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Activity and thermoresistance of some *Amoeba proteus* enzymes with special reference to thermal adaptation of the amoebae

Yulia I. Podlipaeva and Alexander L. Yudin

Laboratory of Cytology of Unicellular Organisms, Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia

Summary

Activity and thermoresistance of the *Amoeba proteus* enzymes – thermostable water-soluble esterases and thermolabile water-soluble glucose-6-phosphatede hydrogenase (G6PDH) – were studied in two amoebae strains (clones) which differed in their optimal temperature of multiplication (thermophility). In one of the clones, these characters were followed during its acclimation to relatively low temperature 10°C within the temperature tolerant range of the clone. It was shown that specific activity and thermoresistance of both enzymes could be regarded as strain-specific characteristics. Positive correlation between the thermoresistance of both enzymes and the thermophility of the amoeba clone was demonstrated, representing an example of genetic temperature adaptation at the intraspecific level. No changes in thermoresistance of esterases of the two clones were revealed during the "cold" amoeba acclimation, whereas the activity and thermoresistance of G6PDH in cold-acclimated amoebae was increased. Electrophoretic spectra of G6PDH were identical in both amoebae clones and at both temperatures studied. Minor fractions of the enzyme seemed to be more thermoresistant than the major one. The higher level of G6PDH activity is presumed to be connected with activation of the antioxidant protective system. The increase of G6PDH thermoresistance at lower temperature supports the suggestion that any temperature which disturbs the metabolic homeostasis of the organism, may result in changes of enzyme thermoresistance which do not necessarily coincide with the same direction as the temperature changes.

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

Introduction

Adaptation is one of the fundamental aspects of evolution and the subject under research by various biological disciplines including molecular and cell biology. Among numerous environmental factors affecting the vital functions of an organism, temperature is one of the most important and thoroughly investigated.

Ectothermal organisms have various biochemical devices to minimize the damage caused by temperature alterations (Hochachka and Somero, 1984). Traditionally, special attention in this field is paid to proteins and to enzymes first of all, because the enzyme systems ensure the necessary metabolism intensity during the temperature changes. In addition, the enzyme activity is a convenient marker for experimental measurement. Thermostability (thermoresistance) of enzymes has often been used to characterize the adaptative biochemical events (Alexandrov, 1985; Ushakov, 1989). Biochemical systems of organisms usually respond to alterations in ambient temperature conditions by both modifications and genetic variations. The latter are connected with the prolonged processes of the species evolution.

Protozoans are ectotherms which combine the features of the cell and the whole organism in "one"; therefore, the peculiarities of their thermal adaptations are of special interest (Sukhanova, 1968; Poljansky, 1973). Thermoresistance of protozoans was studied in terms of comparative biochemistry – in various species (clones, populations), as well as in the representatives of the same taxon during their thermal acclimation. In comparison with multicellular organisms, data concerning the enzyme thermostability in Protozoa are rather scarce. In general, these data show a positive correlation between the thermostability of investigated enzymes and thermophility (the thermal optima of habitation or multiplication) of species

(Janovy, 1972) or intraspecies groups (Lozina-Lozinsky, 1961; Sopina and Podlipaeva, 1984; Sopina, 1986; Podlipaeva, 1992, 1997). No common rules were revealed in the behaviour of enzyme activity and thermoresistance during the thermal acclimation of one species or clone (Seravin et al., 1965; Kovaleva, 1968; Berezina, 1970; Sopina, 1987, 1991, 1997). Nevertheless, the changes in enzymes thermostability of protozoans are usually believed to occur in the same direction as the environmental or cultivation temperature changes do, though the amount of data supporting such an observation are somewhat sparse. It is quite probable that such an opinion resulted from the unwarranted *a priori* extrapolation of the laws of genetic adaptation to the process of physiological acclimation.

Free-living freshwater amoebae, that lack sexual processes in their life-cycle, may serve as a good model for investigating the temperature adaptations. Their culture represent the cell and organism population simultaneously. The absence of a sexual process allows the ready separation of physiological modifications caused by temperature changes from genetic variations.

