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ACTIVITY OF ALKALINE PHOSPHATASE IN SOME SEAWEEDS UNDER THE INFLUENCE OF HYDROSTATIC PRESSURE AND TEMPERATURE

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Abstract

The combined effect of hydrostatic pressures varying from 200—800 atm and temperatures ranging from 5°—25°C was studied on the activity of alkaline phosphatase in *Delesseria sanguinea* and *Fucus vesiculosus*. At temperatures between 5° and 15°C the hydrostatic pressure caused a decrease in the enzymic activity, at higher temperatures however the activity was accelerated under the same pressure. The changes in the enzyme activity brought about by increased hydrostatic pressure at temperatures other than 15°C were greater in *D. sanguinea* as compared to *F. vesiculosus*. Enzmye activity in *Enteromorpha linza*, *Laminaria saccharina* and *Phycodrys sinuosa* could not be detected.

Introduction

Hydrostatic pressure and temperature are the most important abiotic factors in both the thermosphere and psychrosphere. They have a great bearing on the mode of life in pelagic as well as benthic environments of marine hydrosphere, which constitutes about two-third of the total biosphere. Informations regarding the enzyme activities of marine organisms under the influence of high hydrostatic pressure and temperature are very scanty (Hochachka, 1971; ZoBell & Kim, 1972; Shameel, 1975b). A few attempts have been made to study the effects of these ecological parameters on the activity of FDPase in abyssal fishes (Hochachka *et al.*, 1970, 1971a,b), pyrophosphatase in marine microbes (Morita & Mathemeier, 1964), myosin ATPase in benthic fishes (Dreizen & Kim, 1971), phosphatases in marine invertebrates (Napora, 1964; Ponat & Theede, 1973) and bacteria (Morita & Howe, 1957). There does not appear to be any report on the phsophatase activity of marine algae (Shameel, 1973b; Stewart, 1974).

In the present study the effect of hydrostatic pressure on the activity of alkaline phosphatase in *Delesseria sanguinea*, *Enteromorpha linza*, *Fucus vesiculosus*, *Laminaria saccharina* and *Phycodrys sinuosa* at different temperatures is reported. The selection of these seaweeds was made due to the interesting differences shown by some of them in the sensitivity of their thallus-weight (Shameel & Ohno, 1972), rates of respiration and photosynthesis (Shameel, 1973a) and the rate of growth (Shameel, 1975a) against hydrostatic pressure and temperature.

Materials and Methods

Enteromorpha linza (L.) J. Ag. and *Fucus vesiculosus* L., the intertidal seaweeds were collected from boulders and pebbles near Mö.tenort; *Delesseria sanguinea* (L.) Lamour., *Laminaria saccharina* (L.) Lamour. and *Phycodrys sinuosa* (Good et Woodw.) Kütz., the sublittoral macroalgae were dredged from a depth of 8—10 m near "Tonne C", Kieler Aussenfo.de (western Baltic Sea). These algae were determined after

Pankow (1971). The thalli were kept in double-filtered sea water (adjusted to 150/00 S) containing 420 mg $NaHCO_3$/l at 5°C for a few days (Shameel, 1975a). before conducting the experiments.

Pieces of algal thalli (50—100 gm) were minced and homogenized in Ultra Turrax for 1/2 min. The homogenate was centrifuged at 20,000 x g for 20 min, filtered through glass wool, and 5 ml of the supernatant thus obtained was added to 50 ml of 0.05 M glycin-NaOH buffer (pH 10.5) containing 5.5 x 10^{-3} M *p*-nitrophenyl-phosphate and 5 x 10^{-4} M $MgCl_2$. The enzyme solution was then filled in 8 PVC tubes of 6.5 ml capacity, half of the tubes served as control and others were subjected to high hydrostatic pressure. The details of the pressure apparatus and the techniques employed have been described previously (Shameel, 1973a). Five experimental series for 5°, 10°, 15°, 20° & 25°C under pressure levels of 200, 400, 600 & 800 atm were set up. The enzyme reaction was stopped after 1 hr in both the pressure treated as well as control tubes by the addition of 10 ml of 0.02 N NaOH/ml enzyme solution. The activity of alkaline phosphatase was measured following the break down of *p*-nitrophenyl-phosphate at 400 nm on Zeiss PMQ II spectrophotometer as described by Morita & Howe (1957). Merck test rate specifications of No. 3304 were used.

