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ACTIVITY OF PHOSPHOHYDROLYTIC ENZYMES IN WATERS

AKTYWNOŚĆ ENZYMÓW FOSFOHYDROLITYCZNYCH W WODACH

Abstract: Phosphorus occurs in mineral and organic forms in aquatic environments. Much of the available phosphorus resources occur in organic forms which are impossible to use directly by living organisms – cytoplasmic membranes of microorganisms' cells transport almost exclusively orthophosphates. Biological decomposition of organic phosphorus combinations is one of the key stages of phosphorus cycle in surface waters. This process is possible due to existence of a group of phosphohydrolytic enzymes, such as: 5'-nucleotidase, endo- and exonucleases, phytase and phosphatases. Occurrence or activity of these enzymes was found in all components of plankton: bacteria, cyanobacteria, algae, fungi, single- and multicellular plankton animals. Activity of phosphohydrolytic enzymes directly affects productivity of aquatic ecosystems.

Keywords: phosphorus, organic matter, activity of enzymes, water

Phosphorus is one of the elements that are strictly necessary for life of all living organisms. Although, in comparison with carbon and nitrogen, aquatic organisms require small amounts of this nutrient for growth and development (for example molar ratio of C:N:P in biomass of algae equals 106 : 16 : 1), its role in a cell is versatile. It is one of a cell building materials, *eg* phospholipids that build cytoplasmic membrane, compounds which store and transfer energy, *eg* ATP, phosphoenolpyruvic acid, many coenzymes or structures that store and express genetic information – DNA and RNA [1, 2].

Phosphorus occurs in mineral and organic compounds (as dissolved organic phosphorus or phosphorus enclosed in molecular matter) in aquatic environments. Fraction of mineral phosphorus is composed mainly of PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$ ions, various indecomposable orthophosphates or their condensates. Definite majority of organic derivatives of phosphorus consists of various monoesters and diesters of orthophosphoric acid (phosphatides). The compounds that contain P-N binding (phosphocreatine or phosphoarginine) are the only exceptions. Availability of phosphorus resources in Earth hydrosphere is limited. Firstly, phosphorus consisting minerals are

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weakly soluble and relatively rare, so supply of this element to aquatic environments as an effect of erosion and weathering of rocks is minimal. Secondly, global transport of phosphorus is directed almost one-way. This element moves from land ecosystems to inland waters and finally to seas and oceans, where it is practically immobilised for a long time in sedimentary rocks formed in these ecosystems. Duration of phosphorus in these reservoirs equals on average several thousand years [3].

Currently, many various mineral and organic substances enter aquatic ecosystems as a result of human activity. Among them nitrogen and phosphorus, nutrient sources for organisms living in water, can be found. Apart from metabolites of living organisms, soil erosion and weathering of rocks, also various mineral and organic combinations of phosphorus that do not occur in natural state, *eg* synthetic polyphosphates incorporated in detergents or pesticides occur in the pool of phosphorus containing compounds [4–6].

Common presence of phosphorus in many cell structures and important role of this element in metabolism caused that content and transformations of phosphorus became of particular interest of scientists and main objective of research projects within last few decades. Principal conclusion of these studies was a statement that content of phosphorus and its derivatives is a basic factor that controls biological production and evolution [7–12].

Phosphorus is a major nutrient that affects algae growth in surface waters. Biological decomposition of organic combinations of phosphorus is one of key stages of phosphorus cycle in surface waters, because cytoplasmic membranes of microorganism cells transport almost exclusively orthophosphates (PO_4^{3-}) and small amounts of phosphoglycerol and exceptionally phosphopentoses. The rate of mineral phosphorus release determines $P\text{-}PO_4$ assimilation rate by specified biocenoses. Thus, processes that aim at making phosphorus resources available for growth and development of living organisms are extremely important. Enzymatic decomposition of organic phosphorus carried out by four major groups of phosphohydrolytic enzymes is one of these processes [13].