In this work, we have studied the activity and thermoresistance of *Amoeba proteus* enzymes from different thermostability groups: thermostable esterases and thermolabile glucose-6-phosphate dehydrogenase (G6PDH) were studied in two amoeba clones differing by their temperature optima of multiplication (TOM); in one of the clones, these characteristics were followed during its acclimation to lower temperature within the temperature tolerant range of the clone. These findings are summarized in relation to the results of previous studies (Sopina and Podlipaeva, 1984; Podlipaeva, 1992, 1994, 1997).

Material and Methods

The **B**, **Da** and **Petrozavodsk** strains (clones) of *Amoeba proteus* from the amoeba culture collection maintained in the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology, Russian Academy of Sciences, were used in this study. The amoebae were cultivated according to the method of Prescott and Carrier (1964), and fed with *Tetrahymena pyriformis* GL every 48 hr. (Yudin, 1990). The **B**, **Da** and **Petrozavodsk** clones were cultured at 25 and 10 °C. The temperature optimum of multiplication (TOM) for clone **Da** is 22°C (Sopina, 1986), for clone **B** TOM lies within the range 25–28 °C (Sopina, 1976), and for clone **Petrozavodsk** it was not determined.

For enzymes assays, cells from a mass culture after 72 hr. of starvation were precipitated with a low speed centrifuge and then homogenized with a teflon pestle in a glass homogenizer. To determine the activity and thermoresistance of esterases, homogenates were stored in the refrigerator (4°C) for 12 hr. and then centrifuged at 33000 rpm and 4°C during 1 h. In the case of G6PDH, homogenates were immediately centrifuged at 12000 rpm and 4°C during 30 min. In both cases, the supernatant fractions were used for further assays. The protein content in the supernatants was determined by the Lowry method (Lowry et al., 1951).

The activity of water soluble esterases was determined colorimetrically according to a modified Gomori method (Gomori, 1952; Pravdina, 1970; Sopina and Podlipaeva, 1984) and expressed as mg of β-naphtol produced after 20 min incubation at 37°C (AU, arb. units). The activity of water soluble G6PDH 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 (U, units). The reaction mixture was adjusted experimentally (Podlipaeva, 1992).

The thermoresistance of water soluble esterases was evaluated as residual enzyme activity after experimental heating of the supernatant samples at 45, 50, 55 and 60°C for 30 min, and that of water soluble G6PDH, after the heating of samples at temperatures 39, 42, 45 and 48°C for 10 min. Thereafter, the thermoresistance of both enzymes was expressed as a percentage of enzyme activity in the unheated control samples. Thermoresistance was represented in this way only in tables and plots; for statistical data handling, it was expressed as a "decrease of enzymatic activity" (difference between the enzyme activity in unheated control samples and in samples after experimental heating), to avoid operating with the relative values (Zaidel, 1985). The SYSTAT program was used to evaluate differences between the means of enzyme activity, as well as of thermoresistance, at the 95% level of significance. The program EXCEL was applied for plotting.

The electrophoresis of water-soluble G6PDH was carried out in slabs of 7% PAA gel (90 x 120 x 1.5 mm). The unheated control sample together with the samples heated at various temperatures were placed at the start of the same gel as previously described (Podlipaeva, 1992). This approach provided the opportunity to evaluate the thermoresistance of various electrophoretic forms of G6PDH. 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.

Results

Activity and thermoresistance of water-soluble esterases and G6PDH in two amoebae strains cultured at the same temperature 25 °C.

There are numerous studies on the activity and thermoresistance of esterases from closely related animal species living at different temperature conditions; much less data exist concerning such enzyme characteristics in different subspecies or populations of the same species and extremely little is known about intraspecies differences in activity and thermoresistance of esterases in the various strains (clones) of agamic Protozoa, these strains having different temperature optima for their multiplication. The measurements of the specific esterase activity in two amoeba strains (**B** and **Da**) showed that the difference between the average levels of the esterase activity in **B** (87.5±2.7 AU; n=16) and **Da** (78.1±2.0 AU; n=12) amoebae is statistically significant. Thus this value may be interpreted as a strain-specific biochemical characteristic. The same interpretation may also be applied to the activity of G6PDH which is 33.1±0.9 U (n=10) in the **B**-strain amoebae and 40.1±0.8 U (n=10) in the **Da**-strain amoebae, the difference being statistically significant. It should also be noted that K_m for the substrate (G6P) is lower in the **Da**-strain amoebae (5.7×10^{-6}M/ml) than in **B**-amoebae (7.5×10^{-6}M/ml) (Fig. 1).

