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Effects of Temperature on the Activities of Glucose-6-phosphate, 6-phosphogluconate and Isocitrate Dehydrogenases from Culture Form of *Trypanosoma cruzi*

YOSHIMASA KANEDA* AND SACHIO MIURA
Department of Parasitology, School of Medicine, Keio University, Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

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In free-living ciliates, *Paramecium* and *Tetrahymena*, thermal adaptation was accompanied by changes in metabolic pathways which, in turn, reflected in alterations in the enzymic patterns (Irlina, 1967; Levy, 1973). Ghosh and Honigberg (1976) also observed the effects of environmental temperature on the activities of enzymes from two strains of *Leishmania donovani* grown at 25 and 37 C.

Trypanosoma cruzi occurs in three morphologically distinct forms—amastigotes, epimastigotes and trypomastigotes, depending on the environment in which it is found (Hoare, 1972). The trypomastigotes are found in the blood of infected mammals, and the amastigotes are found in the tissues of the mammals. The epimastigotes are found in the digestive tract of the triatome vector as well as in cultures on artificial culture media. The temperature of the body of the triatome vector and of the artificial cultivation are often considered to be lower than mammalian ones. It has been postulated that the morphological changes from epimastigotes to trypo- or a- mastigotes may be a result of exposure to mammalian body temperature. Then, in order to determine whether or not the elevated temperature of cultivation correspond to the morphological changes, we examined the effects of elevated temperature on the proportion of morphological types in the cultures. Furthermore, we observed changes of the enzymic activity at different temperatures of cultivation. The activities of glucose-6-phosphate (G6PD) (EC No. 1.1.1.49), 6-phosphogluconate (PGD) (EC No. 1.1.1.44) and isocitrate (ICD) (EC No. 1.1.1.42) dehydrogenases were assayed in the course of this study. G6PD and PGD are the enzymes mediating the first 2 reactions of the hexose monophosphate shunt which appears to be operational in *T. cruzi* (Raw, 1959). ICD is an important enzyme involved in anaplerotic reactions. All of these 3 dehydrogenases are NADP-linked enzymes. In view of the likely involvement of lipid metabolism changes in thermal adaptations, the activities and thermal stabilities of these dehydrogenases seemed to be of considerable interest.

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* Present address: *Department of Parasitology, School of Medicine, Tokai University, Isehara-shi, Kanagawa-ken 259 11, Japan*

Materials and Methods

The parasites.—Tulahuen strain of *Try-*

panosoma cruzi has been serially maintained at 29 C in liver-infusion-tryptose culture medium (LIT medium) (Kaneda, 1972). The organisms grown at 29 C were transferred to 33 and 36 C, and used for the experiments 7 days after inoculation or after 10 serial passages every seven days at 36 C.

The proportion of morphologic types in the cultures was estimated by the smear of cultures which was stained by Giemsa.

Preparation of extracts for enzyme assays.—Seven days after cultivation at three different temperatures (29,33, and 36 C), the parasites were harvested by centrifugation at 3,500 ×g for 10 minutes and washed 3 times with tris-buffered 0.25 M sucrose pH 7.8. The washed cells were suspended in 10 ml of 0.25 M sucrose and disrupted with sonic vibrator at 100 V for 2 minutes. The resulting homogenates were centrifuged at 12,000 ×g for 20 minutes at 4 C in Kubota refrigerated centrifuge (Model KR/180 FA) and the nuclei and sedimentary cellular debris were discarded. The supernatant fluid, corresponding to the soluble fractions was used for the enzyme assays.

Enzyme assays.—Activities of all of the enzymes studied were determined spectrophotometrically at 340 nm by following the NADP reduction rate in the presence of excess substrate and cofactors. Assays were carried out with Hitachi 556 recording spectrophotometer. The cell compartment of the spectrophotometer was maintained at the desired temperature (15, 25 and 35 C) by Komatsu-Yamato water circulator (Model CTE 21).

The assay method for ICD was that of Kornberg and Pricer (1951). The reaction mixture contained 2.6 ml of 0.1 M tris-buffer (pH 7.8), 0.1 ml of 0.1 M $MgCl_2$, 0.1 ml of 0.006 M NADP (Sigma Chemical Company), and 0.1 ml of cell-free extract of the trypanosome. The reaction was started by the addition of 0.1 ml of 0.015 M DL-isocitrate (Sigma).

Activity of G6PD was assayed according to the method of Glock and McLearn (1953). The reaction mixture consisted of 2.6 ml of 0.1 M tris-buffer (pH 7.8), 0.1 ml of 0.1 M $MgCl_2$, 0.1 ml of 0.006 M NADP and 0.1 ml of the cell-free extract. The reaction was started by the addition of 0.1 ml of 0.1 M glucose-6-phosphate (Sigma). The activity of G6PD was corrected for 6-phosphogluconate dehydrogenase activity by subtracting the latter's NADP reduction rates from the combined activity.

The assay of PGD was based on Glock and McLearn (1953). The reaction mixture was the same as in G6PD except that G6P was replaced by 6PG. The reaction was started by the addition of 0.1 ml of 0.1 M 6PG (Sigma).