For every temperature-pressure combination 8 independent experiments were conducted, the mean value of enzyme activity obtained at one temperature and hydrostatic pressure was compared with the values measured at the same temperature and atmospheric pressure set at 100. The relative enzyme activity was thus expressed as "percent of control".

Results and Discussion

The relative activities of alkaline phosphatese obtained from *Delesseria sanguinea* and *Fucus vesiculosus* indicate that the hydrostatic pressure and temperature have antagonistic effects (fig. 1). With the increasing pressure at 15°C the seaweeds exhibited a very little change in their enzyme activity. A temperature of 15°C appears to be optimal as it just balances the pressure action. The pressure could demonstrate its action only slightly at 800 atm. At temperatures lower than 15°C the increasing pressure decelerated the activity of alkaline phosphatase progressively, while at higher temperatures i.e. 20° and 25°C the enzyme activity was accelerated by step-wise increase of hydrostatic pressure from 200 to 800 atm. A gradual decrease in the pressure action with the rise of temperatures from 5° to 30°C is known to affect the rates of respiration and photosynthesis in *D. sanguinea* and *F. vesiculosus* (Shameel, 1973a). In *Ulva lobata* the temperature increase appeared to counterbalance the inhibitory effect of pressure rise, and hydrostatic pressure tended to antagonize high temperature inhibition of steady rates of photosynthesis (Vidaver, 1972). *D. sanguinea* exhibited a gradual decrease in the pressure induced action on the cell viability of thin leafy fronds (Shameel, 1973a) as well as on the rate of growth of young adventitious leaflets with the temperature increasing from 5° to 22°C (Shameel, 1975)a. Studies on alkaline phosphatase activity in gill homogenates of marine bivalves, *Cyprina islandica* and *Mytilus edulis* also indicated that pressure has a decreasing effect at low temperatures and an increasing effect at high temperatures (Ponat & Theede, 1973). Similar results were obtained on aspartase from *Escherichia coli* (Haight & Morita, 1962) and malate dehydrogenase from *Bacillus stearothermophilus* (Morita & Mathemeier, 1964).