Fig. 2 shows the dependence of the residual activity of esterases of both amoeba strains on the test-temperature. The water soluble esterases of **B**-strain amoebae are more thermoresistant than those of **Da**-strain amoebae. The average time for 50% esterase inactivation was also determined for these two strains after experimental heating for 10 – 60 min at 50 (Fig. 3a) and 55°C (Fig. 3b). It appeared to be about twice as large in **B**-strain amoebae as in amoe-

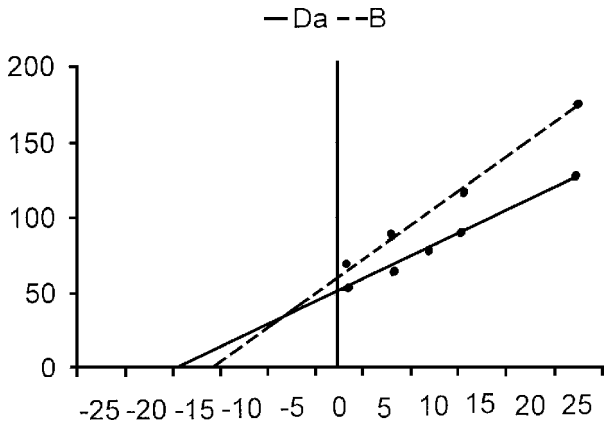

Fig. 1. Determination of the Michaelis constant (K_m) for substrate G6P according to the Lineweer-Berk equation in **B** and **Da**-strain amoebae. *Abscissa* – 1/G6P concentration [$(mM/ml)^{-1}$]; *ordinate* – 1/reaction velocity (optical density per 1 mg of protein per 1 min). Constants determined from the plot are: $-1/K_{mDa}= -1/17.3$; $K_{mDa}=5.7\times10^{-6}$M/ml; $-1/K_{mB}=-1/13.3$; $K_{mB}=7.5\times10^{-6}$M/ml.

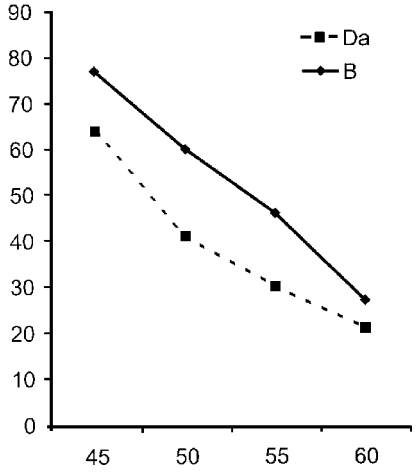

Fig. 2. Thermoresistance of water-soluble esterases of **B** and **Da**-strain amoebae cultured at 25°C. *Abscissa* – test temperature, °C (time of heating – 30 min); *ordinate* – enzymatic activity, % of unheated control; every point is the average of 10–12 measurements.

