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**Sections:**

1. Biomaterials
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4. Gene Expression
5. Metabolites and Correction of Metabolic Processes
6. Proteomics and Protein Functions
7. Molecular Basis of Physiological Functions

## ORTHOPHOSPHATE EFFECT ON PROTEOLYTIC ACTIVITY OF SUPERNATANTS OF *CHLORELLA* *VULGARIS* CELL HOMOGENATES

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It was earlier shown that the brain and liver mitochondrial fraction of mice did not cleave fibrin. The fibrinolysis was shown in the presence of inorganic orthophosphate – Pi. The increase of proteolytic activity in the presence of Pi was also demonstrated on some lymphoblast cell lines. And, judging by data of the inhibitory analysis, this effect was not bound to a resynthesis of the ATP. It allowed us to put forward the idea about existence of the ATP-independent pathway of proteolysis stimulation by Pi – "phosphatic effect". Further it was shown that inorganic orthophosphate (0.001-0.06 M) increased the activating function of streptokinase, urokinase, or tissue activator of a plasminogen by 50-250% and, in general, – 1.2-12.0 times lysis of a number of proteins by trypsin (T),  $\alpha$ -chymotrypsin (CT), subtilisin (S), papain (Pap), metalloproteinase of bacilli (MP), and at  $\leq 0.004$  M pepsin (Pep) as well. In higher concentration phosphate activity of Pep was sharply decreased. It suppressed lysis of Pap gelatin, gelatin and casein of MP by 40-50%. It turned out that fibrinogenolytic activity of a number of opportunistic microorganisms strains was shown only in the presence of inorganic orthophosphate.

The aim of the present work – manifestation of the feature of Pi effect on proteolytic activity of cell water-soluble fraction of a photosynthesizing alga *Chlorella vulgaris*.

Researches are executed on *Ch. vulgaris* cells, the strain of IBCE C-19 (algas' collection of Institute of Biophysics and Cell Engineering of NAS of Belarus). *Ch. vulgaris* grew up in the conditions of periodic culture on the Tamiyya medium at the continuous bubbling of suspension of cells air – 25 l/h;  $t = 25-26$  °C; illuminating intensities on a vessel surface – 32 W/sq.m; to a photoperiod (light/darkness) – 12 h/12 h. After the 7<sup>th</sup> day of culture growth,

we measured the cells concentration, selected their aliquotas, washed with distilled water three times. Cells were homogenized with bidistilled water on ice, homogenates were centrifuged within 10 min, at 8000 rpm, at 4 °C. Proteolytic activity was determined by lysis of a fibrinogen or casein in a thin agar layer as it was earlier described. Concentration of proteins was 10 g/l, and agar – 10 g/l. As solvent for preparation of protein-agar plates we used deionized water to which  $\text{Na}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$  aliquots were added. All experiments were made no less than five-fold. Results are processed statistically with calculation of a *t*-student criterion (Statistica-6).

Supernatants of homogenates of *Ch. vulgaris* cells were capable of cleavage of both proteins and in the absence of inorganic orthophosphate though the casein was hydrolyzed less intensively, than fibrinogen:  $20.3 \pm 0.9$  and  $24.5 \pm 0.9$  mm<sup>2</sup>, respectively. However, in the presence of inorganic orthophosphate the proteolysis significantly differed. At 0.001-0.009 M Pi concentration the fibrinogenolytic activity of supernatant was reduced by 12-37% whereas at effector concentration of 0.15 and 0.45 M it increased by 21 and 27%, respectively. Changes of caseinolytic activity had a three-phase character. At concentration of an effector of 0.001 and 0.003 M this activity increased by 68 and 84%, respectively, at concentration of Pi of 0.009 M it decreased by 37%. It was noted that at Pi concentration between 0.03-0.06 M the second phase of activity increase by 51–63% .

Therefore, as well as it is shown earlier, the effect of Pi depends on substrate protein. In this case, the complex concentration dependence on the effector action and the zone of proteolytic activity inhibition was observed. The causes of such picture need further researches.