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THE EFFECT OF METHYLGLYOXAL ON ACTIN

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SUMMARY Studying the gelation of Ehrlich ascites tumor cell extracts at various methyglyoxal concentrations, an increase of the gelled protein fraction, composed mainly of actin, was found at 10^{-7} M to 10^{-5} M. When methylglyoxal was added to intact tumor cells, the filamentous portion of cytoplasmic actin was increased at 10^{-7} M to 10^{-6} M concentrations. Furthermore, certain functional properties of purified skeletal muscle actin were also affected by μM concentrations of methylglyoxal; the speed of actin polymerization was facilitated and more filamentous actin formed in polymerizing conditions. The possible mode of methylglyoxal action is discussed.

It has been proposed by Szent-Györgyi (1) that methylglyoxal and related aldehydes, which are normal metabolite products, may have a regulatory effect on the electronic state of cellular proteins and, as a consequence, cell division. Methylglyoxal may act as electron acceptor with regard to proteins, thus converting proteins into conductors as has been demonstrated by direct conductivity measurements of bovine serum albumin, casein, lysozyme, and collagen, which have shown an increased conductivity in the long frequency range if they were complexed with methylglyoxal (1-5). It has been asumed that the change in electronic properties of proteins is reflected in their functional behavior. The effect of methylglyoxal and other aldehydes on several biochemical mechanisms of the cell have been already investigated (6), showing the impairment of protein synthesis, protein secretion, tubulin functions, the respiratory rate of mitochondria, etc., at rather high concentrations of methyglyoxal (mM range). Since high concentrations of methylglyoxal are not likely to occur in the living cell, the search for other biochemical processes which may be

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affected or regulated by lower concentrations of methylglyoxal is warranted. The purpose of our present study was to investigate whether methylglyoxal can influence the functional behavior of actin, the main component of the contractile apparatus (7), or not. The results show μM concentrations of methylglyoxal exert quantitative changes in some of the characteristic functional properties of actin.

MATERIALS AND METHODS

Methylglyoxal was obtained from several sources and checked by ^{13}C nuclear magnetic resonance. The purest, purchased from ICN Pharmaceutical (Cleveland, Ohio), was used throughout the experiments. All other chemical were reagent grade or the best available.

Ehrlich ascites tumor cells (ETC) were propagated in Balb/c mice. After 10-13 days of *in vivo* culture, the cells were harvested from the abdomen and washed with 0.15 M NaCl containing 15 mM sodium citrate. The washed cells were either used for the preparation of cell extract or suspended in Eagles minimum essential medium containing 10% fetal calf serum and antibiotics and then kept in a humidified atmosphere of 95% air and 5% CO_2 at 37° C until use (within 4 hours). The viability of cells was assessed by the trypan blue exclusion test.

The preparation of ETC extract and the temperature-dependent gel-sol transformation of ETC extracts, as well as the quantitative measurement of the gelled protein fraction, was carried out according to the method of Ishiura and Okada (8). Briefly, 4 ml ETC extract (9.8 mg/ml extract protein in 0.34 M sucrose and 20 mM Tris-maleate, pH 7.0, containing 0.25 mM ATP, 0.5 mM EGTA, 0.5 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride) was warmed to room temperature after addition of various concentrations of methylglyoxal. One hour later the extracts were reproducibly separated into gel and liquid layers by centrifugation at 1,150 x g for 30 minutes and the total protein (9) and percentage of actin (10) in the gel determined.

The ratio of monomeric to filamentous actin in ETC was measured using the method of Blikstad *et al.* (10) which is based on the selective inhibition of deoxyribonuclease I by monomeric actin and assayed following lysis of the cells by Triton X-100 under appropriate conditions.

Purified G-actin was prepared from an acetone powder of rabbit skeletal white muscle by the method of Spudich and Watt (11). F-actin was polymerized from G-actin in the presence of 0.1 M KCl, sedimented at 176,000 x g for 1 hour, and then dissolved in 1 mM $MgCl_2$ by homogenization. When the kinetics of actin polymerization was followed, the increase in A_{232} of polymerizing actin samples versus a matched G-actin blank was recorded at room temperature in a Beckman Acta III spectrophotometer (12). The deoxyribonuclease I inhibition assay (10) was used for measuring the degree of actin polymerization as well as the functional change of G-actin.

RESULTS

The assembly and disassembly of actin-containing filaments can be studied *in vitro* based on the observation that actin filaments form a gel when extracts of various kinds of non-muscle cells are warmed to room temperature in the presence of ATP, EGTA, and KCl (13, 14). In our experiments, the gel-sol

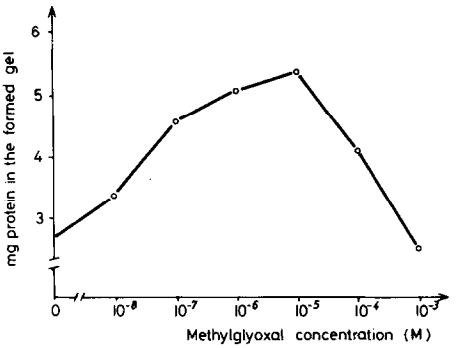

FIG. 1. The effect of methylglyoxal on the temperature-induced gelation of ETC extracts. The percentage of actin in the gel fractions are as follows (methylglyoxal concentration in parenthesis): 29.5 (0); 34.8 (10^{-8} M); 37.1 (10^{-7} M); 46.9 (10^{-6}); 53.2 (10^{-5}); 29.7 (10^{-4}); 14.9 (10^{-3} M). Data represent typical results of several experiments.

transformation was studied quantitatively by determining the total protein of pellets of gels obtained by low-speed centrifugation (8). Adding various concentrations of methylglyoxal (neutralized to pH 7.5 before use, as in all the subsequent experiments) to the cytoplasmic extract of ETC before warming it up, resulted in a substantial increase of the gelled protein fraction as well as its actin content at 10^{-7} M to 10^{-5} M concentrations of methylglyoxal (Fig. 1). The gelled fraction is reduced at higher methylglyoxal concentration.