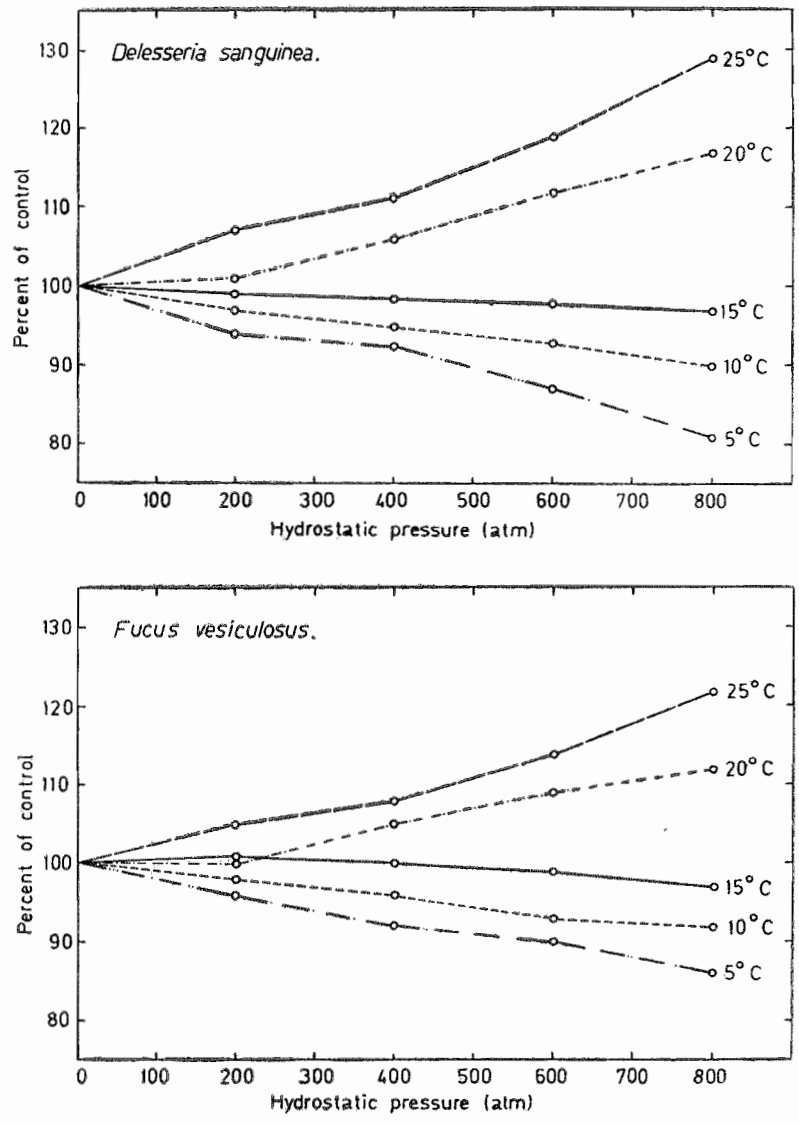

Fig. 1. Relative activity of alkaline phosphatase (in percent of control) from *Delesseria sanguinea* (L.) Lamour and *Fucus Vesiculosus* L. as affected by high hydrostatic pressure at various temperatures.

The observed results could be explained on the basis of the theory of absolute reaction rates (Johnson & Eyring, 1970). Hydrostatic pressure and temperature

affect on one hand the speed of the reaction catalyzed by an enzyme and on the other influence the balance between active and inactive conformations of the enzyme. During the formation of enzyme-substrate-complex the enzyme is to undergo unfolding to accept the substrate, which involves a positive $\triangle V$ i.e. volume increase. When the volume of this activated ES-complex is greater than that of the reaction partners i.e. positive $\triangle V$, pressure will decrease the speed of the reaction; but when its volume is smaller than the volumes of the individual reaction partners i.e. negative $\triangle V$, pressure promotes the reaction. Increase in temperatures accelerates the rate of enzyme activity, when the activated ES-complex produces a more energy rich configuration than that of its individual components. At low temperatures the condition gets reverse and thus the pressure action becomes acute.

It is interesting to observe that the acceleration or deceleration in the activity of alkaline phosphatase caused by increasing pressure at temperatures other than 15°C was comparatively greater in *D. sanguinea* than in *F. vesiculosus* (fig. 1). It has also been observed previously that the effect of hydrostatic pressure on the rates of respiration and of photosynthesis are more influenced by increase in temperatures in *D. sanguinea* than in *F. vesiculosus*, which appears to be baroduric (Shameel, 1973a, b). This is prabably due to the fact that *F. vesiculosus* is a littoral, eurythermal and shallow-water seaweed, while *D. sanguinea* is a sublittoral, cold stenothermal and deep-water alga. As *F. vesiculosus* experiences direct sunlight, dessication and high temperature variations during long periods of exposure, it has possibly developed the adaptive control mechanisms which protect its enzymes from thermal denaturation. The enzyme-substrate affinities often decrease as thermal kinetic energy increases, and, therefore, the temperature induced changes in the reaction velocity are compensated at low substrate concentrations. The adaptive mechanisms against pressure and temperature at enzymatic level have been studied by following the activity of phosphofructokinase and FDPase in off-shore benthic organisms (Hochachka, 1971).

It is probable that regulatory control systems are present in *F. vesiculosus* which enable it to counter balance the danger of metabolic disturbances, as this resistant alga has to maintain changes in the enzyme activity induced by hydrostatic pressure within a limit (fig. 1). The functional solution for that is to elaborate those enzymes whose affinities for key metabolites are independent of pressure. It was similarly expected that *Porphyra perforata*, an intertidal alga may posses regulatory rate control systems which serve to keep the pressure induced responses in its photosynthetic reactions within certain limits despite temperature extremes (Vidaver, 1972). The pressure sensitivities of enzyme catalysis and control of catalysis at physiological substrate concentrations appear to be held at a minimum.

All attempts to study the activity of alkaline phosphatase in *Enteromorpha linza*, *Laminaria saccharina* and *Phycodrys sinuosa* failed. It appears that the alkaline phosphatase assay method used in this investigation is not suitable for all macroalgae and is to be modified for other seaweeds.

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