Influence of environmental factors on the contents of free amino acids in *Fucus vesiculosus* in the Barents Sea during the day

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Abstract. Influence of main environmental factors (temperature, intensity of photosynthetically active radiation and tidal cycle) on the contents of free amino acids (FAAs) in *Fucus vesiculosus* in the Barents Sea during the day was investigated. An undular change in the content of FAAs occurs during the day, associated with the phases of the tidal cycle. Temperature and photosynthetically active radiation influenced the content of FAAs, but their relation probably is a nonlinear. The content of most of FAAs and their total amount reached the highest values at a minimum water level. The lowest values were determined when algae were in seawater. The difference in the content of individual FAAs and their common amounts averaged 1.5-3 times during the day. The reason for the changes in the content of FAAs is probably related to the metabolic processes and their switching during the transition of algae from an aquatic environment to an air and vice versa.

1 Introduction

All living organisms have rhythms of biological activity, which allow them to adapt to environmental factors, such as the change of day and night, the intensity of solar radiation, temperature, humidity, etc. Diurnal rhythms of photosynthesis and respiration intensity, water potential, nitrogen compounds content, enzymatic activity, gene expression, etc. were revealed in plants and algae [1, 2, 3, 4, 5, 6]. Higher plants have shown the presence of circadian rhythms of the content of free amino acids (FAAs): their concentration increases during the daytime [1, 2].

For organisms living in the intertidal zone of seas, one of the main environmental factors is the tidal cycle. Submersion of algae to seawater while high tide as well as emersion during low tide lead to changes the environment factors such as illumination, temperature, salinity, availability of minerals and nutrients, etc. Obviously, this affects their physiological processes and biochemical composition. Experimental studies on macroalgae have shown that the presence of algae in the air during low tide leads to a decrease in the intensity of photosynthesis, nitrate reductase activity, the content of inorganic nitrogen forms and an increase in the content of protein, pigments, polyamines and glutathione, the synthesis of protective proteins and enzymes involved in the biosynthesis of antioxidant compounds and

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amino acids [7, 8, 9, 10]. Desiccation of Ulva lactuca within 24 hours leads to an increase in the content of amino acids, metabolites of the enzymatic pathway and antioxidants in its cells [10].

It is likely that the eulittoral macroalgae Fucus vesiculosus will also have changes in metabolic activity and biochemical composition during the day. Therefore, the purpose of our investigation was to analyze (1) the daily dynamics of the content of FAAs in this species and (2) the influence of environmental factors (tidal cycle, photosynthetically active radiation (PAR) and temperature) on the content of FAAs. The data obtained probably will allow to evaluate the participation of FAAs in the adaptation of macroalgae to environmental conditions.

2 Materials and methods

Sapling was carried out during the polar day from July 14 to July 15, 2020 in the Zelenetskaya Bay of the Barents Sea (69° 07.1' N, 36° 04.6' E) every 2 hours. The object of the study was brown algae *Fucus vesiculosus* L. with 5-7 dichotomous branches. For the analysis of amino acids, 1.5 g of apical parts from 5-6 thalli were taken, which were fixed with 96% ethanol and stored in a dark and cool place until the studies had been carried out. While sampling, the environmental temperature (water or air, depending on the phase of the tidal cycle) was measured with a mercury thermometer (model TL-4, Russia, TU 25-2021.003-88), the intensity of PAR with a photometer LI-185A (LI-COR, Lambda Inst., Nebraska, USA), the height of the water with the measuring rail, the phase of the tidal cycle was determined visually.

Determination of the content and composition of FAAs in algal samples was carried out by capillary electrophoresis according to the previously described method [11]. The data (mg/g dry weight) are presented as averages of 4 biological replicas with an indication of the standard deviation. The dry matter content in algae (n = 30) was determined by the standard method [12].

To identify environmental factors that can influence the content of FAAs, a one-way analysis of variance (ANOVA) was performed. According to the results of the analysis of variance between the factors and the amino acid content, the presence of a linear correlation was determined using the Pearson correlation coefficient ($p<0.05$). Statistical data processing was carried out with Microsoft Excel 2010 (Microsoft Corporation, USA, 2010) and NCSS 11 Statistical software (LLC, USA, 2016).

3 Results

During the study period, a polar day was observed. But due to heavy cloud cover at night period, a significant change in PAR intensity was observed: on the daytime values reached 3500 $\mu Mol \times m^{-2} \times sec^{-1}$, and at night it decreased to 9 $\mu Mol \times m^{-2} \times sec^{-1}$. The environment temperature (water and air) varied from 7 to 19 °C: maximum was recorded during the day and minimum at night. The water level ranged from 1.4 to 3.4 m from sea level. The duration of the submergence of algae in seawater during high tide was about two hours. The rest time they stayed in the air (Figure 1A). The water content in the apical parts of *F. vesiculosus* varied within 71-86% of the wet weight.

