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SOME FACTORS AFFECTING THE INTERACTION BETWEEN ACTIN IN LEUKEMIC L1210 CELLS AND DNASE I

Maria Malicka-Blaszkiewicz[1] and Jay S. Roth[2]

Section of Biochemistry and Biophysics, Biological Sciences Group,
University of Connecticut, Storrs, Connecticut 06268

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SUMMARY

Total actin content and F/G actin ratios were determined in L1210 leukemic cell cytosol based on inhibition of DNase I by actin. DMSO, when present in the assay mixture, inhibited the interaction between actin and DNase I. Ca^{++} and pCMB were also inhibitory with maximum effect at 1.25 μM and 2.5 μM, respectively. For expression of maximum inhibition of DNase I by L1210 cytosol, 0.25 μM ATP is necessary in addition to proper dilution. Preincubation of purified chicken muscle actin or actin in L1210 cytosol with double stranded DNA inhibited the subsequent interaction between DNase I and the actin.

INTRODUCTION

Actin is an interesting protein which now is recognized to play an important role in forming the cytoskeleton of cells, in cell movement and division (1,2). It occurs in several forms in cells. The common muscle form α is different in amino acid sequence from two cytoplasmic forms β and γ found in other cell types and another distinct actin variant has been reported to occur in the nucleus (3,4). Lazarides and Lindberg (5) showed that actin was the DNase I inhibitor which had been studied by a number of investigators. Actin is of potential importance, therefore, as a regulator of intracellular DNase I activity. Recently, several investigators have shown that transcriptionally active genes are sensitive to DNase I which degrades them, in part

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[1]On leave from Institute of Biochemistry-University of Wroclaw, Poland.
[2]Career Fellow of the National Cancer Institute, National Institutes of Health.

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at least, from the 5' end (6). Inactive genes are not affected. It has been suggested from this evidence that DNase I may function to inactivate genes under some circumstances, possibly during differentiation and transformation of cells.

It is clear, therefore, that the study of some factors affecting the interaction between various DNase I enzymes and different forms of actin might help to determine whether these factors play a role in regulating the action of cell DNase I on cellular DNA. In this report the effects of some factors on the interaction of crystalline bovine pancreatic DNase and actin from chicken muscle or L1210 cytosol were investigated.

MATERIALS AND METHODS

Leukemic Cells: L1210 leukemia is carried in DBA mice by intraperitoneal transfer once a week. Ascites fluid is removed and immediately diluted with 15 volumes of cold 0.9% NaCl solution. The sample is centrifuged at low speed, the supernatant fluid discarded and the red cells lysed by addition of 12 volumes of ice-cold distilled water to the cell pellet and shaking for 30 sec. The liquid is immediately made up to 0.9% saline again by adding concentrated NaCl solution and the sample centrifuged to obtain the white cells. They are rewashed 3 or 4 more times in 0.9% saline. Freshly prepared cells were used.

Cell Homogenates: These are prepared using a 1:3 ratio of cells to extracting buffer [Buffer A, 10 mM Tris-HCl, pH 7.4 in 0.25 M sucrose containing 0.1 mM ATP, 0.1 mM $CaCl_2$ and 1 mM dithiothreitol (DTT)]. The cells are broken by shearing in a small close-fitting Dounce homogenizer. Completeness of homogenization is always checked microscopically.

High Speed Supernatant Fraction (hss): Obtained by centrifugation of the cell homogenate at 105,000 g for one hour. The pellet is discarded and the hss used immediately for analysis or frozen.

DNase Activity: Estimations are done at 37° using 0.2 ml of 0.2 M HEPES buffer pH 7, 0.1 ml 50 mM Mg^{++}, 0.2 ml DNA (1 mg/ml), 0.2 ml DNase (stock 1mg/100 ml of 0.9% NaCl + 0.1% gelatin, diluted 100-fold with the same solution). The volume of reaction mixture is adjusted to 1 ml with water. The reaction is started by addition of DNA and after 15 min stopped with 0.5 ml 15% $HClO_4$. The mixture is allowed to stand in ice for 15 min and precipitated DNA is removed by centrifugation at 10,000 g for 10 min. The absorbance of the nucleotides in the clear supernatant fluid is measured at 260 nm and is a measure of the DNase activity. Calcium ion did not improve the activity of DNase I and was not used.

Actin Content: The inhibition of crystalline bovine pancreatic DNase I is a measure of G actin monomer and can be obtained directly. Total actin is usually determined after dilution of sample with an equal volume of depolymerizing buffer, prepared according to Blikstad et al. (8). It contains 1.5 M guanidine hydrochloride, 1 M sodium acetate, 1 mM ATP and 1 mM $CaCl_2$ in 20 mM Tris-HCl buffer pH 7.5. For cytosol we use 40-fold dilution of the sample with buffer A. (See 1 and Fig. 2.) By subtracting the amount of G actin from the amount of total actin the amount of F actin is calculated.

