# CONCANAVALIN A INDUCED CHANGES IN ACID PHOSPHATASE AND N-ACETYL-β-GLUCOSAMINIDASE ACTIVITY IN MOUSE LYMPHOCYTES

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The study of biochemical processes taking place in lymphocytes on activation and transformation is very interesting for modern medicine. Stimulation of the various stages of transition of the lymphocyte into the transformed state, by means of mitogenic agents *in vitro*, may be very useful not only to assist our understanding of the mechanisms of changes in the state of function of the cell but also for helping with the search for ways of early diagnosis of diseases accompanied by lymphocyte proliferation.

The action of plant lectins [concanavalin A (con A), phytohemagglutinin, etc.] on lymphocytes is known to be accompanied by bilateral movement of the cell surface receptors carrying molecules of the lectin, followed by their endocytosis [9, 13]. The subsequent fate of the endocytosed structures is linked with the participation of the lysosomal apparatus of the cell [5, 9]. Existing data in the literature on the role of lysosomal hydrolases in these processes are very contradictory and relate mainly to the late stages of lymphocyte activation (36 h or later) [5, 6]. Meanwhile the discovery of early changes in activity of these enzymes during the first 24 h after the mitogenic stimulus, i.e., before the appearance of morphological signs of blast transformation, could give new information on the mechanism and dynamics of lymphocyte activation.

The object of this investigation was to study activity of lysosomal hydrolases (acid phosphatase and N-acetyl-β-glucosaminidase) during the first 24 h of action of con A on mouse lymphocytes.

## EXPERIMENTAL METHOD

Lymphocytes isolated from the spleen of CBA mice were used. The lymphocyte population obtained contained 70-72% of T cells, as shown by the cytotoxic test with anti-ν-AKR-anti-CBA mouse sera. Lymphocytes were isolated by the method in [7] after preliminary removal of adherent cells from the suspension of splenocytes by passage through thermostatically controlled columns packed with cotton wool [8]. Erythrocytes were removed by hemolysis in 0.83% $NH_4Cl$. The lymphocytes were washed twice with Hanks' solution containing 5% bovine serum, and then transferred to growth medium containing RPMI, embryonic calf serum, L-glutamine, HEPES, 2-mercaptoethanol, penicillin, and streptomycin. Lymphocytes were activated with con A (5 μg/ml) and with growth medium from a mixed lymphocyte culture (MLCGM). The mitogenic effect of con A and MLCGM was judged from the degree of incorporation of $^3$H-thymidine, added to the cell culture 72 h after the beginning of incubation with it. At each stage of isolation and culture of the lymphocytes their viability was determined: It was 80-85% after incubation for 15 h and 70-75% after incubation for 22 h.

Acid hydrolase activity was determined in cell extracts [1, 3]. For this purpose the cells were destroyed in a glass disintegrator and the resulting homogenate was centrifuged. Total protein in the cell extracts was determined by Lowry's method [10].

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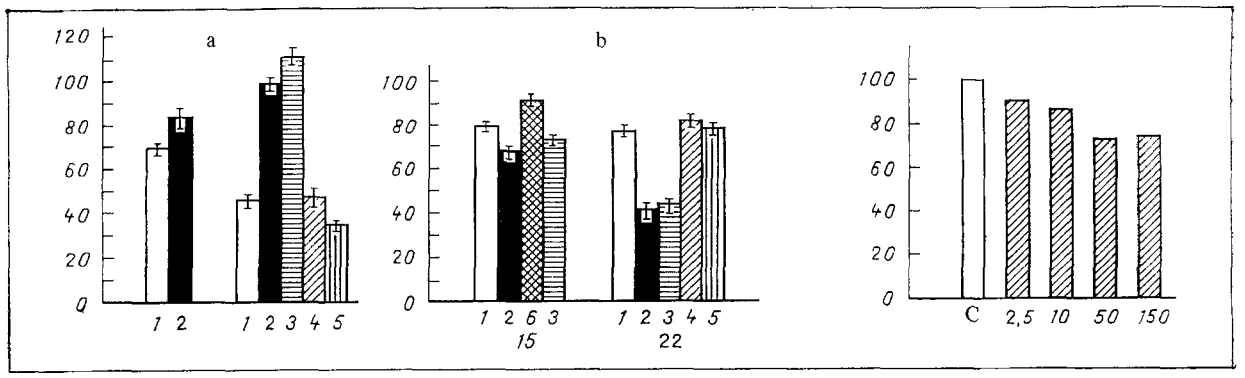


Fig. 1 Fig. 2

Fig. 1. Time course of acid phosphatase (a) and N-acetyl-β-glucosaminidase (b) activity in mouse lymphocytes under influence of con A (5 μg/ml). 1) Control; 2) con A; 3) con A + MLCGM; 4) α-methylmannoside (20 mM); 5) washed; 6) MLCGM. Abscissa, time of incubation of lymphocytes (in h): ordinate, enzyme activity (in μmoles *p*-nitrophenyl/mg protein).

