive triphosphates (The Radiochemical Centre Ltd., Amersham, Bucks., U.K.) was measured by the method of Hooten and Hoffbrand.[13] The reaction mixture contained in a total volume of 150 µl 17 µM each of dGTP, dCTP and dATP, 7 mM $MgCl_2$, 0.1 M NaCl, 10 mM ATP, 0.7 mM EDTA, 1 mM β-mercaptoethanol, 0.17 M Tris-HCl, pH 7.8, nuclei corresponding to 75 µg DNA and 3.3 µM [*methyl*-^3H] dTTP (TRK. 354) or 0.2 µM [^3H] CTP (TRK. 339). When [^3H] CTP was used as the precursor for RNA the washed nuclei were hydrolyzed in 0.5 ml of 0.3 M KOH overnight at 37 °C. The hydrolyzate was precipitated by adding 0.3 ml of 1.04 M perchloric acid and centrifuging at 1000 *g* for 15 min. The supernatant was decanted into a scintillation vial, the pellet was washed once with 0.5 ml of 0.2 M perchloric acid and the washings were combined with the first supernatants.

The degradation of DNA in isolated nuclei was studied by labeling them with [*methyl*-^3H]-dTTP for 10 min, cooling on ice, diluting with 5 ml of TEM buffer, centrifuging at 800 *g* for 5 min and washing once with fresh medium. The nuclei were then incubated further with 1.5 ml of fresh medium under the conditions described above. This contained 250 µl of soluble fractions from untreated or SiO_2-treated subcellular particles of macrophages or Tris-HCl, pH 7.8, distilled water or purified macrophage RNase (0.7 µg protein in distilled water; gift from Sirpa Aho, Ph.Lic.). Aliquots (150 µl) from the media were then taken at different time intervals and put into 1 ml of 0.8 M perchloric acid in 10 mM $Na_4P_2O_7$. After centrifugation at 1000 *g* for 15 min the supernatants were decanted into scintillation vials. The precipitates were treated as described by Hooten and Hoffbrand.[13]

Assay of radioactivity. The radioactivities were assayed with a Packard Tri-Carb liquid scintillation spectrometer Model 3320 (Packard Instrument Company Inc., Downer's Grove, Ill., U.S.A.). For the measurement of the radioactivity the scintillant consisted of 15 g of 2,5-diphenyloxazole (Packard No. 6002026) and 50 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene (Packard No. 6002030) in 1000 ml of distilled toluene and 677 ml of Tergitol^R (Union Carbide 15-S9, Turun Saippua Oy, Turku, Finland). The counting efficiency for tritium was 25 %.

RESULTS AND DISCUSSION

Time course of the incorporation of precursors into nucleic acids in isolated nuclei. The data presented in Fig. 1 indicate that the incorporation of [*methyl*-^3H]dTTP into isolated nuclei reached a maximum value at 5 min, and that the radioactivity decreased slowly when the incubation was continued. The amount of [^3H]CTP incorporated into nuclear RNA was maximal at 10 min, after which the radio-

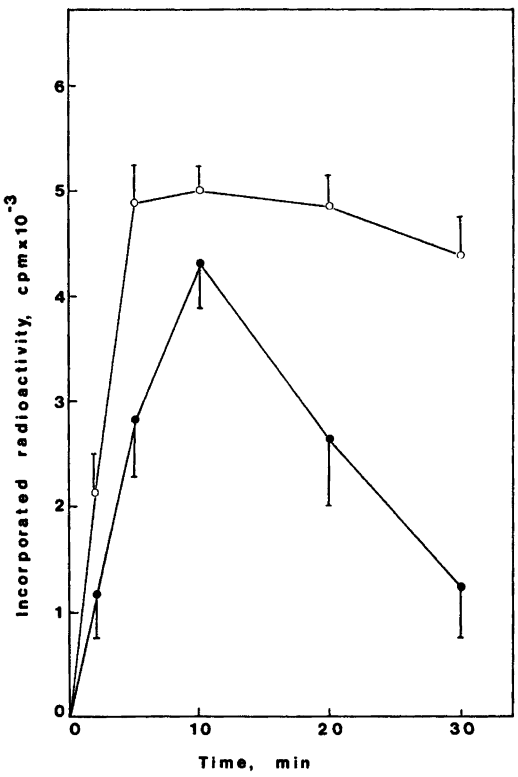

Fig. 1. Time course of the incorporation of radioactive precursors into nucleic acids in isolated nuclei. No additions to the incubation mixture were made. The incorporation of [*methyl*-^{3}H]dTTP into DNA (○) and of [^3H]-CTP into the nuclear RNA (●) are presented. Means ± S.E.M. ($n = 4$) are presented.

activity of RNA decreased rapidly. After a 30–40 min incubation the nuclei began to aggregate presumably because of changes in nuclear membranes. The shapes of the curves were independent of the concentration of nuclei in the incubations and were not essentially changed by soluble fractions.