Unit: One unit of actin inhibitor is the amount which decreases the activity of 20 ng of DNase I by 10% (or 10 ng by 20%).

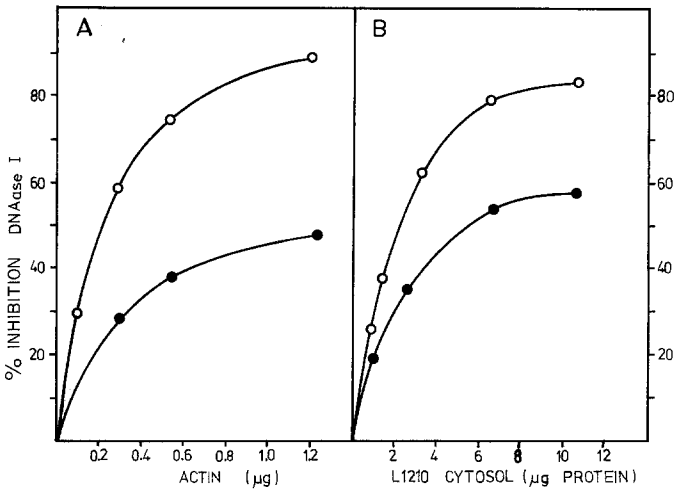

Fig. 1. Effect of DMSO on inhibition of DNase I by purified chicken muscle actin (A) and L1210 cytosol (B). Inhibition performed in standard assay conditions described in Materials and Methods. 20 ng crystalline DNase I was used. Chicken muscle actin and L1210 cytosol were diluted with fresh buffer A to the proper concentrations; o—o inhibition of DNase I without DMSO, •—• inhibition of DNase I in the presence of 10% DMSO.

Crystalline DNase I and purified chicken muscle actin were obtained from Sigma. Purified actin was prepared by dissolving 1 mg in 2 ml buffer A and diluting to the proper concentration with buffer A.

Protein: Estimated by the standard Lowry procedure (7).

RESULTS AND DISCUSSION

1. Actin content in L1210 cytosol - Effect of dilution: Inhibition of DNase I by purified actin from chicken muscle is shown in Figure 1A and by actin in L1210 cytosol in Figure 1B, (top curves). From the amount of protein in curves 1A and 1B which gives 50% inhibition, it appears that about 10% of the cytosol protein in L1210 is actin. No free DNase I or acid DNase activity was found in L1210 cytosol.

To determine total actin, preparations are generally treated with depolymerizing buffer of Blikstad et al. (8) which contains 1.5 M guanidine. This buffer converts, presumably, all F actin into G actin. We have found, however, that simple dilution of cytosol with buffer A will convert all the actin in the sample to G actin. Maximum amount of actin is reached with 40-fold dilution. A typical experiment is illustrated in Figure 2. The circle marked A is the actin content of undiluted hss from L1210 cells as determined by

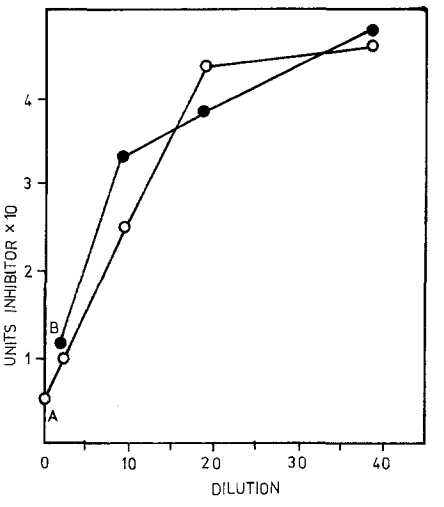

Fig. 2. Effect of dilution on the quantity of actin measured in L1210 cytosol. o—o dilution with buffer A, •—• dilution with depolymerizing buffer.

DNase I inhibition. The circle marked B is the total actin content determined by addition of an equal volume of depolymerizing buffer. A similar value is obtained by dilution with an equal volume of buffer A and this is the case for all of the dilutions with either depolymerizing buffer or buffer A up to the maximum 40-fold. The ratio of F/G actin after 40-fold dilution is about 10, while with the 1:1 dilution the ratio is around 3.

It appears that, with respect to L1210 cytosol, use of depolymerizing buffer with a 1:1 dilution gives values for total actin which are much too low. This buffer with its strong denaturing agent is not required since a 40-fold dilution with buffer A will give maximum values for total actin (Figure 2).

It seems likely that high dilution favors the depolymerization of the short-chain actin polymers present. We do not claim that this dilution procedure would necessarily depolymerize high molecular weight or branched-chain polymers which might be found in the sediment from high speed centrifugation, or in cell lysates.