Fig. 2. N-acetyl-β-glucosaminidase activity on addition of various concentrations of con A to incubation medium. Abscissa, concentration of con A (in μg/ml); ordinate, enzyme activity (in % of control). C) Control.

## EXPERIMENTAL RESULTS

To study acid hydrolase activity, a population of mouse lymphocytes consisting chiefly of T cells was chosen as test material. This choice was based on data in the literature indicating that one of the enzymes to be determined, namely acid phosphatase, is present in 80% of T lymphocytes, by contrast with B lymphocytes, in only 45% of which is this enzyme found. In T lymphocytes, acid phosphatase also was distinguished by its higher inducibility [11]. Tetravalent con A, which has a mitogenic action on T lymphocytes but no such action on B cells, was used in the experiments. The process of lymphocyte activation by con A is known to consist of a series of repeated cycles of stages of binding and interaction of the lectin with receptors and the disappearance of these complexes from the surface by means of endocytosis. The effect of con A thus depends not only on dose, but also on duration of incubation with it. The time course of acid phosphatase activity in mouse lymphocytes under the influence of the optimal dose of con A (5 μg/ml) is illustrated in Fig. 1a.

It will be clear from Fig. 1 that activity of this enzyme increases considerably after the first 15 h, and after 22 h it is doubled. Addition of 1% MLCGM to the growth medium together with con A potentiated induction of acid phosphatase activity. Stopping the action of con A by means of α-methyl-mannoside or by washing out 1.5-2 h after its addition to the growth medium, i.e., after the end of the first cycle of interaction between con A and the cells, abolished the inducing effect on lymphocytes. This was confirmed by the stimulation index (SI — the ratio of label incorporated into cells of experimental samples to the control), which amounted to 18, 31, 1.9, and 1.3 for the second (with con A), third (with con A + MLCGM), fourth (with α-methylmannoside), and fifth (washed) samples respectively. These data thus show that resynthesis of acid phosphatase is not triggered during the first cycle of interaction between con A and T lymphocytes. Activation of the enzyme requires longer interaction between lectin and cell. Resynthesis of the enzyme probably begins after about 12 h, i.e., when the cells have already moved into the $G_1$ phase.

Unlike acid phosphatase activity, N-acetyl-β-glucosaminidase activity was reduced by con A (Fig. 1b). This decrease was already statistically significant after 15 h, and after 22 h activity in samples with con A and with con A + MLCGM was reduced by half. Washing the cells to remove con A or addition of α-methylmannoside to the samples with con A, just as in the previous experiments, abolished the action of the lectin on N-acetyl-β-glucosaminidase activity. It can be tentatively suggested that the inhibitory action of con A on the activity of this enzyme is connected with its direct interaction with the enzyme molecule. According to data in the literature, lysosomal enzymes belong to the glycoprotein class and, on combining with con A, they may lose their activity [12]. The principal sugar residues to which con A binds are α-mannopyranosyl and glucopyranosyl [4]. This mechanism of the inhibitory action of con A on N-acetyl-β-glucosaminidase activity is confirmed by two groups of facts: first, dependence of activity on time is clearly exhibited and, second, the

addition of different concentrations of con A directly to the incubation medium when determining activity of the enzyme revealed a well-marked dose-dependent effect (Fig. 2).

It will be clear from Fig. 2 that in doses of con A of 2.5-50 μg/ml activity gradually declined. A larger dose of con A (150 μg/ml) caused no further decline in activity. The ratio between activities of N-acetyl-β-glucosaminidase and acid phosphatase can be used, in the writers' opinion, to deduce an enzymic index of lymphocyte transformation under the influence of con A. Whereas for the control it was 1.14 after 15 h and 1.65 after 22 h, for the experimental samples it was 0.8 and 0.4 respectively, significantly under 1.

The results may be of definite importance in the search for markers of activation of lymphocytes and their transition into the transformed state under the influence of mitogenic agents.

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