Microorganisms, particularly heterotrophic bacteria, play crucial role in cycles of elements and organic matter decomposition. Majority of these processes depends on microorganisms abilities to produce ectoenzymes. Phosphohydrolases produced by microorganisms are very important for phosphorus mineralisation and supply of inorganic phosphates. Also large number of labile organic compounds that act as bacterial substrates is formed in a cycle of gradual enzymatic reduction of polymeric organic matter. Proteins, polysaccharides, lipids or nucleic acids belong to this category of polymers. They are a significant fraction of dissolved organic matter which is the most important source of nutrients and is hydrolysed and then used by aquatic micro-heterotrophic organisms [7, 13].

Phosphohydrolases as ectoenzymes

Microorganisms that produce ectoenzymes are probably the most likely to win the competition for inorganic as well as for organic nutrients in aquatic environment. Ectoenzymes transform unavailable components of organic matter into easily adoptive and easily penetrable through cellular membranes substrates for microorganisms, also called UDOM fraction (*ultrafiltered dissolved organic matter*). Because ectoenzymes are located on the cell surface, they make UDOM fraction components easily available for the cell transport system. It increases chance of microorganisms to survive in aquatic environment, where availability of energy and nutrients is a factor that limits growth and proliferation of population. Heterotrophic bacteria associated with organic matter particles are the most effective in ectoenzymes production. Specific activity of some ectoenzymes calculated for bacteria cells may be 2 to 20 times higher for *fraction associated with cells* (ectoenzymes) than for "free" enzymes [14].

Production of ectoenzymes which degrade substrates resistant to decomposition by other microorganisms may give their producers advantage in nutrients availability and let dominate the ecosystem. What is more, ectoenzymes producing bacteria are the significant part of aquatic ecosystem, in which they not only make carbon, phosphorus and nitrogen available for themselves by organic matter decomposition, but also influence count of algae by supplying them with nutrients [15].

Four major groups of phosphohydrolytic enzymes produced by aquatic organisms take part in processes of decomposition of organic compounds of phosphorus: 5'-nucleotidase, endo- and exonucleases, phytase and phosphatases. Occurrence or activity of these enzymes was found in all components of plankton: bacteria, cyanobacteria, algae, fungi and single- and multicellular plankton animals [16–19].

The majority of above-mentioned enzymes are functionally ectoenzymes. Only some of them occur in water as free enzymes or enzymes adsorbed on seston particles or on mineral particles and particles associated with biofilm "matrix". Ectophosphohydrolases of eukaryotic microorganisms are located on external surfaces of cytoplasmic membrane, in Gram-positive bacteria in cell wall whereas in Gram-negative bacteria in periplasmic space or in its boundary structures [20, 21].

Activators and inhibitors of phosphohydrolases

Activity of ectoenzymes in waters mostly depends on environmental factors, such as: temperature, pH, inorganic and organic compounds, UV-B radiation and presence of activators and inhibitors or lack of inhibitors. Divalent metal ions are activators for alkaline phosphatases. Alkaline phosphatase activity is stimulated by magnesium, which binds with active site of every part of enzyme. This site differs from the active site for zinc. Magnesium ion causes allosteric modulation, which stimulates dephosphorylation process. If the site provided for magnesium is bound with zinc cation, with higher affinity than magnesium, it will cause decrease of enzyme activity. Inhibitors of alkaline phosphatase are chelates of bivalent ions, such as EDTA and fluoride ions showing uncompetitive effects. On the other hand, acid phosphatase activity is often inhibited by fluorides. Also in presence of heavy metal ions rapid decrease of phosphatase activity occurs (acid as well as alkaline). Product of phosphatase activity – phosphate ion – is competitive inhibitor, similarly to other multivalent anions of inorganic and organic acids, similar in size and charge distribution to phosphate ion [22–24].

