Note

Effect of Sodium Nitrite on Ribosome Distribution in Chick Embryo Liver

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The transformation and mutation of Syrian hamster cells by sodium nitrite were reported in *in vitro* or transplacental systems.^{1,2)} In such cases various cellular components seem to be influenced simultaneously by nitrite before the final effect can be scored as a genetic alteration. Actually dimethylnitrosoamine and aflatoxin B₁, a powerful carcinogen and a mutagen, have been known to modify both DNA and translational steps in liver.^{3~5)} It would be of interest to know whether nitrite can influence translational steps. Nevertheless, the change of these steps following administration of nitrite has remained obscure. In this paper we described the effect of nitrite on ribosome distributions which are considered to reflect changes in protein synthesis.^{6,7)}

Fertilized white-leghorn chicken eggs were incubated at 37°C for 16 days and sodium nitrite was injected into these eggs (2.0 mg/egg). After 3 hr, the embryos were dissected and livers were removed for preparation of ribosomes. Livers were homogenized with 2.5 volumes of 0.35 M sucrose in buffer A (0.05 M Tris-HCl (pH 7.5), 0.01 M magnesium chloride, 0.025 M potassium chloride) and centrifuged at 15,000 × *g* for 10 min. Five ml of the supernatant (post-mitochondrial fraction) was layered over a two-layer discontinuous gradient, with 2.5 ml of 1.5 M sucrose in buffer A on top of 2.5 ml of 2.0 M sucrose in buffer A. The gradients were centrifuged at 150,000 × *g* for 3 hr and yielded a free ribosomes pellet. The pellet was dissolved with 2 ml of 0.25 M sucrose in buffer A and 1.0 ml was layered on 30 ml of a linear gradient (15~40% sucrose in buffer A). The tubes were centrifuged at 90,000 × *g* for 3 hr and sedimentation profiles were recorded at 260 nm. Total ribosomes were obtained as described above except that a sodium desoxycholate treated post-mitochondrial fraction was used (1.0 ml of 1.2% sodium desoxycholate was applied to 4 ml of post-mitochondrial fraction). Ribonuclease-A (Sigma type 1-A) treatment of ribosomes was done at 1 U/ml for 10 min at 37°C. To prepare microsomes, livers were homogenized with 5 volumes of 0.25 M sucrose and centrifuged at 10,000 × *g* for 10 min. Eight ml of the supernatant was centrifuged at 120,000 × *g* for 60 min. The precipitate was

suspended with 8 ml of 0.25 M sucrose and centrifuged at 120,000 × *g* for 60 min. The precipitate (microsomes) was suspended with 0.2 M Tris buffer (pH 8.0) and the concentration of protein was determined by Bio-Rad protein assay. To estimate the endogenous ribonuclease activity of microsomes, the following components were incubated at 38°C for 30 min in a final volume of 1.25 ml: 0.15 ml of 0.2 M Tris buffer (pH 8.0), 0.1 ml of the suspension of microsomes, and 1.0 ml of 0.18 M sodium chloride in 0.025 M Tris buffer (pH 8.0).⁸⁾ The same experiments were done using a solution of 0.18 M sodium chloride containing 1.5% serum albumin or 0.01 M magnesium chloride. The reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid and centrifuged at 3,000 rpm for 10 min. The optical density of the supernatant was measured at 260 nm and the result was expressed as optical density per milligram of protein per 30 min.⁸⁾ All operations for the isolation of ribosomes and microsomes were done at 0~2°C. Control animals received sodium chloride solution which contained the same concentration of sodium as that of the sodium nitrite treatment.

FIG. 1. Sedimentation Profile of Ribonuclease-A Treated Ribosomal Fraction.

Ribonuclease-A treated ribosomes were overlaid on a sucrose gradient system and spun for 3 hr at 90,000 × *g*. After the centrifugation, each fraction was collected from the bottom of the tube.

FIG. 2. Effect of Nitrite on Ribosome Distributions.

Embryos were killed 3 hr after injection of nitrite and ribosomal fractions were obtained and analyzed by the sucrose gradient system. A, nitrite (2.0 mg/egg); B, control.

FIG. 3. Effect of Nitrite on Desoxycholate Treated Ribosomes Distributions.

The experimental conditions were the same as described in the legend of Fig. 2, except that desoxycholate-treated ribosomes were used. A, nitrite (2.0 mg/egg); B, control.

TABLE I. EFFECTS OF NITRITE TREATMENT ON RIBONUCLEASE ACTIVITY IN MICROSOMAL FRACTION

Activity is expressed as optical density at 260 nm per milligram of protein per 30 min. Values are expressed as the mean $\pm$ S.E. of 8 experiments.

Assay condition	Control	Nitrite-treated
Microsomes alone	0.40 $\pm$ 0.03	0.27 $\pm$ 0.03
Microsomes + magnesium	0.22 $\pm$ 0.03	0.17 $\pm$ 0.01
Microsomes + serum albumin	0.79 $\pm$ 0.04	0.73 $\pm$ 0.05

The sedimentation profile of ribonuclease-A treated ribosomes showed that monosomes were situated at fraction number 18 to 22 (Fig. 1). Figure 2 shows the ribosome distributions of nitrite-treated and control animals. This result indicated that monosomes are increased by the nitrite treatment, and the augmentation of monosomes is dependent on the dose of nitrite (50% augmentation in 1.0 mg nitrite, 93% augmentation in 2.0 mg nitrite). It is

unknown what is the primary effect of nitrite which induces the augmentation of monosomes. It is interesting, however, to examine the participation of microsomal membranes and microsomal endogenous ribonuclease in the augmentation of monosomes. Figure 3 shows desoxycholate treated ribosome distributions. The augmentation of monosomes is also recognized in nitrite-treated ribosomes. This decreases possibility that the increments of monosomes are supplied by the release of monosomes from microsomal membrane as a result of treatment of nitrite. Table I shows nitrite treatment decreases the release of acid-soluble 260 nm absorbing material, whereas the release is promoted by serum albumin and inhibited by magnesium as described by Mycek.⁸⁾ This indicates that nitrite treatment decreases the activity of microsomal endogenous ribonuclease, and consequently denies the possibility that the augmentation of monosomes is supplied by degradation of polysomes according to activation of microsomal endogenous ribonuclease. We are further studying the reason why the administration of nitrite augments the monosomes, and the relationship between ribosome distributions and ribonuclease.

REFERENCES

- 1) H. Tsuda, N. Inui and S. Takayama, *Gann.*, **67**, 165 (1976).
- 2) N. Inui, Y. Nishi, M. Taketomi and M. Moli, *Mutation Res.*, **66**, 149 (1979).
- 3) Y. Kawazoe and M. Kouda, "New mechanism and evaluation of carcinogenesis," ed. by U. Hayashi and T. Kuroki, Science Forum, Tokyo, 1984, pp. 16~31.
- 4) L. N. Vernie, W. S. Bont and P. Emmelot, *Cancer Res.*, **31**, 2189 (1971).
- 5) Y. Umemura, T. Inoue and M. Takagi, *Agric. Biol. Chem.*, **37**, 219 (1973).
- 6) O. Nygard and T. Hultin, *Biochem. J.*, **194**, 469 (1981).
- 7) A. J. Munro, R. J. Jackson and A. Korner, *Biochem. J.*, **92**, 289 (1964).
- 8) M. J. Mycek, *Biochem. Pharm.*, **20**, 325 (1971).