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Thermoresistance and electrophoretic spectrum of glucose-6-phosphate dehydrogenase in *Amoeba proteus* after thermal acclimation

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Summary

The cultivation of amoebae (*Amoeba proteus*) of Da strain at 28°C – the upper border of the temperature tolerance range of this strain – does not bring about any changes in thermoresistance of its glucose-6-phosphate dehydrogenase (G6PD), the chief enzyme of pentose phosphate shunt, but alters the electrophoretic pattern of the enzyme. A statistically significant increase in G6PD thermoresistance was observed after the amoebae were returned from 28 back to 25°C. It may be associated with epigenetic variability, a special variability type (Yudin, 1982). The temperature of 28°C might presumably be considered as one of the destabilizing factors (together with some antibiotics, X-rays, micrurgical procedures) which cause the expression of newly inherited phenotypes in amoebae.

Key words: amoebae, *Amoeba proteus*, thermoresistance, glucose-6-phosphate dehydrogenase

Introduction

Temperature is one of the most important and thoroughly investigated environmental factors affecting vital functions of an organism. Biochemical systems of organisms, especially those of ectotherms, usually respond to temperature alterations by both modifications and genetic adaptations. The enzyme systems ensure the necessary metabolism intensity during temperature changes. Enzyme activity is a convenient marker for experimental measurements and consequently special attention in this field was traditionally paid to the enzymes behaviour. Thermoresistance (thermostability) of enzymes has often been used to characterize adaptative biochemical events (see: Alexandrov, 1975, 1985; Ushakov, 1989).

In general, the data concerning evolutional (genetic) adaptation of ectotherm organisms, including unicellular ones, show a positive correlation between the thermoresistance of enzymes investigated and thermophility (the thermal optima of habitation or multiplication) of species (Janovy, 1972) or intraspecies groups (Lozina-Lozinsky, 1961; Sopina and Podlipaeva, 1984; Podlipaeva, 1992; Podlipaeva and Yudin, 2001). As for the modifications (physiological acclimation) in Protozoa, it was a priori expected that ''cold'' acclimation must cause decreasing, and the ''warm'' one, increasing of thermoresistance of this or

that enzyme. However, almost no common rules were revealed in the behaviour of enzymes activity and thermoresistance during the thermal acclimation of one species or clone, either warm or cold (Seravin et al., 1965; Kovaleva, 1968; Berezina, 1970; Sopina, 1987, 1991, 1997).

Earlier it was shown that the cold acclimation of free living freshwater amoebae *Amoeba proteus* within the temperature tolerance range (TTR) of the studied strain (clone) Da resulted in a reliable increase of the activity and thermoresistance of pentose phosphate shunt enzyme glucose-6-phosphate dehydrogenase (G6PD). It was also shown that the electrophoretic patterns of this enzyme were the same at both temperatures: 25 (cultivation temperature) and 10°C (temperature of acclimation, the lowest border for the clone Da TTR) (Podlipaeva, 1994; Podlipaeva and Yudin, 2001). The same results were obtained for the electrophoretic spectrum of tritonesoluble G6PD of another amoeba clone B (Sopina, 1991).

The upper border of strain Da TTR (28°C) was used in this study to examine the G6PD behaviour in the process of warm physiological acclimation of the amoebae.

Material and methods

Da strain (clone) of *Amoeba proteus* from the amoebae culture collection of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology, Russian Academy of Sciences, were used in this study. The amoebae were cultivated at 25°C according to the method of Prescott and Carrier (1964) and fed with *Tetrahymena pyriformis* GL every 48 hr (Yudin, 1990). Experimental amoebae were acclimated to 28°C during no less than 30 days. Some of the cultures were then brought back to 25°C and tested. The changes caused by thermal acclimation were registered in mass culture without recloning of amoebae after the treatment.

For enzymatic assays, cells from a mass culture after 72 hr of starvation were precipitated in a low speed centrifuge and then homogenized with a teflon pestle in a glass homogenizer. To determine the activity and thermoresistance of G6PD, homogenates were immediately centrifuged at 12,000 rpm and 4°C for 30 min. The supernatant fraction was used for further assays. Protein content in the supernatants was determined by the Lowry method (Lowry et al., 1951).