Brief Characteristic of phosphohydrolytic enzymes

5'-nucleotidase is located only in surface structures of bacteria and cyanobacteria. It is more specific than phosphatases, because it can only hydrolyse 5'-mono, di- and triphosphates of nucleotide. This enzyme is used by bacteria, similarly to alkaline phosphatase, to supplement intracellular pool of orthophosphate during deficiency of this compound in environment. Free nucleic acids – DNA and RNA are the most important reservoir of substrates for 5'-nucleotidases in lake waters. They belong to *dissolved organic phosphorus* (DOP) fraction particularly rich in this element, because they contain 9 % of phosphorus, whereas dry mass of algae contains only 2 % P, and dry matter of bacteria – 3 % P [25, 26].

Nucleases take part in pre-hydrolysis of DNA (dDNA) and RNA (dRNA) dissolved in water. Due to methodological reasons it is very difficult to define meaning of nucleic acids, and therefore also meaning of nucleases [27].

Phytase is an adaptive enzyme of bacterial origin, with maximum activity at pH 4–6. Effects of its activity are particularly visible in naturally acid or artificially acidulated waters, where it takes part in releasing phosphates from macromolecular organic phosphorus combinations which are mostly products of decomposition of plant material – from inositol phosphates combined with proteins, lipids and fulvic acid [28].

Phosphomonoesterases also called phosphatases are the most common and usually most active phosphohydrolytic enzymes in water. These enzymes catalyse reactions of splitting phosphate rest from proteins, fats, nucleotides and other compounds. Bacterial phosphatases are intracellular enzymes or they are secreted into environment. It depends on construction of external cellular structures, because Gram-negative bacteria with multilayer cell wall secrete small amount of periplasmic enzymes, whereas Gram-positive bacteria secrete more extracellular enzymes due to lack of one or a few layers of cell wall [23, 29].

Research on *Pseudomonas* bacteria showed [30] that phosphatases are located in periplasmic space. Touati et al [31] described acid phosphatase located in periplasm, produced by *Escherichia coli* (Gram-negative bacteria) in alkaline environments. Extracellular secretion of phosphatases from living cells was found by Glew and Heath [32] from *Micrococcus* (Gram-positive bacteria) and Kobori and Taga [33] from *Pseudomonas* (Gram-negative bacteria). Kobori et al [34] proved presence of constitutive high activity phosphatases in bacteria occurring in coastal waters. Cotner and Wetzel [35] found presence of *Acinetobacter* bacteria in pelagic zone of Third Sister Lake. This organism produces adaptive alkaline phosphatase; activity of this phosphatase increased even a thousand times under conditions of severe shortage of orthophosphate. If high concentration of orthophosphates is found in waters (higher than 140 μgPO_4^{3-} dm^{-3}), then pelagic bacteria produce alkaline phosphatase, which activity is inhibited by shortage of organic carbon rather than by phosphorus [36].

Phosphatases are also synthesised by most algae species. In algae they are located on the cell surface, in cellular membrane or secreted outside the cell [37–39].

There is also a part of the fraction of enzymes called "free" enzymes. This fraction consists of dissolved enzymes actively secreted by living algae cells, zooplankton and bacteria; they constitute an important part of total phosphatase activity. Part of activity of this fraction also comes from leakage of contents of aging cells or due to their damage by herbivorous organisms, decomposition of dead cells, their fragmentation, autolysis or lysis of living algae cells by bacteria or non-bacteria parasites. Free enzymes, unlike ectoenzymes do not have contact with cellular structures as a result of physico-chemical processes or after lysis of cells that produce them. Usually their activity, in comparison with the one observed in ectoenzymes is small and is usually a few percent high. Alkaline phosphatase, which connection with cytoplasmic membranes is often weak, may be an ectoenzyme as well as a free enzyme. In turn, 5'-nucleotidase does not occur in environment in "free" state [20, 40, 41].