Effects of the soluble fractions from untreated and SiO_2-treated subcellular particles of macrophages. The soluble fraction from SiO_2-treated subcellular particles of macrophages stimulated the relative incorporation of [^{3}H]CTP into nuclear RNA. The maximum increase, 26 %, was observed already after a 5 min incubation (Fig. 2). After 10 min there was no stimulation

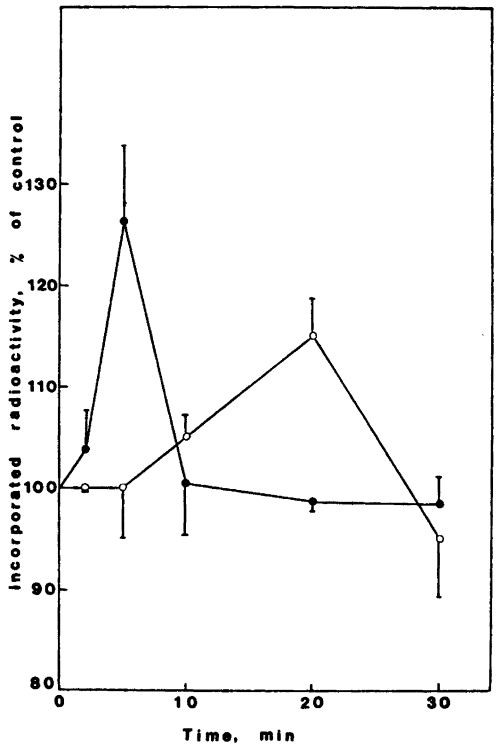

Fig. 2. Effect of the soluble fraction from SiO_2-treated subcellular particles of macrophages on the relative incorporation of radioactive precursors into nucleic acids in isolated nuclei. Effect on the incorporation of [^3H]CTP into the nuclear RNA (●) and effect on the incorporation of [*methyl*-^3H]dTTP into DNA (○) are presented. Results from the incubations with soluble fractions from untreated subcellular particles have been taken as the reference (100 %). The means ± S.E.M. ($n=4$) are presented.

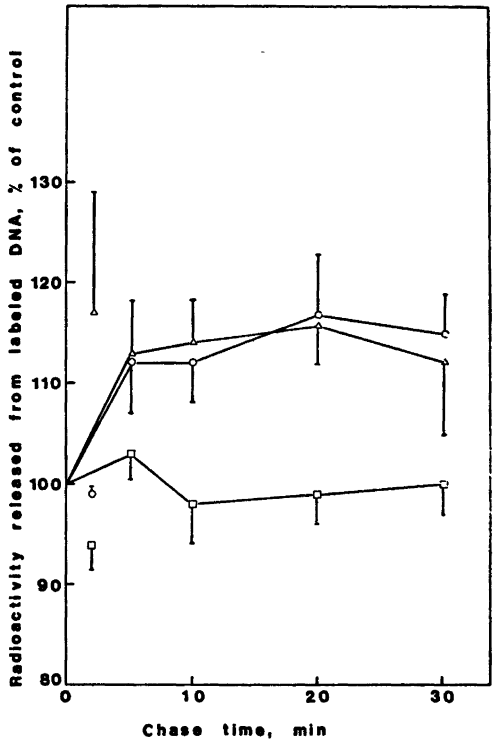

Fig. 3. Effects of the soluble fractions from untreated and SiO_2-treated subcellular particles of macrophages and the macrophage RNase on the release of radioactivity from labeled DNA. Effects of the soluble fractions from untreated (○), and SiO_2-treated (□) subcellular particles and the macrophage RNase (△) are presented. Values have been calculated as percentage from the untreated control incubations. Means ± S.E.M. ($n=4-6$) are presented.

when compared to the control. The corresponding stimulation of [*methyl*-^3H]dTTP incorporation into DNA was not observed until after 10 min, and the maximum effect (+16 %) was reached after 20 min (Fig. 2). Simultaneously the degradation of DNA was retarded significantly (Fig. 3).

The degradation of RNA could not be investigated due to difficulties in separating rapidly CTP-labeled RNA from DNA. UTP does not incorporate into the isolated nuclei of the experimental granulation tissue until destabilization of the nuclear membrane occurs.