Eighteen FAAs were identified in the algae samples, of which sixteen are proteinogenic (glutamic acid, aspartic acid, alanine, phenylalanine, serine, threonine, glycine, arginine, valine, lysine, isoleucine, leucine, proline, tyrosine, histidine, methionine), and two are non–proteinogenic (ornithine, taurine). The dominants were glutamic acid (53.7-61.2% of the total

amount), aspartic acid (17.7-28.2%), alanine (2.2-7.2%) and phenylalanine (2.3-5.3%). The relative content of the remaining amino acids did not exceed 2%.

During the day, the content of FAAs changed undularly with a period of 4-6 hours, with maximum changes and maximal content during the emersion of algae at low tide. Such dynamics was revealed for the total amount of FAAs, as well as for all the dominant amino acids (alanine, aspartic acid, glutamic acid, proline and phenylalanine), valine, glycine, leucine with isoleucine, methionine, tyrosine and threonine. An increase in the content of the remaining amino acids was recorded only at low tide in the morning (histidine, lysine, taurine) or it also happened during the high tide (arginine, ornithine, serine). Interestingly, at evening low tide (10 p.m.), the increase in proline content was more significant (3.8 times) than at morning low tide (only 1.8 times). During the day, the difference in the content of FAAs averaged from 1.5 to 3 times for different amino acids. The total content of FAAs ranged from 2.3 to 6.3 mg/g of dry weight (Figure 1B, C).

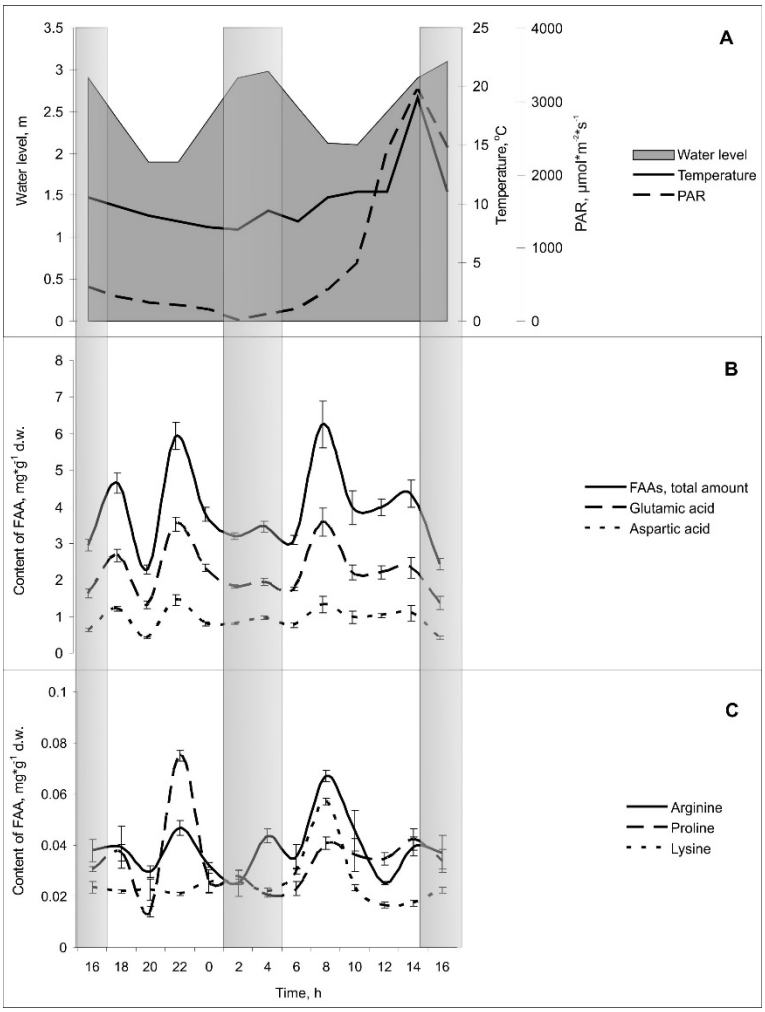

Fig. 1. Changes in environmental parameters (A), the content of total amount of FAAs and some individual FAAs (B, C) in F. vesiculosus during the period of investigation. Gray rectangular areas indicate the periods when algae were submerged during the high tide.

Significant fluctuations in the ambient temperature were recorded from 12 a.m. to 4 p.m. When the temperature increased by 7 °C for two hours, the content of most FAAs, as well as their total amount, did not significantly change. A significant increase of 1.4-1.5 times was found only for arginine, methionine, serine and threonine. While algae submerging and environmental temperature decreasing from 19 °C (air) to 11 °C (water), the total content of FAAs and dominant amino acids (glutamate, aspartate, alanine), as well as serine, tyrosine and threonine, significantly decreased. At the same time, the content of lysine and taurine increased. The contents of remaining FAAs did not change.

