Mycosporine, the component of UV-protection system in lichen *Cladonia arbuscula* (Wallr.) Flot thalli – physico-chemical properties and ecological function

The ultraviolet radiation (UV) is considered as one of the main environmental factors harmful to many organisms. Consequently, with the constant threat posed by recurring exposure to the high doses of UV, they have developed, in a process of evolution, some very effective UV-protective mechanisms. These include biosynthesis and accumulation of UV-screening mycosporine-like amino acids (MAAs). MAAs belong to the group of secondary metabolites identified so far in the cells of cyanobacteria, algae, fungi, bacteria, as well as in the tissues of fish and invertebrates. The occurrence of MAAs has also been reported in the thalli of lichens containing cyanobacterial photobionts.

Due to the occupied habitats, lichens with eukaryotic component are also exposed to the deleterious effects of UV. Nevertheless, non-enzymatic mechanism developed by these symbiotic organisms to protect against UV radiation has so far remained unclear. The lack of literature information on the nature of MAAs produced by green algal lichens and the available data showing that both lichen partners, green algae and fungi, as independent organisms are able to MAAs synthesis, were crucial to undertake the intensive research. Their main goal was the identification and detailed characterization of MAAs synthetized by *Cladonia arbuscula* (Wallr.) Flot subsp. *squarrosa* (Wallr.) Ruoss (the most common species of *Cladonia* on the Polish territory).

The need to acquire knowledge on the nature of UV-absorbing compounds seems to be urgent in view of the fact that, nowadays, the progressive depletion of the ozone layer is observed. It is also very important issue, especially as the potential application of MAAs is extensively considered recently. These substances are promising candidates for use in pharmaceutical and cosmetic industry, as efficient UV-protectors.

In the extract obtained from *C. arbuscula* thalli, the presence of eight MAAs was detected, out of which seven showed a maximum absorption in the UV-B and one in the UV-A region. Methods of their extraction and purification using high performance liquid chromatography were optimized. A detailed qualitative

identification was performed for one of the isolated MAAs, which was quantitatively dominant in the extract and in addition not described in the literature. To obtain as much information as possible on its structure and properties, high-resolution methods, such as mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR), were used. This compound showed a strong polarity and a maximum absorption at a wavelength of 310 nm. Analysis MS helped to determine its molecular weight of 303 Da and molecular formula $C_{12}H_{17}N_1O_8$. Based on the results obtained by applying the NMR spectroscopy its structural formula and name, mycosporine-aspartate, were proposed.

Identification of new compounds entails the need for further analysis aimed to determine *i.a.*, their physico-chemical properties. In the next stage of the dissertation process, the attention was concentrated on the research designed to describe the physico-chemical properties of the newly identified MAA. This compound's detailed characteristics included the evaluation of its stability under the influence of various abiotic factors. Mycosporine-aspartate molecule under different conditions of pH remained stable for 2 weeks. After this period of time it has been shown that at an acidic pH of 3 the molecule underwent slow degradation. However, an alkaline pH of 10 as well as a neutral pH had no effect on its stability. Mycosporine-aspartate was also insensitive to the effects of temperature of 21±1°C or 40±1°C regardless of the pH of the solution. The accelerated degradation of this compound was observed after 2 h of incubation at the boiling temperature (100±1°C) at acidic pH. At similar temperature conditions other tested pH values did not affect the durability of its molecule. Under UV-B irradiation a degree of mycosporine-aspartate degradation depended mainly on the value of applied radiation intensity. The solutions of mycosporine-aspartate irradiated with photosynthetically active radiation (400-700 nm, PAR) at intensity of 2000 $\mu mol\ m^{-2}\ s^{-1}$ or UV-B at intensity of 9 $\mu mol\ m^{-2}\ s^{-1}$ were characterized by a pH-independent high resistance for 10 h. On the other hand, the exposure to UV-B at intensity of 18 $\mu mol\ m^{-2}\ s^{-1}$ caused a slight decrease of its concentration in all tested solutions with different pH values. A significant reduction in concentration was observed only under the influence of UV-B radiation at intensity of 36 $\mu mol\ m^{-2}\ s^{-1}$. Mycosporine-aspartate characterized also a high resistance to the presence of oxidizing substances, 4% hydrogen peroxide and molecular oxygen, in the reaction environment.

The results of further studies indicated that the kinetics of mycosporine-aspartate content in *C. arbuscula* thalli in one calendar year of 2014 exhibited a strong seasonality. In the summer season, its content was at least 2-fold higher compared to the winter months. *In vivo* experiments showed that UV-B played a crucial role in the control of mycosporine-aspartate synthesis process. Irradiation with UV-B at intensity of 5 $\mu mol\ m^{-2}\ s^{-1}$ enhanced production of studied metabolite. After 7 weeks of cultivation under these conditions its content in the tips and stems parts of podetia increased more than 7-fold and almost 6-fold, respectively. PAR radiation was also one of the conditions determining the synthesis of mycosporine-aspartate in *C. arbuscula*, but the kinetics of this process depended on the applied intensity. The highest increase of the metabolite content was observed after 49 days of PAR irradiation at intensity of 1000 $\mu mol\ m^{-2}\ s^{-1}$. With the increase of this radiation intensity up to 1500 or 2000 $\mu mol\ m^{-2}\ s^{-1}$ the rate of mycosporine-aspartate production underwent a slowdown. In turn, dark or PAR irradiation conditions with simultaneous deprivation of CO_2 in the atmosphere surrounding *C. arbuscula*, as well as dark conditions coupled with the continued access of atmospheric CO_2 levels, did not induce any changes in the content of mycosporine-aspartate in the lichen thalli during its cultivation. Therefore, the results showed that the synthesis process of the analysed compound depended on the availability of light and the presence of CO_2.

To verify the application potential of mycosporine-aspartate its biological activity has been assessed. It has been shown that this compound is characterized by antioxidant properties providing protection against damage from reactive oxygen species generated during excessive UV exposure. In addition, mycosporine-aspartate has a high UV-protective activity expressed by efficient inhibition of UV-B and UV-A transmission. The results obtained from tests carried out on selected cell lines demonstrated also that this metabolite has no significant cytotoxic activity relative to normal cells, such as: human keratinocytes as well as Chinese hamster lung fibroblasts V-79 and ovary cells CHO. Moreover, it exerts the promoting effect on their proliferation. This compound is also highly effective anticancer agent due to the antiproliferative activity on melanoma tumour cell lines: human SKMEL and BLM, murine B-16 F10 and S-91, as well as on the human lung adenocarcinoma epithelial cell line A-549. Moreover, tested metabolite has a clear inhibitory effect on the

activity of enzymes, collagenase and elastase, that cause a degradation of collagen and elastin fibers in fibroblasts and lead to unfavourable, from our point of view, photoaging process.

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