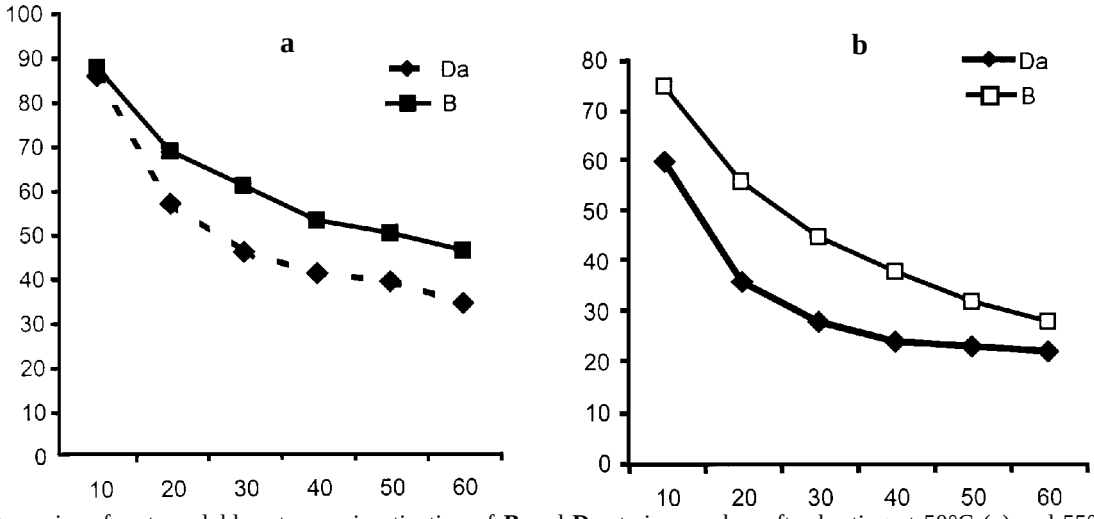

Fig. 3. Dynamics of water-soluble esterases inactivation of **B** and **Da**-strain amoebae after heating at 50°C (**a**) and 55°C (**b**). *Abscissa* – time of heating, min; *ordinate* – enzymatic activity, % of unheated control; every point is the average of 2–4 measurements.

bae of **Da** strain (Fig. 3). Thus, thermoresistance of water-soluble esterases determined by these different methods is higher in amoebae of the more thermophilic **B** strain.

Water-soluble G6PDH is much more thermolabile in comparison with esterases. When measuring the residual activity of this enzyme, temperatures in the range 39–48°C were used for experimental heating (see Fig. 4). The G6PDH thermoresistance of **B**-amoebae, after heating at most test temperatures, appeared higher than that of **Da** amoebae, both clones being cultured at the same temperature. The thermoresistance of G6PDH in the investigated clones does not correlate with the activity of the enzyme in the unheated control.

The electrophoretic patterns of water-soluble G6PDH of **B** and **Da** amoebae do not differ from each other and consist of similar set of fractions — namely, one major (Figs 5a and 5b; n 4) and three minor ones (Figs 5a and 5b; nn 1, 2, 3). In amoebae of both strains cultured at

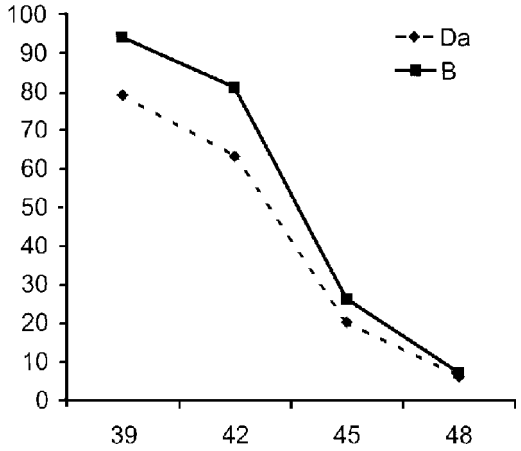

Fig. 4. Thermoresistance of water-soluble G6PDH of **B** and **Da**-strain amoebae cultured at 25°C. *Abscissa* – test temperature, °C (time of heating – 10 min); *ordinate* – enzymatic activity, % of unheated control; every point is the average of 9–10 measurements.

