EFFECT OF TRIFLUPHENAZINE ON Ca-ATPase ACTIVITY OF FIBROBLASTS AND EHRLICH'S ASCITES CELLS

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Calcium ions are intracellular mediators of many functionally important processes. Explanation of the specific changes in calcium metabolism in transformed cells and the study of the properties of the proteins and enzymes involved in malignant change of cells are topics for investigation in modern membrane biology of tumor cells. Transport of calcium ions through the plasma membrane is the basic mechanism regulating and maintaining a low concentration of calcium ions in the cell, and is responsible for the normal course of metabolic processes [1, 3]. An important role in the regulation of work in the calcium–ATPase system for calcium transport through the plasma membrane is played by calmodulin [2]. It was discovered previously that active transport of calcium, coupled with increased Ca-ATPase activity, is observed in tumor cells [4].

The aim of this investigation was to explain the mechanism lying at the basis of increased activity of this enzyme in tumor cells, which is not observed in the fibroblasts.

EXPERIMENTAL METHOD

The test objects were preparations of plasma membranes of fibroblasts and of Ehrlich's ascites cells. The homogenization medium contained 0.25 M sucrose, 0.004 mM NaCl, 0.1 mM KCl., 0.005 mM $MgSO_4$, 0.02 mM Tris-HCl buffer (pH 7.6). The method of obtaining preparations was that suggested by Molnar et al. [11] and by Wallach and Verlay [15]. The protein concentration was determined by Lowry's method. Ca-ATPase activity was determined by the method of Fiske and Subbarow.

EXPERIMENTAL RESULTS

To elucidate the cause of the differences observed during the study of Ca-ATPase activity in normal and tumor cells, kinetic investigations of this enzyme were undertaken. The Ca-ATPase of plasma membranes is activated by the multifunctional protein calmodulin, which increases the affinity of the enzyme for calcium ions [5, 6, 14]. It has been suggested that two forms of calmodulin interact with Ca-ATPase. One form of this protein binds very firmly with the ATPase molecule and becomes a component of the subunitary structure of the enzyme, whereas the other – an easily translocated and mobile form of calmodulin – interacts with Ca-ATPase only under certain conditions of cellular metabolism [7]. Considering that the quantity of Ca-binding proteins, including calmodulin, increases in tumor cells, it might be expected that with an increase in the extracellular calcium concentration (to 9.5 mM) the ATPase of tumor cells will work on account of the mobile form of calmodulin. To test this hypothesis a kinetic analysis was undertaken on the enzyme in cells of both types (normal and tumor cells) in the presence of trifluphenazine (TF), a specific calmodulin inhibitor [8].

Substrate dependence of Ca-ATPase of fibroblasts and of Ehrlich's ascites cells in the presence of TF is shown in Fig. 1. Analysis of the curves indicates the different character of the inhibitory action of TF in cells of the two types. TF is a typical noncompetitive inhibitor of fibroblast Ca-ATPase, but in ascites cells it induces inhibition of mixed type. The

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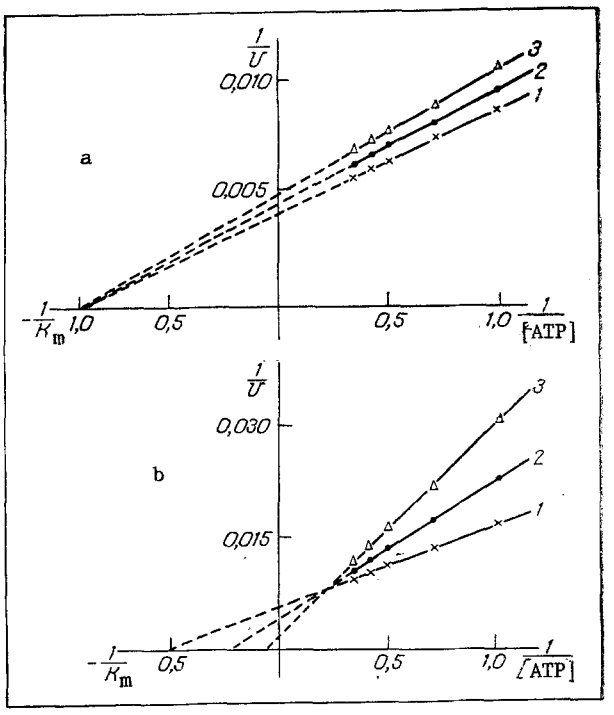

Fig. 1. Dependence of Ca-ATPase activity on different concentrations of ATP in absence (curve 1) and after addition of trifluphenazine 10^{-6} M (curve 2) and 2×10^{-6} M (curve 3), between Lineweaver–Burk coordinates. Here and in Fig. 2: a) fibroblasts; b) Ehrlich's ascites cells.

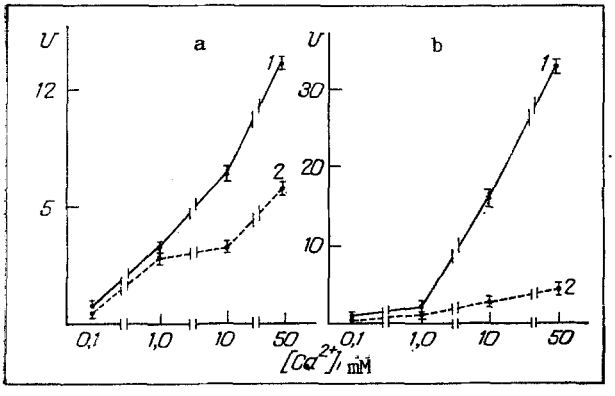

Fig. 2. Dependence of Ca-ATPase activity on different calcium ions concentrations. Calcium ion concentration: 1) in absence of extracellular calcium; 2) in presence of 2×10^{-6} M TF. a) Fibroblasts; b) Ehrlich's ascites cells.

Michaelis constant for ATP in fibroblasts is 1.1 mM and in ascites cells 2.2 mM. Incidentally fibroblasts have higher affinity for the substrate than ascites cells. In the last case, an increase in concentration of TF to 2×10^{-6} M not only caused a decrease in the maximal reaction velocity, but also reduced the affinity for ATP to K_m = 20 mM.

Evidently there is only one form of calmodulin in the plasma membranes of fibroblasts, connected with the Ca-ATPase molecule, whereas in ascites cells there are at least two forms of calmodulin, differing in their affinity for TF.

The study of dependence of Ca-ATPase activity on different calcium ion concentrations (0.1-50 mM) in fibroblasts (Fig. 2a) and ascites cells (Fig. 2b) revealed that the constant of semimaximal activation of Ca-ATPase was 5-6 mM, whereas in ascites cells it was 16-18 mM Ca^{++}. Analysis of the experimental results suggests that the difference in the catalytic

properties of Ca-ATPase of fibroblasts and ascites cells may be due to the action of different forms of calmodulin in these formations.

Tumor cells are known to contain large quantities of Ca-binding proteins, including calmodulin [12]. However, against the background of high concentrations of Ca-binding proteins, the malignant cell loses its sensitivity to calcium ions and its ability to regulate oriented Ca-dependent processes [10]. It has been suggested that disturbance of these processes is connected, not with physicochemical changes in the Ca-binding proteins and calmodulin, but with destruction of the principal calmodulin-binding elements of the cell [9].

There is evidence that the role of calmodulin in cell proliferation may lie in morphological changes in the cytoskeleton [13]. The change in the catalytic properties of Ca-ATPase observed in the present experiments, leading to a disturbance of normal calcium homeostasis in the transformed cells, and the compensatory increase in enzyme-bound calmodulin prevent normalization of the process.

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