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Heat-Sensitive Spermine-Binding Factor from Peripheral Human Lymphocyte (41343)

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Abstract. Human peripheral lymphocytes contain a cytosolic factor able to bind noncovalently spermine with high affinity. Other polyamines bind much less effectively and the order is: spermidine, putrescine, cadaverine. The factor is heat sensitive, and appears to be a protein on the basis of nucleolytic and proteolytic digestion. It seems to be an intracellular specific binder for spermine.

Aliphatic polyamines occur widely among mammalian cells, and in high concentrations during cell growth and development (1, 2). Numerous observations indicate that these compounds, in all probability, have essential functions in cellular metabolism even though the precise mechanism of their action is not yet well understood (1, 2). These compounds behave as cations at physiological pH and therefore can link through ionic forces to negatively charged substances in the cells. It is possible that some of the biological effects exerted by these amines can be ascribed, at least in part, to this kind of interaction. Recently, specific extracellular polyamine binding proteins have been described in rabbit and human serum (3, 4), and covalent complexes have been described in human amniotic fluid (5). An androgen sensitive spermine-binding protein has been identified and purified to homogeneity from the cytosol of rat ventral prostate (6). In a previous paper we reported that chick duodenal mucosa contains a cytoplasmic protein capable of binding selectively spermine with high affinity (7). In this paper we report the presence of a cytoplasmic spermine-binding protein in human peripheral lymphocytes as a preliminary and new approach to the study of polyamine metabolism and significance in normal and abnormal growth.

Materials and Methods. *Chemicals.* [^{3}H]Spermine tetrahydrochloride (44.3 Ci/mmole) and other radioactive polyamines were obtained from New England Nuclear. Electrophoretically pure nucleases and crystalline trypsin, obtained from Worthington, were always desalted before use. Whole tRNA from rat liver was obtained from General Biochemicals.

Freshly drawn heparinized human blood from voluntary donors was mixed with 1/10th volume of 6% dextran in physiological solution. The supernatant after sedimentation was carefully removed and centrifuged at 600*g* for 10 min. The white cells pellet was resuspended in Eagle's minimal essential medium (MEM) purchased from Laboratoires Eurobio, Paris (France), 20 bd. Saint-Germain, containing autologous plasma (20%). Each 20 ml of the mixture was layered on 20 ml of lymphocyte separation medium obtained from Flow Laboratories, and centrifuged at 400*g* for 30 min in an International 269 rotor. Lymphocytes and monocytes in the interface were carefully removed and diluted with Eagle's MEM containing autologous plasma (20%). The cells were then pelleted and resuspended in a small volume of 40 m*M* glycine buffer, pH 8.7, containing 0.25 *M* sucrose. After counting the cells were disrupted by homogenization in Potter-Elvehjem homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 300,000*g* for 1 hr, the upper three-fourths of the supernatant was taken and used as the cytosol fraction after exhaustive dialysis against 40 m*M* glycine buffer, pH 8.7. The yield was about 3 mg protein/100

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TABLE I. SENSITIVITY OF SPERMINE-BINDING ACTIVITY FROM HUMAN LYMPHOCYTES TO NUCLEOLYTIC AND PROTEOLYTIC DIGESTION[a]

Treatment	Percentage of binding		
	Buffer alone	Liver tRNA	Lymphocytes cytosol
None	0	100	100
RNAse (pancreatic A + T_1)	0	0.02	65.7
Trypsin	0	—	35.2
RNAse + trypsin	0	—	0.02

[a] Aliquots (0.7 mg of protein) of the cytosol preparation were incubated at 20° for 10 min with 50 μg of RNAse mixture (pancreatic RNAse A + RNAse T_1, 20:1) and/or with 500 μg of trypsin for 4 hr at 4°. These preparations were thereafter assayed for their spermine-binding activity as described under Materials and Methods. Data are reported as percentage of the activity found in untreated sample, and represent the average of at least five determinations performed on different occasions on freshly drawn blood pooled from at least two volunteers.

ml of the original fresh blood (average of 20 donors).

The sample was then incubated with 0.25 μCi of 10 μ*M* [^{3}H]spermine in 0.35 ml of 40 m*M* glycine buffer, pH 8.7, at 0° for 10 min. After incubation the reaction mixture was passed through a Sephadex G-25 column (0.7 × 16 cm) to measure the amount of radioactive spermine bound to the macromolecules in the void volume of the effluent. Fractions (0.5 ml) were collected and the spermine-binding activity eventually present was estimated from the area of the peak of radioactivity corrected for the protein present in the assay (7).