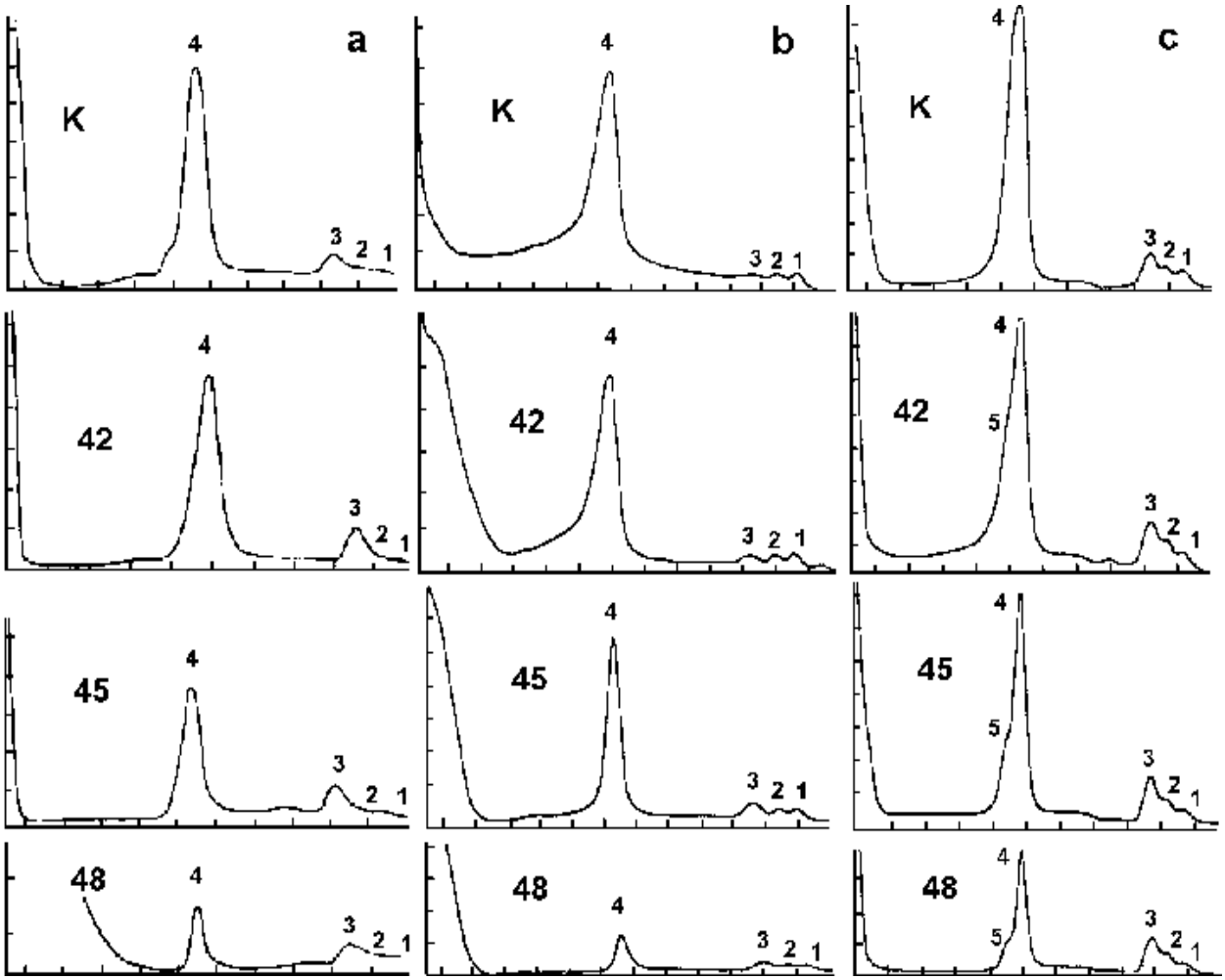

Fig 5. Densitograms of water-soluble G6PDH electrophoretic fractions of **B** and **Da**-strain amoebae after the electrophoresis of unheated (control) and heated samples in 7% PAAG. **a** – strain **B**-amoebae cultured at 25°C, **b** – strain **Da**-amoebae cultured at 25°C, **c** – strain **Da**-amoebae cultured at 10°C. *Abscissa* – the distance from the start of the gel, arb. units; *ordinate* – optical density, % of total absorption; **c** – control unheated sample; 42, 45, 48 – samples heated at 42, 45 and 48°C (time of heating – 10 min) respectively; 1 – 3 minor fractions, 4 – major fraction, 5 – subfraction; at the start of every gel – 150 μg of protein.

25°C, loss in G6PDH activity after experimental heating mostly occurs due to inactivation of the major electrophoretic fraction, while the minor fractions seem to remain quite intact. Therefore, after experimental heating, total enzyme activity is redistributed between the major and the minor fractions in favour of the latter ones (Table 1). In control samples, the share of the minor, presumably more thermoresistant fractions in the total G6PDH activity is higher in the **B**-strain, than in **Da**-strain amoebae (Table 1); this fact could account for higher thermoresistance of water soluble G6PDH in **B**-strain amoebae.

The interstrain differences in the G6PDH thermoresistance show the same trend as those in water soluble esterases of the same clones, the thermoresistance of both enzymes – thermostable and thermolabile – being positively correlated with the temperature optimum of multiplication (thermophility) of the amoeba clone.