The thermoresistance of water soluble G6PD was evaluated as residual enzyme activity after experimental heating of the supernatant samples at temperatures 39, 42, 45 and 48°C for 10 min. Thermoresistance was expressed as a percentage of enzyme activity in the unheated control samples. The activity of water soluble G6PD was measured with a spectrophotometer (Specol-211) at 340 nm by the rate of NADP reduction and expressed in nM of NADP•H per 1 min per 1 mg of protein. The reaction mixture was adjusted experimentally (Podlipaeva, 1992). No less than three experiments with no less than three measurements at each point were carried out. We did not specially study the absolute values of specific G6PD activity and used these values only for obtaining relative ones (thermoresistance). Everywhere, speaking about ''reliable'' difference between average values we mean statistically significant difference at the 95% level of significance. Statistical data were processed as described earlier (Podlipaeva and Yudin, 2001). EXCEL was used for plotting.

The electrophoresis of water-soluble G6PD was carried out in slabs of 7% PAAG (90 x 120 x 1.5 mm). The unheated control sample and the samples heated at various temperatures were placed at the start of the same gel as described previously (Podlipaeva, 1992). After electrophoresis, gels were incubated for 30 min at 37°C in standard reaction mixture (Serov et al., 1977) for the detection of G6PD activity, then fixed with 7.5% acetic acid and scanned on MD-100 microdensitometer (Carl Zeiss - Jena). The peaks on densitograms were numbered in order of decreasing electrophoretic mobility. This approach provided an opportunity to evaluate the relative thermoresistance of various electrophoretic forms of G6PD.

Results

Amoebae of Da strain were cultivated at 28°C during 30 days. Then the thermoresistance of G6PD was determined as a residual enzyme activity after experimental heating at four test temperatures (39, 42, 45 and 48°C) and expressed as a percentage of enzyme activity in the unheated control samples. The average experimental values of G6PD thermoresistance of such amoebae did not differ from those of amoebae cultivated at 25°C (Fig.1).

The analysis of G6PD electrophoretic spectrum of the amoebae cultivated at 28°C showed that the number of minor fractions of the enzyme decreased to 1 (Fig. 2, B) in comparison with their number (3) in amoebae cultivated at 25°C (Fig. 2, A). We did not reveal the full set of electrophoretic fractions (one main and three minor ones) in the amoebae, cultivated at 28°C. The main loss of the G6PD activity after experimental heating is due to inactivation of the main fraction (Fig. 2, B; 42, 45, 48°C). As for the minor fraction, its peak height even slightly increases after the experimental heating similarly to the case of amoebae cultivated at 25°C. The reasons for this increase were

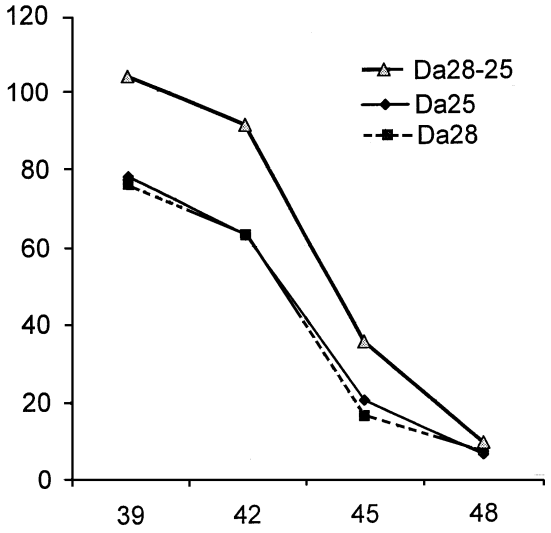

Fig. 1. Thermoresistance of water-soluble G6PDH of Da strain of *Amoeba proteus* cultured at 25 and 28°C. Abscissa – test temperature, °C (time of heating - 10 min); ordinate – enzymatic activity, % of unheated control; every point is the average of 9-12 measurements.

discussed in previous articles and may be associated with tetrameric-dimeric-monomeric interactions of G6PD subunits after heating (Podlipaeva, 1992; Podlipaeva and Yudin, 2001).