Results and Discussion. When [^{3}H]-spermine is incubated with cytosol prepared from human lymphocytes and then chromatographed on a Sephadex G-25, a portion of the radioactivity is eluted in the void volume of the effluent. This is due to the binding of the [^{3}H]spermine to macromolecular factor(s) present in the cytosol fraction and it is not observed when the lymphocyte preparation is omitted. In order to understand the chemical nature of the macromolecular component we have performed studies based on extensive nucleolytic and/or proteolytic digestion of the factor.

Table I correlates these treatments with the spermine-binding activity shown by the treated and untreated lymphocyte preparations. Extensive treatment with a RNAse solution (pancreatic A + RNAse T_1,20:1) resulted in a loss of about 35% of binding, probably due to the breakage of a specific bindings with cytoplasmic RNA species. The effectiveness of this digestion was checked by the capability to suppress totally [^{3}H]spermine binding by high amounts of rat liver tRNA. From the experiments

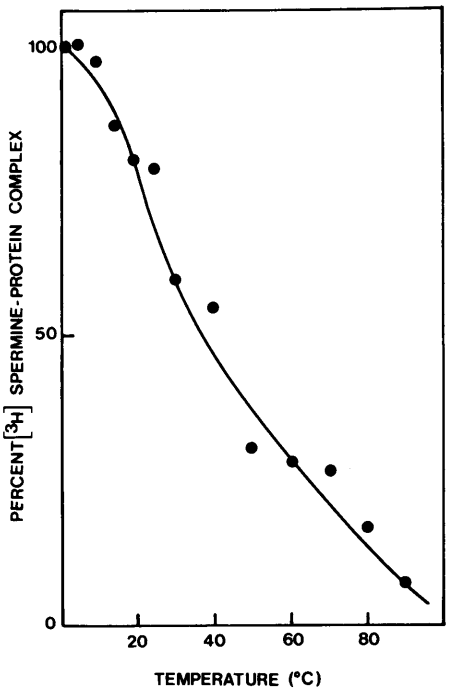

FIG. 1. Heat sensitivity of the binding protein in the crude cytosol. One milligram of dialyzed cytosol protein pretreated with RNAse as described in the legend to Table I was incubated with 0.25 μCi of 10 μ*M* [^{3}H]-spermine. The mixtures were analyzed by gel filtration as described under Materials and Methods. The plot has been drawn by reporting the data obtained from four experiments performed on different occasions.

reported it appears that the binding factor is a protein, in fact the radioactive RNAse-resistant spermine-binding complex is abolished only after extensive trypsin digestion. In the absence of the cytosol preparations the nucleolytic and proteolytic enzymes incubated with radioactive spermine did not give any detectable binding. Further proof of the protein nature of the binder was sought by its sensitivity to temperature (Fig. 1). In fact about 50% of the binding activity of the fresh preparation is lost within 15 min at 40°, and essentially all of the activity is destroyed by heating the sample at 80° for 15 min.

It is known that human lymphocytes contain intracellular soluble transglutaminase activity (8) and that transglutaminase in mammalian reproductive tissues and fluids is able to catalyze the Ca^{2+}-dependent covalent incorporation of polyamines into proteins (9). To understand whether the above-mentioned spermine–protein complex could represent the product of transglutaminase activity, we have studied the effect of Ca^{2+} ions on the formation of the complex itself. The results are reported in Fig. 2 and show that 5 m*M* $CaCl_2$ is able to inhibit the formation of the complex by 70%, indicating that transglutaminase activity is not involved in the reaction and suggesting the noncovalent nature of the binding.

The experiments depicted in Fig. 3 show that with increasing concentrations of cold spermine in the assay the amount of radioactive spermine bound decreased as expected if a binding substance was approaching saturation. On the other hand this protein seems to bind selectively only