Resume

Aquatic microorganisms produce a range of hydrolytic enzymes. Activity of this enzymatic potential, particularly bacterial but also algal, influences rate of organic matter assimilation in aquatic environments, which directly influences productivity of ecosystems [42]. Heterotrophic activity of multi-species bacteriocenoses is an indicator that well reflects degree of intensity of organic matter transformation processes in natural ecosystems [43].

Conclusions

1. Availability of phosphorus in aquatic environments is limited, therefore processes that aim at making it available for aquatic microorganisms are crucial.
2. Organic phosphorus decomposition is carried out by four groups of phosphohydrolytic enzymes: 5'-nucleotidase, endo- and exonucleases, phytase and phosphatases.
3. Activity of phosphohydrolytic enzymes was found in all components of plankton: bacteria, cyanobacteria, algae, fungi, single- and multicellular plankton animals.
4. Activity of phosphohydrolytic enzymes in waters is affected by the following environmental conditions: temperature, pH, inorganic and organic compounds, UV-B radiation and presence or lack of activators and inhibitors or lack of inhibitors.
5. Activity of phosphohydrolytic enzymes determines rate of organic matter assimilation in aquatic environments, which directly influences productivity of ecosystems.

References

[1] Siuda W, Chróst RJ. Polish J Environ Stud. 2001;10(6):475-483.
[2] Chróst RJ, Siuda W. In: Burns R, Dick R, editors. Enzymes in the environment: Activity, ecology and applications. New York: Marcel Dekker Inc.; 2002.
[3] Harrison AE. Soil organic phosphorus. Wallinford, UK: C.A.B. Int.; 1987.
[4] Carr OJ, Goulder R. Water Res. 1990;24(5):631-638. DOI: 10.1016/0043-1354(90)90196-D.
[5] Tabata M, Ozawa K, Ohtakara A, Nakabayashi H, Suzuki S. B Environ Contam Tox. 1990;44(6):892-899.
[6] Hajda P, Novotny V. Water Sci Technol. 1996;33(4-5):153-158.