Activity and thermoresistance of esterases and G6PDH after the amoebae acclimation to relatively low temperature 10°C.

B and **Da** amoebae were acclimated for 10°C for 14 days. Then the specific activity of their water soluble esterases was measured with the following results:

B 25°C	**B 10°C**	**Da 25°C**	**Da 10°C**
87.5±2.7	80.7±2.4	78.1±2.0	110.1±3.9

The difference in the average value of esterase activity at 25 and 10°C was statistically significant (Tuky-test: $p << 0.01$) only in the **Da** amoebae, and in **B** amoebae it was unreliable ($p = 0.062$). Table 2 shows the thermoresistance of esterases in both strains cultured at different temperatures.

All differences in thermoresistance of water soluble esterases between the amoebae strains cultivated at 25 and 10°°C were statistically insignificant, with one exception, namely the **Da**-amoebae samples heated at 60°C Amoebae from 25 and 10°C also did not differ from each other by the average time of 50% inactivation of their esterases (Table 3). Thus no difference in the thermoresistance of water-soluble esterases between amoebae acclimated to 25 and 10°C was revealed.

When studying the activity and thermoresistance of G6PDH during "cold" acclimation, we increased the time of acclimation up to 30 days. As it was not possible to obtain sufficient material for biochemical research when cultivating **B** amoebae at 10°C, amoebae of **Da** and **Petrozavodsk** strains were used to determine the activity of G6PDH at both temperatures of cultivation. The specific G6PDH activity of both strains at 10°C was significantly higher than that at 25°C.

Da 25°C	**Da 10°C**	**Petrozavodsk 25°C**	**Petrozavodsk 10°C**
45.6±0.8	55.6±1.4	35.2±1.3	41.4±0.9

Table 1. Distribution of G6PDH activity between the major and minor electrophoretic fractions in **Da** and **B** amoebae

Temperature of heating, °C	Portion of G6PDH activity in different electrophoretic fractions, % of total activity			
	Da strain		**B** strain	
	major fraction	**minor fractions**	**major fraction**	**minor fractions**
Control (without heating)	91	9	85	15
42	88	12	73	27
45	80	20	72	28
48	50	50	50	50

Table 2. Thermoresistance of esterases in two amoeba strains acclimated to different temperatures

Strain	Temperature of cultivation, °C	Residual enzymatic activity of samples (% of unheated control) after heating at various test-temperatures			
		45°C	**50°C**	**55°C**	**60°C**
Da	10	64.1	39.9	30.7	22.9
	25	64.3	40.7	29.6	20.8
B	10	74.7	57.6	45.6	28.6
	25	76.7	60.2	45.9	27.0

Table 3. Time of 50% esterases inactivation of two amoebae strains acclimated to different temperatures

Strain	Temperature of acclimation, °C	Temperature of heating, °C	Time of inactivation, min
Da	25	50	24
	10	50	25.5
	25	55	13.5
	10	55	14.0
B	25	50	46.5
	10	50	48.0
	25	55	25.0
	10	55	24.0

Table 4. Thermoresistance of G6PDH of amoebae of **Da** strain acclimated to different temperatures

Temperature of cultivation, °C	Residual enzymatic activity of samples (% of unheated control) after heating at various test-temperatures			
	39°C	**42°C**	**45°C**	**48°C**
10	90	81	31	9
25	78	63	20	6
10⟶25	79	66	23	7

The thermoresistance of water-soluble G6PDH of Da amoebae acclimated to 10°C appeared to be higher than that of amoebae from 25°C (Table 4).

Amoebae acclimated to 10°C were returned to 25°C. After 30 days of cultivation, their G6PDH thermoresistance did not differ from that of the amoebae constantly cultured at 25°C (Table 4).

The patterns of electrophoretic forms of water-soluble G6PDH in **Da** amoebae from 25 and 10°C were identical and in both temperatures the significant loss of the G6PDH activity after test-heating occured due to inactivation of the major electrophoretic fraction (Figs 5b and 5c; n 4), the minor fractions appeared to be more thermoresistant (Figs 5b and 5c; nn 1,2,3). The subfraction n 5 originated from fraction n 4 after test-heating of the 10°C-amoebae samples (Fig.5c).