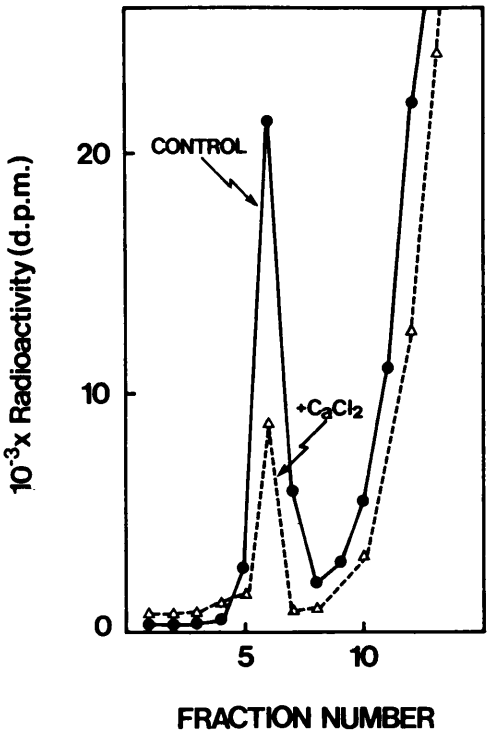

FIG. 2. Effect of Ca^{2+} ions on [^{3}H]spermine-binding activity in crude cytosol. Dialyzed cytosol protein, 0.75 mg, pretreated with RNAse as described in the legend to Table I was incubated with 0.25 μCi of 10 μM [^{3}H]spermine in the absence or in the presence of 5 m*M* $CaCl_2$. The mixtures were analyzed by gel filtration as described under Materials and Methods. Each point represents the average of three separate experiments.

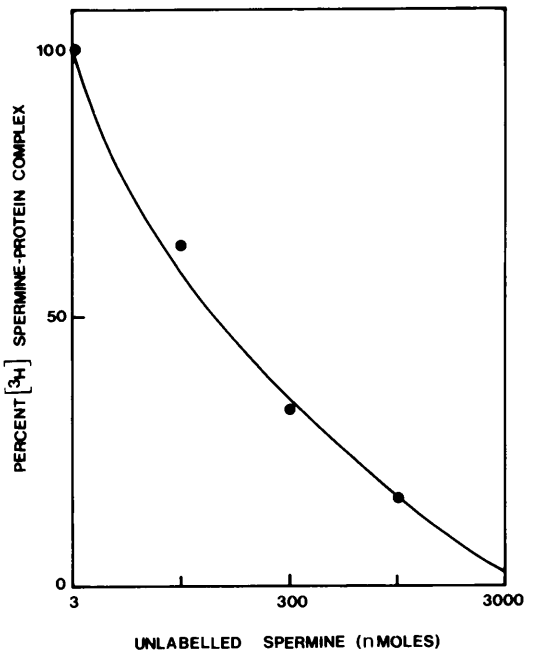

FIG. 3. Competition of [^{3}H]spermine binding by nonradioactive spermine. One milligram of dialyzed cytosol protein pretreated with RNAse as described in the legend to Table I was incubated with 0.25 μCi of 10 μM [^{3}H]spermine in the presence of the indicated amounts of nonradioactive spermine. The mixtures were analyzed by gel filtration as described under Materials and Methods. Each point represents the average of three separate experiments.

TABLE II. STRUCTURAL FORMULAS AND RELATIVE-BINDING ACTIVITIES OF DI- AND POLYAMINES[a]

	Amine	Relative binding activity
Spermine	H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH_2	1.0
Spermidine	H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2	0.003
Putrescine	H_2N-$(CH_2)_4$-NH_2	0.001
Cadaverine	H_2N-$(CH_2)_5$-NH_2	0.0009
Ornithine	H_2N-$(CH_2)_3$-$CHNH_2$-COOH	<0.0009

[a] Aliquots (0.7 mg of protein) from cytosol of human lymphocytes were incubated with 0.25 μCi of the indicated polyamine at the final concentration of 1 μM. The mixture was thereafter assayed for its amine binding activities as described under Materials and Methods. Data are reported as relative binding activities taking the binding activity of spermine as 1, and represent the average of at least four determinations performed on different occasions on freshly drawn blood pooled from at least two volunteers.

spermine. In fact, other radioactive polyamines of various size and charge tested under the same experimental conditions were not so effective in binding (Table II).

The results presented in this paper show that cytosol from human lymphocytes which is rich in polyamines (10) is also rich in spermine-binding protein(s). The physiological role of this protein and of the others described in rat ventral prostate (6) and in chick duodenal mucosa (7) is still obscure. The biological importance of the protein–polyamine interaction will be clarified when the cellular function of the binding protein will be identified. If the binding protein is an enzyme or a regulatory part of an enzyme, however, the enzyme activity may be altered by polyamines. Another possible role of this protein as a cytoplasmic spermine binder responsible for the intracellular compartmentation and transport of spermine has already been suggested (6). In physiologically growing tissues such as duodenal mucosa of developing chick embryo (7) or prostate from castrated rats injected with androgen (6) it has been found that polyamine binding proteins respond to the growth stimulus by rapidly modifying their activity. In this light it seems to us that the study of polyamine concentrations and/or metabolism performed in parallel with the evaluation of the activity of specific polyamine binders can be a useful approach to study the role of polyamine in normal and abnormal growth in humans.

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