Several reports have suggested that the cellular RNase/RNase inhibition system plays a key role in controlling protein synthesis.[14,15]

Earlier works [4–9] in our laboratory have suggested that macrophages can regulate protein synthesis in adjacent connective tissue cells through stabilization of their RNA. SiO_2 particles disturb this regulatory mechanism which can be observed as enhanced protein synthesis. The decrease in RNase activity may be due to some protein inhibitor released by SiO_2 particles [16] or that the phagocytized SiO_2 particles absorb RNase and perhaps other proteins.[9] Previously the soluble fraction from SiO_2-treated subcellular particles of macrophages has been shown to stimulate the incorporation of radioactive precursors into DNA of granuloma slices and isolated nuclei [5,6] and the medium of SiO_2-treated cultured macrophages consistently increased incorporation of thymidine into cultured granulation-tissue cells.[17] On the basis of these results the soluble fractions from subcellular particles of macrophages can regulate DNA metabolism either through RNA metabolism or through a direct effect on the DNA itself. The present results show that the soluble fraction from SiO_2-treated subcellular particles of macrophages stimulates the incorporation of precursors into DNA mainly through a decreased degradation of DNA. Interestingly, the macrophage RNase and the soluble fraction from untreated subcellular particles of macrophages released radioactivity from labeled DNA similarly (Fig. 3). Thus the macrophage RNase seems to have some nuclease activity on DNA, too. It has been reported before that there are RNases in eukaryotic cells which cause a destabilization of the DNA double helix [18] and RNA-DNA hybrids.[19] It remains to be seen whether there are also other factors in these soluble fractions that can regulate the DNA metabolism of the experimental granulation tissue.

Acknowledgements. I wish to thank Professor Eino Kulonen from the Department of Medical Chemistry, University of Turku, Turku, Finland, for useful discussions and help during the experiments and preparation of the manuscript. I am also indebted to Sirpa Aho, Ph. Lic., from the Department of Medical Chemistry, University of Turku, for the macrophage RNase and to Mrs. Terttu Jompero for technical assistance. Institutional grants from the Medical Research Council of Finland and from the Emil Aaltonen Foundation are gratefully acknowledged.

REFERENCES

1. Heppleston, A. G. and Styles, J. A. *Nature 214* (1967) 521.
2. Allison, A. C. In Bendz, G. and Lindqvist, I., Eds., *Biochemistry of Silicon and Releated Problems*, Plenum, New York 1978, pp. 337–380.
3. Burrell, R. and Anderson, M. *Environmental Res. 6* (1973) 389.
4. Aalto, M., Potila, M. and Kulonen, E. *Exp. Cell. Res. 97* (1976) 193.
5. Lehtinen, P. and Kulonen, E. *Upsala J. Med. Sci. 82* (1977) 141.
6. Lehtinen, P. and Kulonen, E. *6th Colloquium of the Federation of European Connective Tissue Clubs, Biochemistry of Normal and Pathological Connective Tissue*, Ed. du CNRS, Paris, Vol. I (1978), pp. 288–290.
7. Aho, S. and Kulonen, E. *Exp. Cell. Res. 104* (1977) 31.
8. Aho, S. and Kulonen, E. *Upsala J. Med. 82* (1977) 118.
9. Aho, S. and Kulonen, E. *6th Colloquium of the Federation of European Connective Tissue Clubs, Biochemistry of Normal and Pathological Connective Tissue*, Ed. du CNRS, Paris, Vol. I (1978), pp. 266–267.
10. Viljanto, J. and Kulonen, E. *Acta Pathol. Microbiol. Scand. 56* (1962) 120.
11. Lampiaho, K. and Kulonen, E. *Biochem. J. 105* (1967) 333.
12. Hershey, H. V., Steiber, J. F. and Mueller, G. C. *Eur. J. Biochem. 34* (1973) 383.
13. Hooten, J. W. L. and Hoffbrand, A. V. *Biochim. Biophys. Acta 477* (1977) 250.
14. Liu, D. K., Williams, G. H. and Fritz, P. J. *Biochem. J. 148* (1974) 57.
15. Liu, D. K. and Matrisian, P. E. *Biochem. J. 164* (1977) 371.
16. Aalto, M., Turakainen, H. and Kulonen, E. *Scand. J. Clin. Lab. Invest. In press.*
17. Kulonen, E., Aalto, M., Aho, S., Lehtinen, P. and Potila, M. In Viidik, A., Ed., *Proc. Int. Symp. Biol. Collagen*, Aarhus 1978, Academic, London. *In press.*
18. Dezelee, S., Wyers, F., Darlix, J.-L., Sentenac, A. and Fromageot, P. *J. Biol. Chem. 252* (1977) 8935.
19. Huet, J., Buhler, J. M., Sentenac, A. and Fromageot, P. *J. Biol. Chem. 252* (1977) 8848.

Received October 16, 1978.