Discussion

The activities of water-soluble esterases and G6PDH has been shown to be strain specific characters as well as the thermoresistance of both these enzymes. The collection of the amoeba strains of the Laboratory of Cytology of Unicellular Organisms contains strains (clones) of free-living amoebae isolated from nature and/or received from other laboratories. The interstrain differences in activity and thermoresistance of investigated enzymes between amoeba clones of various origin are retained during prolonged period of cultivation under standard laboratory conditions. It allows consideration of these differences so as to reflect the biochemical diversity of amoeba clones and supplements the knowledge about the intraspecific biochemical polymorphism of *Amoeba proteus*. The interstrain polymorphism of some amoebae physiological characters has previously been shown (Yudin and Sopina, 1970) together with that of Tritone-soluble esterases and G6PDH electrophoretic spectra (Sopina, 1989, 1994).

The temperature optimum for multiplication in **B**-strain amoebae is higher (Sopina, 1976) than in amoebae of strain **Da** (Sopina, 1986). Thus the higher thermoresistance of both investigated enzymes – thermostable esterases and thermolabile G6PDH – in **B**-strain amoebae may point to the existence of a positive correlation between thermoresistance of amoebae enzymes (proteins) and thermophility of the amoeba clone. Numerous examples of such correlation at the interspecies level exist for multicellular organisms (see: Alexandrov, 1985; Ushakov, 1989) and for their esterases in particular (Kusakina, 1962, 1967, 1973; Pravdina, 1970; Ivanenkov and Korobtzov, 1976). At the intraspecific level, no differences were revealed in the thermoresistance of various classes of esterases in subspecies and populations of some ectotherm animals differing by their optimal temperature conditions (Kusakina, 1965; Glushankova and Kusakina, 1967).

Some contradictive data were obtained for differences in esterase thermoresistance of **B**- and **Da**-amoeba strains. On the one hand the thermoresistance of Tritone-soluble esterases evaluated by the method of microelectrophoresis was also shown to be higher in **B** strain amoebae than in amoebae **Da** due to the differences in their electrophoretic spectra and thermoresistance of some fractions

(Sopina, 1986). However, in the same work it was demonstrated that the difference in thermoresistance of water-soluble esterases measured colorimetrically is much less than that presented in Fig. 2 and it was absolutely absent in Tritone-soluble esterases of **B** and **Da** amoebae (Sopina, 1995). We (Sopina and Podlipaeva, 1984) used ultracentrifugation (33000 rpm, 1 h) of amoeba homogenates which were stored for 12 hr. at 4°C. Sopina (1986) centrifuged amoeba homogenates, which had been stored for 3 hours, at 16000 rpm for 30 min and homogenates, stored for 30 min, at 12000 rpm for 10 min (Sopina, 1995) and explained the contradictions in the data by the time that had passed between the processes of amoebae homogenization and homogenate centrifugation – the shorter this period, the smaller was the difference in esterases thermoresistance. We believe that the contradictions result from the different centrifugation regimes applied in these three works. To solve this problem it would be useful to know the electrophoretic spectrum of water-soluble esterases from the supernatant after ultracentrifugation at 33000 rpm, but unfortunately such data is not currently available.

As for the positive correlation between the thermoresistance of G6PDH and the organism thermophility, the example of such correlation in Protozoans concerns thermoresistance of G6PDH in three *Leishmania* species. G6PDH from *Leishmania tarentolae* – the parasite of reptiles – was more thermosensitive than the same enzyme from *L. mexicana* and *L. donovani*, both parasites of mammals (Janovy, 1972).

It is noticeable from our data that the more thermosensitive G6PDH of **Da**-strain amoebae has the higher initial level of activity compared with the more thermoresistant **B**-amoebae enzyme. This feature together with the higher thermoresistance of G6PDH from the more thermophilic amoeba strain may be qualified as genetic adaptation at intraspecific level in its classic form (Hochachka and Somero, 1984).

The analysis of behaviour of G6PDH electrophoretic fractions after test heating revealed the differences in thermoinactivation between the major and the minor fractions. Minor fractions may be supposed to be more thermoresistant. However, such a conclusion must be made very carefully because of the following reasons. Polymorphism of G6PDH may be connected with various numbers of subunits which comprise the molecules of each electrophoretic G6PDH fraction (Schmukler, 1970). It may be presumed that the major fraction is a tetramer and the minor fraction N 3 – a dimer. Thus the number of dimeric molecules in the sample after experimental heating may not decrease and may even increase as a result of two processes: 1) dissociation of some tetramer molecules to fermentative-active dimeric ones and 2) final denaturation of dimers (Podlipaeva, 1992).