Surprisingly, when the amoebae were returned from 28 to 25°C, the values of G6PD thermoresistance became different both from those of amoebae constantly cultivated at 25°C and from those of amoebae cultivated at 28°C. Even after three months of cultivation of amoebae from 28°C at 25°C (further we designate them as ''28>25 amoebae''), their G6PD thermoresistance after the experimental heating at three of four test temperatures (39, 42 and 45°C) was reliably higher than that of amoebae constantly cultivated at 25°C (Fig. 1). The temperature of 28°C seems to somehow affect the amoebae of Da strain, and, as a result, the G6PD thermoresistance increases

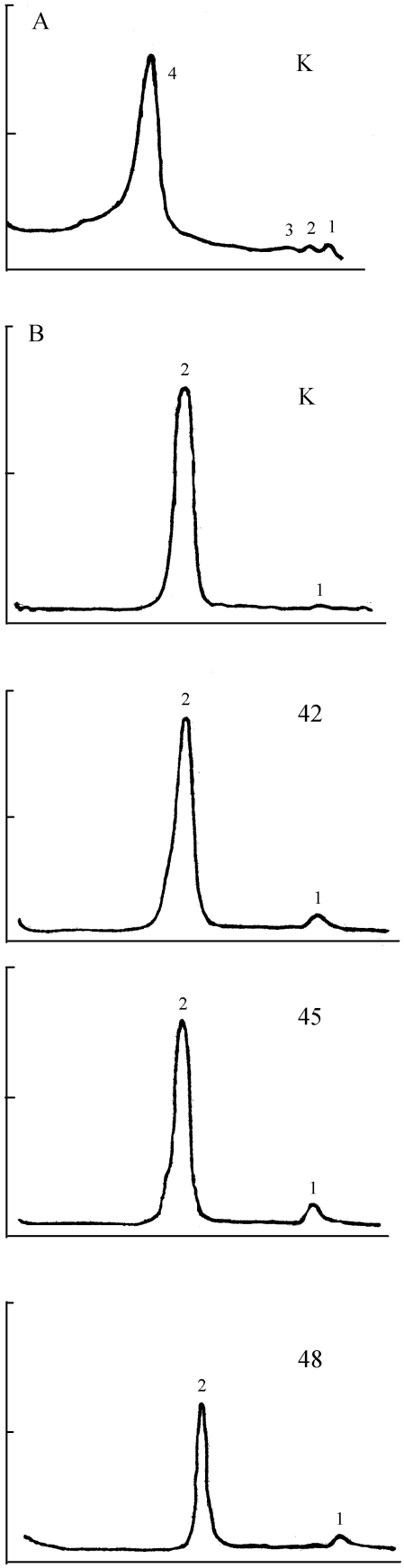

Fig. 2. Densitograms of water-soluble G6PDH electrophoretic fractions of Da strain of *Amoeba proteus* after the electrophoresis of unheated (control) and heated samples in 7% PAAG. A – amoebae cultured at 25°C; B - amoebae cultured at 28°C. Abscissa – the distance from the start of the gel, arb. units; ordinate – optical density, % of total absorption; K – control unheated sample; 42, 45, 48 - samples heated at 42, 45 и 48°C (time of heating - 10 min) respectively; 1 – 3 minor fractions, 4 (2) - major fraction, at the start of every gel - 140 mg of protein.

in ''28>25 amoebae'' in such a way that the temperature of 39°C does not bring about thermal inactivation of the enzyme. This characteristic is obviously inherited, as at 25°C amoebae undoubtedly multiply.

Thus, it seems probable that amoebae of Da strain, cultivated at 28°C for no less than a month and then returned to 25°C, acquire several new characters. These characters are inherited; their emergence results from the influence of 28°C, this influence being displayed in the process of cultivation at 25°C.