In another series of experiments, methylglyoxal was added to intact ETC (2×10^6/ml) kept under cell culture conditions. Two hours later, the cells were processed (10) to determine the cellular ratio of monomer to filamentous actin. In control ETC, to which methylglyoxal was not added, 52% of the total actin content was in polymerized (filamentous) form (Table I). The presence

TABLE I. Polymerized (filamentous) portion of the actin pool in Ehrlich ascites tumor cells 2 hours after the addition of methylglyoxal

Methylglyoxal concentration (M)	0.0	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Percent of polymerized (F) actin	52.3	51.7	63.9	69.8	59.4	49.1	32.2
Percent of still viable cells	97.0	95.6	96.8	95.5	94.5	91.5	74.2

The data are typical representatives of several experiments.

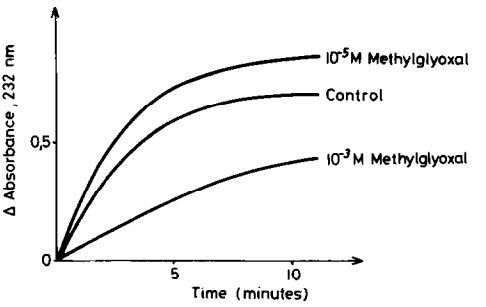

FIG. 2. Kinetics of actin polymerization induced by 5 mM $MgCl_2$ in the absence and presence of methylglyoxal. The change of absorbance at 232 nm is plotted versus time. The actin concentration of all samples was 0.3 mg/ml, and all samples contained 0.2 mM ATP, 0.1 mM $CaCl_2$, 5 mM imidazole, and 0.8 mM β-mercaptoethanol at pH 7.5.

of 10^{-7} M to 10^{-5} M methylglyoxal during the incubation period resulted in a shift of monomer to filamentous form of cellular actin. Meanwhile, the cells remained viable; that is, no significant change in the proportion of viable cells could be observed using the trypan blue exclusion test (Table I). 10^{-3} M methylglyoxal was found to be toxic, with a concomitant depolymerization of actin inside the cells (Table I).

Studying the effect of methylglyoxal on highly purified actin, the following observations were made:

Adding methylglyoxal (10^{-8} M to 10^{-4} M final concentrations) at 37° C to G-actin samples (2.5 mg/ml in 0.5 mM ATP, 0.1 mM $CaCl_2$, 5 mM imidazole, and 1.5 mM β-mercaptoethanol, pH 7.5), there was no change in its ability to inhibit DNAse activity measured at any time in a 4-hour incubation period. At 10^{-3} M concentration, a decrease (22% in 4 hours) of the inhibitor activity of G-actin was found.

The speed of actin polymerization (G-F transition) induced by the addition of 5 mM $MgCl_2$ to G-actin samples was not affected at 10^{-8} M to 10^{-6} M, but stimulated at 10^{-5} M to 10^{-4} M methylglyoxal concentrations (Fig. 2). Furthermore, at 10^{-5} M to 10^{-4} M methylglyoxal concentrations, the proportion of F-actin in the polymerized actin samples was also higher (by 26% and 21%,

respectively) as measured by the DNAse inhibition assay (results not included in the figure) 1 hour following the addition of $MgCl_2$. 10^{-3} M methylglyoxal concentrations resulted in an impaired polymerization; that is, the speed of actin polymerization (Fig. 2) as well as the proportion of F-actin (only 49% of control) were decreased.

DISCUSSION

The observed effects of methylglyoxal, namely, an increased amount of the gelled protein fraction of ETC extract (composed mainly of actin), the higher proportion of filamentous actin inside the cells, and the facilitation of the polymerization of purified actin suggest that an interaction takes place between methylglyoxal and actin such that the latter undergoes a conformational change favoring polymerization.

We did not observe the development of the characteristic brown color which has been shown by others in the case of an interaction between methylglyoxal and a number of proteins, such as bovine serum albumin, casein, collagen, and lysozyme (2-5), nor could we detect any change in the absorption spectra of either G- or F-actin under a series of various conditions tried so far. However, this does not exclude the possibility that these observations are related to a charge transfer reaction between methylglyoxal polymers and the polypeptide chain accompanied by a charge transfer reaction (15, 16).

Several authors have reported that the organization of actin filaments is disturbed in malignant cells (17-19). In our experiments, methylglyoxal facilitated the organization of actin both in purified systems and in cell extracts of intact tumor cells, suggesting that the methylglyoxal formed in the cell may have a regulatory influence on the organization of the contractile system. The fact that the effects were observed at rather low (10^{-7} M to 10^{-5} M) concentrations of methylglyoxal also make the results relevant to the possible situations in the living cell. Toxic effects, observed at 10^{-3} M methylglyoxal concentrations, are reminiscent of those observed by Dianzani (6). Considering the fact that a powerful enzyme, glyoxalase, is present in

tissues (20), the concentration of methylglyoxal is probably very low inside the cells. However, this small amount of methylglyoxal might be quite enough to produce definite changes in the functional properties of cellular proteins such as actin.

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