[7] Berman T, Wynne D, Kaplan B. Hydrobiol. 1990;207:287-294.
[8] Miyabara Y, Watanabe H, Suzuki J, Suzuki S. Jpn J Limn. 1993;54(3):171-178.
[9] Jamet D, Amblard C, Devaux J. Hydrobiol. 1997;347:185-195.
[10] Nausch M. Aquat Microb Ecol. 1998;16(1):87-94.
[11] Rose C, Axler RP. Hydrobiol. 1998;361:145-156.
[12] Gambin F, Boge G, Jamet D. Marine Environ Res. 1999;47(5):441-456.
[13] Siuda W. Post Mikrob. 2001;40(2):187-217.
[14] Hoppe HG. In: Microbial enzymes in aquatic environments., Chróst RJ, editor. New York: Springer--Verlag; 1991.
[15] Chróst RJ, Overbeck J. Microbial Ecol. 1987;13:229-248.
[16] Degobbis D, Homme-Masłowska E, Orio AA, Donazzolo R, Pavoni B. Estuar Coast Shelf Sci. 1986;22(4):425-437.
[17] Boavida MJ, Heath RT. Arch Hydrobiol. 1988;111:507-518.
[18] Jansson M. Hydrobiol. 1988;170:177-189.
[19] Jansson M, Olsson H, Pettersson K. Hydrobiol. 1988;170:157-175.
[20] Chróst RJ. In: Microbial enzymes in aquatic environments. Chróst RJ, editor. New York, Berlin, Heidelberg: Springer-Verlag; 1991.
[21] Chróst RJ. In: Microbial ecology in Lake Pluâsee. Overbeck J, Chróst RJ, edotors. New York: Springer-Verlag; 1994:118-174.
[22] Bretaudiere JP, Stillman T. In: Methods of enzymatic analysis. Bergmeyer HU, editor. Weinheim: Verlag Chemie; 1984;4:75-82.
[23] Cembella AD, Antia NJ, Harrison PJ. CRC Crit Rev Microbiol. 1984;10:317-391.
[24] Siuda W, Chróst RJ. Acta Microb Pol. 1987;36:247-257.
[25] Ammerman JW, Azam F. Science. 1985;227:1338-1340.
[26] Siuda W, Güde H. Arch Hydrobiol. 1994;131(2):211-229,
[27] Siuda W, Chróst RJ. Aquat Microb Ecol. 2000;21:195-201. DOI: 10.3354/ame021195.
[28] Cooper JE, Early J, Holding AJ. Hydrobiol. 1991;209(2):89-94.
[29] Siuda W. Pol Arch Hydrobiol. 1984;31:207-233.
[30] Hassan HM, Pratt D. J Bacter. 1977;129:1607-1612.
[31] Touati ED, Dassa J, Boquet PL. In: Phosphate metabolism and cellular regulation in microorganisms. Torriani-Gorini A, Rothman FG, Silver S, Wright A, Yagil E, editors. Washington D.C.: American Society for Microbiol.; 1987:31-40.
[32] Glew RH, Heath EC. J Biol Chem. 1971;246:1556-1565.
[33] Kobori H, Taga N. Can J Microbiol. 1980;26:833-838.
[34] Kobori H, Taga N, Simidu U. B Jpn Soc Sci Fish. 1979;45(11):1429-1433.
[35] Cotner JB, Wetzel RG. In: Microbial enzymes in aquatic environments. Chróst RJ, editor. New York, Berlin, Heidelberg: Springer-Verlag;1991:187-205.
[36] Chróst RJ, Münster U, Rai H, Albrecht D, Witzel PK, Overbeck J. J Plankton Res. 1989;11:223-242.
[37] Aaronson S, Patni NJ. Limnol Oceanogr. 1976;21:838-845.
[38] Healey FP, Hendzel LL. Freshwat Biol. 1979;9:429-439.
[39] Wynne D. Hydrobiol. 1981;83:93-99.
[40] Chróst RJ. In: Aquatic microbial ecology. Overbeck J, Chróst RJ, editors. New York: Springer-Verlag; 1990: 47-78.
[41] Münster U, Chróst RJ. In: Aquatic microbial ecology. Biochemical and molecular approaches. Overbeck J, Chróst RJ, editors. Berlin, Heidelberg, London, Paris, Tokyo, Hong-Kong: Springer-Verlag; 1990: 8-46.
[42] Chróst RJ, Siuda W, Albrecht D, Overbeck J. Limnol Oceanogr. 1986;31(3):662-667.
[43] Mudryk Z. Bakterie heterotroficzne w procesach transformacji materii organicznej w jeziorach estuariowych. Słupsk: WSP; 1994.

AKTYWNOŚĆ ENZYMÓW FOSFOHYDROLITYCZNYCH W WODACH

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Abstrakt: W środowiskach wodnych fosfor występuje w postaci związków mineralnych i organicznych. Znaczna część dostępnych zasobów fosforu ma postać organiczną, niemożliwą do wykorzystania przez organizmy żywe bezpośrednio. Przez błony cytoplazmatyczne komórek mikroorganizmów są transportowane prawie wyłącznie jony ortofosforanowe. Biologiczny rozkład organicznych połączeń fosforu jest jednym z kluczowych etapów obiegu fosforu w wodach powierzchniowych. Jest to możliwe dzięki grupie enzymów fosfohydrolitycznych, takich jak: 5'-nukleotydaza, endo- i egzonukleazy, fitaza oraz fosfatazy. Występowanie lub aktywność tych enzymów stwierdzono we wszystkich składnikach planktonu: bakteriach, sinicach, glonach, grzybach oraz jedno- i wielokomórkowych zwierzętach planktonowych. Aktywność enzymów fosfohydrolitycznych wpływa bezpośrednio na produktywność ekosystemów wodnych.

Słowa kluczowe: fosfor, materia organiczna, aktywność enzymów, woda