ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Influence of Humic Acid Alkaline Solutions in Khanty-Mansiysk-Yugra Peats on Lipase Activity.

M.P. Sartakov[1]*, V.A. Chumak[2], and V.V. Leonov[3].

[1,2]Yugorsky State University, the city of Khanty-Mansiysk, Chekhova street, 16, Russia.
[3]Hanty-Mansiysk State Medical Academy, Khanty-Mansiysk, Mira street, 40, Russia.

ABSTRACT

The article presents the data about the study of humic acid alkaline solution effects found in various types and kinds of peats in Khanty-Mansiysk Autonomous Region - Yugra on the activity of lipase enzyme. It was found out that all drugs have the biological activity according to lipase inhibition test, but humic acids extracted from the peats with the lowest degree of degradation (5-25%) and more homogeneous by botanical composition exhibit a more potent inhibitory effect.

Keywords: humic acids, biological activity, lipase, botanical composition of peats, Khanty-Mansiysk AR.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

If we consider humic acids as growth substances, their influence on lipid membranes is very important, where usually the lipid layer is exposed continuously to a variety of external factors, which results in the formation of lipid peroxides, which live extremely long and are toxic to plants [1]. The damage of lipid molecules, their oxidation and the obtaining of peroxide lipids occurs under the influence of various stresses. In nature, plants experience repeatedly under some stressful conditions under extreme temperatures and the lack or excess moisture. Membranes are damaged constantly by the accumulation of lipase, an enzyme that destroys a lipid layer [2, 3].

SUBJECTS AND METHODS

We used the preparations of humic acids isolated from the surface layers of upland, transitional and lowland peats of Khanty-Mansiysk-Yugra (Middle Ob region) as the objects of the study. The extraction was performed according to a previously described procedure [4].

There was the study of inhibitory effect of humic acids alkaline solutions with the concentration of 0.001% on the enzyme lipase which is contained in the test specimen TRIGLYCERIDES GRO-PAP method/liguid, produced by Chonolab AG, (Sweden) and may decompose the triglycerides of a standard solution. Besides a working reagent incorporates respiratory enzymes to get closer to natural stable conditions: peroxidase, oxidase and catalase, which are not involved in the breakdown of lipids and transmit O2 and H2 in a plant cell.

The determination of humic acid inhibitory effect on lipase is carried out by spectrophotometry.

Spectrophotometric methods are based on the absorption of light in certain regions of the spectrum by many compounds which are the active groups of enzymes, substrates or reaction products. This method is highly sensitive, has a fast determination, a small expenditure of enzyme and reactants, and allows to monitor the progress of reaction over time. To do this 1 cm (l) of cuvette of sequentially introduced using a 0.1 ml micropipette of one of triglyceride solutions and 2 ml of working reagent from Chronolab kit. The resulting solution is thoroughly stirred with a glass stick, and a stopwatch is turned on immediately. The cuvette is placed in the cuvette compartment of CPK (λ = 505 nm), reduced to zero and the optical density D of test solution is measured against the reference solution. The results re recorded every 30 seconds. The reference solution is prepared by adding 0.1 ml of distilled water to 2 ml of working reagent. Measurement is continued until the optical density value does not change. Similarly, the measurements of all prepared solutions are performed.

This process proceeds according to a free radical mechanism, with the accumulation of exceedingly toxic peroxy radicals with low molecular weight.

RESULTS AND DISCUSSIONS

The macromolecules of humic acids may be regarded as stable free radicals capable of recombination at the cooperation with aggressive lipid peroxides i.e. be their "traps". This effect is manifisted to suppress the activity of the enzyme lipase under the influence of humic acids.

Determining lipase activity inhibition it is possible to talk about the effect of humic acid effect on plants and biological activity.

The idea of the membrane effect is discussed in the scientific literature very often nowadays. Not all authors associate it with free radicals, but it is taken into account that humic acids do not pass into a cell, as their sizes are too large compared with the parameters of the plant pores and make 100-200Å in a minimized as a globular structure.

Humic acids produce the inactivation of a damaged lipid layer. By its spin effect and the system of polyconjugation the electrones influence the reactions that occur on the membrane. Our results confirm these provisions.