To analyse the results of experimental amoebae acclimation to relatively low temperature it should be noted that the 14-days cultivation at 10°C did not give rise to reliable differences in thermoresistance of water-soluble esterases. The same results were obtained by Seravin and co-authors (1965), who showed the absence of the difference in esterase thermoresistance of *Paramecium caudatum* cultivated at 28 and 15°C. The thermoresistance of carboxylesterase was the same in wheat leaves of plants grown at high and low temperatures (Konstantinova, 1983). A slight decrease of tritone soluble esterase thermoresistance after heating at some test temperatures was shown by microelectrophoresis in PAAG for **B**-strain amoebae acclimated to 10°C (Sopina, 1987). In **B**-strain amoebae 17 fractions of Tritone-soluble esterases were revealed and in **Da**-strain amoebae – 16 fractions (Sopina, 1986). The absence of differences in colorimetrically measured esterase thermoresistance in 25 and 10°C – acclimated amoebae may reflect the fact that the summary thermoresistance of this enzyme is the resultant of multidirective changes in thermoresistance of different electromorphs (Sopina, 1997).

As for the higher level of the G6PDH activity in **Da** amoebae from 10°C as compared with the activity of the enzyme in the amoebae from 25, these data show good correspondence with other published studies – the G6PDH activity was higher in the cold-acclimated ectothermal organisms than in the warm-acclimated ones (Sopina, 1991; Jagdale, Gordon, 1997; Seddon, 1997). Some authors connect the higher level of the G6PDH activity in the course of cold acclimation with the increased lipogenesis at low temperatures (Yamauchi et al., 1975; Campbell and Davies, 1978) bearing in mind that reduced NADP (NADP·H) – the product of the reaction catalyzed by G6PDH – is used in the synthesis of fatty acids.

The protective antioxidant system of the organism which neutralizes the damaging effect of various environmental factors temperature changes among them includes some enzymes, for example, gluthatione reductase (Sokolovsky, 1984; Harmann, 1992). To reduce the oxidyzed gluthation the gluthation reductase utilises NADP·H – thus there exists correlation between the levels of the activity of gluthation reductase and G6PDH (Sokolovsky, 1984; Garcia-Alfonso et al., 1998). The increase in the G6PD activity in amoebae cultivated at 10°C might suggest the activation of an adaptive antioxidant system, and thus the increase in the level of the activity of G6PDH may be qualified as an adaptive modification during cold acclimation of amoebae (Podlipaeva, 1994).

The absence of the visible loss of the activity of minor G6PDH fractions after the heating at 42 and 45°C allows us to presume that the thermoresistance of the major electromorph differs from that of the minor ones.

It is hard to explain the nature of increasing G6PDH thermoresistance in **Da** amoebae at low cultivation temperature. It seems that the thermoresistance of this enzyme in the amoebae depends upon the difference between the temperature of cultivation and the temperature optimum

of the organism, but does not depend upon the direction (towards higher or lower temperatures) of the temperature changes (Podlipaeva, 1997). In other words, every temperature which disturbs the metabolic balance (homeostasis) of the organism causes the changes in its proteins (enzymes) characters, including thermoresistance, some of such changes being unexpected.

As for the rules which enzymes activity and thermoresistance follow during thermal acclimation of an organism, it seems that the enzymes differing greatly by their structure, thermolability, conformational potencies and other characteristics are not obligatorily governed by the common scheme in the course of temperature regime changes (Lutova, 1995; Podlipaeva, 2000).

The more different enzymes of different representatives from different thermal conditions are under study, the more chances are available to reflect biochemical diversity of multi-enzyme metabolic systems of the organisms, including unicellular ones.

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Address for correspondence: Yulia I. Podlipaeva, Institute of Cytology, Russian Academy of Sciences, 4 Tikhoretsky Ave., St. Petrsburg 194064, Russia. E-mail: yulia@cit.ras.spb.ru.