2. Effect of Dimethylsulfoxide (DMSO): DMSO has many complex physiological effects on living cells or organisms. Of particular interest is its ability to induce differentiation in a variety of leukemic or proleukemic cells including Friend and Rauscher erythroleukemic cells (10). In addition, it has been observed in a few laboratories (11,12) that in cells treated with DMSO bundles of actin disappear from the cytoplasm and appear in the nucleus. In view of this ability, its effect on the actin-DNase I system is of interest. It was found that DMSO, when included in the reaction mixture reduced the inhibition of DNase I by purified actin or actin in L1210 hss. (Figures 1 (A) and 1 (B) lower curves.) The greatest effect was observed at 10% concentration of DMSO, an amount which did not affect the activity of DNase I itself. No effect of DMSO on inhibition of DNase I was observed when L1210 cell cytosol was preincubated with 5 or 10% DMSO for 1 hr or 16 hrs and then inhibition of DNase I measured.

3. Effect of preincubation of actin with DNA on inhibition of DNase I by actin: Purified chicken muscle actin or L1210 leukemia cell cytosol were preincubated 1 hr at 0° with DNA with or without the components usually present in the reaction mixture for DNase assay (Mg^{++} and HEPES buffer). A standard assay for actin was then run on the preincubated samples.

The results are illustrated in Table I which shows typical results with chicken muscle actin. Preincubation of actin with DNA alone caused the actin to lose the major portion of its inhibitory activity. This effect was diminished by the presence of DMSO and abolished by the presence of HEPES buffer and Mg^{++}. The effect may be a minor one due to the absence of HEPES or Mg^{++} but one other explanation of many possible ones is that actin can bind to DNA and this prior binding to DNA prevents, at least in part, the subsequent binding to DNase I. Some suggestive evidence to support this is the observation that when denatured DNA is used instead of native DNA, there is no suppression of the subsequent interaction of actin with DNase I.

TABLE I

EFFECT OF PREINCUBATION OF ACTIN WITH DNA ON SUBSEQUENT INHIBITION OF DNASE I BY ACTIN

Reaction Started with DNase	Incubated with	DNase Activity	% Inhibition
DNA	0	100	0
DNA	Buffer A	100	0
DNA	HEPES + Mg^{++}	100	0
DNA	HEPES + Mg^{++} + Buffer A	100	0
DNA	Actin	88	12
DNA	Actin + 10% DMSO	60	40
DNA	HEPES + Mg^{++} + Actin	28	72
DNA	HEPES + Mg^{++} + Actin + 10% DMSO	54	46

Standard assay procedure, 200 μg DNA, 20 ng DNase I, Actin 0.5 μg (chicken muscle), 1 hr preincubation at 0°. Similar results were obtained when L1210 cytosol was the source of actin.

4. Effect of ATP: ATP has been shown to be necessary for maintenance of the nature conformation of G actin and for the conversion of F actin to G actin (9). Its role in the actin-DNase I interaction is not known, however.

We have observed that with L1210 cytosol when no ATP is added inhibition of 20 ng of DNase I is 40% while when enough ATP is added to give the reaction mixture an ATP concentration of 0.25 μM the inhibition is increased to 87%. Apparently cytosol ATP concentration is limiting for the reaction. Concentrations of ATP greater than 0.25 μM up to 5 uM do not change the inhibition.

5. The effect of calcium ion: Ca^{++} decreased the inhibitory effect of actin in L1210 leukemic cells on DNase I as illustrated in Table II. Maximum effect was observed at 1.25 mM Ca^{++}, above that there was no further reduction in the inhibition or even, possibly, an increase. Ca^{++} can interact with all three major components in the assay, DNA, actin and DNase I and the basis for its effect is not known.

6. The effect of p-chloromercuribenzoic acid (pCMB): pCMB at a concentration of 2.5×10^{-4} M decreased inhibition of DNase I by L1210 cytosol by about 50%. Higher concentrations of pCMB inhibit DNase I. This result suggests that the integrity of -SH groups, of actin and/or DNase I may be important for optimum interaction between the two.

TABLE II

THE EFFECT OF Ca^{++} ON THE INHIBITION OF DNASE I BY ACTIN IN L1210 CYTOSOL

Added Ca^{++} Concentration mM	Inhibition %
0*	75
0.5	65
1.25	37
2.50	44
5.0	50

Standard assay, 10 µl hss, 10 ng DNase I
*There is probably some free Ca^{++} in the cytosol and 0.001 mM Ca^{++} is present from the buffer used to prepare hss.

7. The effect of temperature: Actin in L1210 leukemia cells was found to be very sensitive to temperature changes. Storage of a sample at 4° for 24 hours decreased the inhibition of DNase I by 50%. The same decrease was observed after 1 hour at room temperature (20-25°) or 5 minutes heating at 50°. After storage for 4 days at 4° the inhibitory effect was destroyed almost completely.

F actin was observed to be more resistant to denaturing conditions than G actin and, in general, it was possible to avoid destruction of actin by freezing or by immediately dialyzing a sample against buffer A.

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