ISSN: 0975-8585

Membrane effects play a very important role. It is recognized that a membrane is composed of lipid and protein layers, but the main role is played by lipids and the change of their structure. The lipid membranes of a cell have a great importance in metabolic processes. They allow to store and extract toxic substances without letting them get inside a cell. Therefore their damage could be a global one.

Humic acids are adaptogens. They act better under stress on plants and as a preventive measure.

The alkaline solutions of humic acid with the concentration of 0.0001%, that were extracted from peats of various botanical composition and the degree of decomposition were used for the inhibition.

During the course of enzymatic hydrolysis the lipid solution turns from slightly yellow color into a red one as the result of enzymatic reaction cascade leading to the formation of colored quinoneimine:

$$\text{triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acid}$$

$$\text{glycerol} + \text{АТП} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{glycerol-3-phosphate} + O^{-2} \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrin} + \text{4- chlorophenol} \xrightarrow{\text{POD}} \text{guinoneimine} + HCl + 4H_2O$$

To detect the inhibition of lipase by humic acid the activity of enzyme was calculated and studied in the presence of humic acid.

Lipase activity was determined by the following formula

$A_{lip} = (c_k\text{-}D_o/D_k \cdot c_k)t_{инкуб.}$,
where A_{lip} – lipase activity;
c_k – control concentration;
D_o – optical density;
D_k – optical density of control;
$t_{инкуб.}$ – incubation period;

The lipase activity without the addition of humic acids was determined by the ratio of the concentration to the incubation period (Table 1).

Table 1: Inhibition of lipase activity

Original peat appearance	R, %	Lipase activity, mmol $l^{-1}min^{-1}$
Without humic acids	--	0,53
Cotton grass-sphagnum	65	0,22
Cottongrass	55	0,16
Buck-bean	60	0,25
Wooden	45	0,16
Sphagnum	15	0,26
Grass	15	0,26
Sphagnum (100% fuscum)	5	0,10
Wooden	10	0,21
Grass-sphagnum	35	0,14
Wooden	25	0,19

Note: R – decomposition degree

The obtained data show that the lipase activity is reduced in the presence of humic acids. The highest inhibitory activity on the lipase preparation had a humic acid preparation extracted from sphagnum peat, consisting entirely of brown sphagnum with the decomposition degree of 5%.

The humic acid of this peat in comparison with other drugs, is characterized by a particular chemical structure - a lower degree of condensation and benzenoid content, a high proportion of aliphatic fragments and the concentration of paramagnetic centers.

SUMMARY

- It was determined that the inhibitory effect of humic acids from Khanty-Mansiysk - Ugra (Middle Ob region) peats on lipase activity, which may be considered as the measure of humic biological activity. The lipase content is reduced by 50- 80%.
- The greatest effect of antiseptic action was detected in the drug, derived from a uniform sphagnum peat (according to botanical composition) with a low degree of decomposition (5%).

CONCLUSION

The investigated samples of humic acids exhibit biological activity according to the test of lipase inhibition, but the humic acids derived from sphagnum and wood peat with the decomposition degree from 5 to 25% inhibit more lipase (about by 75%) than herbal sphagnum and wooded ones with a higher degree of decomposition from 25 to 65%.

ACKNOWLEDGEMENTS

The work was fulfilled under the financial support of the Russian Foundation for Fundamental Research and government of Khanty Mansyisk District and Yugra, agreement N 15-44-00090\15 от 28.04.15г.

REFERENCES

[1] Sartakov M.P. Characteristic of humic acids from Middle Ob region peats: The thesis of biol. Sciences Doctor. - Tyumen, 2013. - 298 p.
[2] Komissarov I.D., A.A. Klimova. The effect of humic acid on biocatalytic processes. // Proceedings of the Tyumen Agricultural Institute. - 1971. - V. 14. - Pp. 225-242.
[3] Khristeva L.A. The influence of humic acids on the growth of plants at various ratios of nutrients during the early development / L.A. Khristeva // The reports of the All-Union Academy of Agricultural Sciences, 1947. - Pp. 23-29
[4] Komissarov I.D. The influence of humic acids extraction method from raw materials on the chemical composition of the obtained preparations / I.D. Komissarov, I.N. Streltsova // Proceedings of the Tyumen Agricultural Institute. - 1971. - V. 14. - Pp. 34-48.