Analysis of variance (ANOVA) revealed that temperature, PAR and water level affect the content of almost all FAAs. But the calculated Pearson correlation coefficients showed the absence of linear dependencies.

4 Discussion

The obtained results revealed that changes in the content of FAAs are associated with a phases of the tidal cycle and the transition of algae from water to air and back. The highest content of the majority of FAAs was recorded at low tide, during algae were emerged. At high tide, while algae were submerged in seawater, there was a decrease in both the total content of FAAs and most of the individual amino acids. Probably, the transition of algae from the aquatic environment to the air and back leads to a change in the direction of metabolic processes: in the seawater, the synthesis and accumulation of carbohydrates occurs (glucose, sucrose), in the air – the synthesis and accumulation of compounds with an amino group (amino acids, proteins) [10]. The signal to start these processes is a change in the concentration of reactive oxygen species (ROS) in algae cells [7, 8, 10].

The accumulation of FAAs at low tide is most likely due to the accumulation of these compounds as an energy substrate and structural elements, as well as antioxidant compounds capable of neutralizing the formed ROS [8, 10, 13]. It was shown that during emersion and desiccation in low tide, the littoral green algal *Ulva prolifera* had a decrease in the content of inorganic forms of nitrogen in the thalli, which may be associated with their use for the synthesis of organic nitrogen-containing compounds [9]. The increase of the contents of FAAs can occur due to their synthesis, as well as due to their transporting from other parts of the thalli.

After 5 hours of emersion and subsequent submergence in seawater, a decrease in the content of almost all FAAs was revealed, which was also observed for *U. lactuca* [10]. A decrease in the content of FAAs during prolonged placement of algae on air may be associated with a decrease in the activity of enzymes involved in their synthesis, as well as with the use of amino acids as an energy source to maintain homeostasis, synthesis and restoration of the pool of antioxidants [9]. The submergence of algae in the aquatic environment after desiccation reduce the concentration of ROS, restore the intensity of photosynthesis and normalize the activity of enzymes in the cells [9, 10, 14, 15]. After submergence, algae can use FAAs for the regeneration of damaged structures, as well as for growth, synthesis of osmoprotectors and other compounds [10]. These processes can involve both newly synthesized amino acids and those obtained as a result of hydrolysis of damaged or unused proteins (enzymes of energetic or antioxidant metabolism, protective proteins) [8].

The high intensity of solar radiation and the temperature of the air during low tide will accelerate the loss of moisture and stimulate the formation of ROS in macroalgae cells [7, 10, 15]. The data obtained showed that the intensity of PAR influenced the accumulation of some FAAs. The increase in the content of alanine, arginine, threonine, leucine with isoleucine, valine, leucine, histidine and taurine was more significant at morning low tide in comparison with evening low tide. This, most likely, may be due to the influence of PAR intensity on the formation of precursors of amino acids (ammonium, pyruvate, oxaloacetate)

[2, 10]. An increase in temperature by 7 °C, which occurred at 2 p.m. during the low tide period, caused significant changes in the content of only 5 of the 18 identified amino acids (arginine, methionine, serine, taurine, threonine). Since high temperature has a negative effect on algae, changes in the content of individual FAAs may be the result of violations of various biochemical processes. The effect of elevated temperature did not last long, therefore, probably, there were no significant changes in the total content of FAAs. Changes in the content of FAAs after algae submerging in seawater can be associated with both the transition of algae from air into an aquatic environment and a decrease in temperature.

Interestingly, a significant increase in the content of proline occurred not at morning low tide, but at evening. This may be due to desalination caused by rain in the evening. As previously have shown, proline takes an active part in the processes of osmoregulation in algae [16, 17, 18], and a decrease in the salinity of seawater leads to an increase in the content of proline in cells of *F. vesiculosus* [19].

No linear relationships between other investigated environmental factors (temperature and PAR) and the content of individual FAAs in *F. vesiculosus* during the day was revealed. It is possible that these factors have a complex effect that has a non-linear character. Therefore, it was not possible to identify a certain factor that can have a strong effect on the content of amino acids.

5 Conclusion

In *F. vesiculosus*, an undular dynamic of changing of the content of FAAs occurs during the day, associated with the phases of the tidal cycle: the maximum content of most FAAs was detected during low tide, the minimum during high tide. The difference in the content of FAAs was 1.5-3 times. The dynamics in the content of FAAs during the tidal cycle is associated with a change in the direction of metabolic processes and their switching from the synthesis and accumulation of carbohydrates in the aquatic environment to the synthesis and accumulation of compounds with an amino group in the air. Identification a certain environmental factor that would have a clear directional effect on the content of FAAs in *F. vesiculosus* has failed.

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