Discussion

We consider the temperature of 28°C to be an upper border of the Da strain TTR because the multiplication rate of amoebae cultivated at this temperature is comparable with the optimum (22°C), but the efficiency of cloning is lower because of increasing number of dying amoebae and subclones (Sopina, 1986). The lower border of TTR of the strain Da is 10°C. At this temperature the amoebae may survive for a long time but do not multiply. So, both temperatures - 10 and 28°C - produce some damaging effect on amoebae of the strain Da.

There are no reasons to consider the reliable increase of their G6PD thermoresistance in the course of cold acclimation as inherited, since after the returning of the Da strain amoebae from 10 back to 25°C thermoresistance of G6PD has reached the initial level of ''25°C''-amoebae (Podlipaeva and Yudin, 2001). As for the upper TTR border of the strain, the temperature 28°C itself did not cause reliable changes in the G6PD thermoresistance when Da amoebae were cultivated at 28°C.

Thermoresistance of G6PD in ''28>25 amoebae'' demonstrates a different behaviour. At first glance, its increase resembles the "temperature hardening", i.e., the increase of thermoresistance of animal or plant cells or thermoresistance of some cell functions after exposure to superoptimal temperatures (Alexandrov, 1975). However, in the case when the heating at 39°C did not cause a decrease of G6PD activity, we can not speak about hardening in the strict sense of the term according to V.Ya. Alexandrov, since the scheme of character testing applied in our work was different from the one that is necessary to reveal the hardening phenomenon.

The temperature of 28°C causes the changes in G6PD electrophoretic spectrum of the acclimated amoebae. Enzyme electrophoretic spectrum alterations caused by environmental temperature conditions can be illustrated on the example of total tritonesoluble esterases in amoebae of B strain, cultivated at 25 and 10°C. It was shown that when α- and β-naphtilacetate were used as substrates, the electromorphs №№ 7 and 10 were absent in amoebae from 25 and №№ 15 and 16, in amoebae from 10°C (Sopina, 1997). Spontaneous disappearance of minor electrophoretic form of tritonesoluble G6PD was observed in A and C strains of *Amoeba proteus* (Sopina, 1989). The absence of minor G6PD electrophoretic forms was also reported in the clones, derived from the intrastrain transplantants of various types, and nevertheless they were present in the initial amoebae strains. It was shown that disappearance of these G6PD fractions might be induced by factors associated with the micrurgic procedure itself (Sopina and Yudin, 1993). Noteworthy, in both above cases tritonesoluble G6PD fractions were revealed with the method of microelectrophoresis with NADP in the cathode buffer and usually contained in the control samples 2 fractions of G6PD, a major and a minor one, whereas the samples of watersoluble G6PD of amoebae, analyzed by electrophoresis without NADP in the cathode buffer, contained one major and three minor fractions (Sopina and Podlipaeva, 1989)

The emergence of an inherited phenotype of amoebae differing from the initial one after the culture has been exposed to the influence of some factor might be associated with a special type of inherited variability, described by A.L. Yudin (1982). It was observed in clones originating from heterocaryons, as a result of genetic interactions of nuclei in heterocaryotic cells, and also in amoebae cells after the so-called destabilizing treatment, e.g., by some antibiotics, by RNA-ase, by micrurgy and by X-rays. Variability like this, demonstrating simultaneously the features of pheno- and genotypic ones, is presumably epigenetic (Yudin, 1982). The phenomenon of long-term modifications, described for protozoans (Poljansky, 1973) and still not fully explained, must be also taken into account.

It seems that the superoptimal temperature of 28°C – the upper TTR border of Da strain amoebae – is one of the destabilizing factors that may cause the epigenetic changes in amoebae of this strain. These changes include the disappearance of two (out of three) minor electrophoretic fractions of watersoluble G6PD, revealed by the electrophoresis without NADP in the cathode buffer and a higher thermoresistance of this enzyme after the transfer of amoebae from 28 back to 25°C.

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