In order to determine the stability of G6PD, ICD and PGD, cell-free extracts which were obtained from the trypanosomes grown at three different temperatures, were incubated at 37 C and samples were retrieved at 30-, 60-, 90- and 120- minute intervals. After the samples were chilled in ice water, the residual activities of the three enzymes were assayed at 20 C. Temperature coefficients (Q_{10}) of G6PD, ICD and PGD obtained from various cultures were determined by measuring the activity of each enzyme at 15, 25 and 35 C.

Protein concentration of the homogenates was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Results

Glucose-6-phosphate dehydrogenase (G6PD).—The activities of G6PD from the organisms grown at three different temperatures intermediated between the ICD and PGD activities irrespective of the assay temperature (Table 1). When the culture temperature was changed from 29 to 36 C, the activity of G6PD assayed at 15 C was enhanced more than 111%, furthermore, it

Table 1 Temperature coefficient (Q_{10}) values for glucose-6-phosphate (G6PD), isocitrate (ICD) and 6-phosphogluconate (PGD) dehydrogenases from *Trypanosoma cruzi* cultured at 25, 33 and 36 C

Temperature of cultivation	Enzyme	Assayed at		Q_{10} (25/15 C)	Assayed at	Q_{10} (35/25 C)
		15 C*	25 C*		35 C*	
29 C	G6PD	1.709±0.036†	3.090±0.109	1.81	4.908±0.036	1.59
	ICD	3.546±0.280	6.907±0.185	1.95	13.453±0.429	1.95
	PGD	0.175±0.002	0.510±0.170	2.91	1.592±0.243	3.12
33 C	G6PD	1.642±0.059	2.902±0.088	1.77	4.544±0.147	1.57
	ICD	2.902±0.088	6.420±0.088	2.21	12.107±0.264	1.89
	PGD	0.157±0.002	0.550±0	3.50	1.179±0.036	2.14
36 C	G6PD	3.616±0.071	5.672±0.142	1.57	6.522±0.002	1.15
	ICD	3.960±0.070	9.495±1.540	2.40	14.103±0.347	1.49
	PGD	0.278±0.001	0.626±0.069	2.25	1.667±0.278	2.66

* Number of individual determination was 4.

† Activity expressed in nmoles of product formed/min/mg protein ±S.D.

was enhanced more than 83% and 32% at 25 and 35 C assay temperature respectively (Table 1). It was revealed that for increase in the culture temperature there was a corresponding increase in the activity of G6PD.

For the three different cultures, Q_{10} values of G6PD in the temperature range below 25 C were higher than those in the range above 25 C (Table 1). And, as the culture temperature was increased, the Q_{10} values of G6PD decreased gradually.

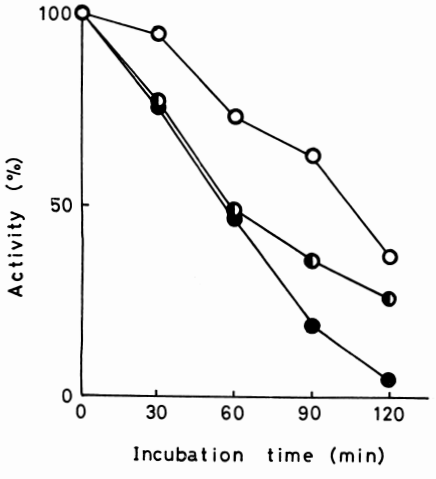

Fig. 1 Stabilities of G6PD obtained from the cultures at 36 (●), 33 (◐) and 29 C (○).

Incubation of the enzymes at 37 C for 2 hrs caused a significant loss in activity (Fig. 1). Such loss in activity was found in the enzyme obtained from all of the cultures grown at three different temperatures.

Isocitrate dehydrogenase (ICD).—The activities of ICD from the cultures grown at three different temperatures were higher than those of either G6PD or PGD (Table 1). When the cultures were transferred from 29 to 36 C, the activity of ICD was enhanced slightly: at 25 C assay temperature the enhancing rate was 37%. The increasing rate of the activity was low as compared with that of G6PD.

The temperature coefficient for ICD in described in Table 1. The Q_{10} values in the temperature range above 25 C decreased according as the temperature of cultivation elevated, while in the range below 25 C it increased gradually.

ICD from the cultures grown at three different temperatures was stable for up to 2 hrs at 37 C, as is shown in Figure 2.

6-phosphogluconate dehydrogenase (PGD).—Irrespective of the assay temperature, the activities of PGD from the cultures grown at three different temperatures were lower than those of either G6PD or ICD and the activity of the 36 C culture was

slightly higher than that of PGD from the 29 C culture (Table 1). When the culture temperature was changed from 29 to 36 C, the activity of PGD increased by 58%, 23% and 4% at 15, 25 and 35 C assay temperature respectively.

The Q_{10} values for PGD in the temperature range below 25 C were lower than those in the range above 25 C, as is shown in Table 1.

PGD from all of the cultures grown at three different temperatures was more labile in extracts prepared in 0.25 M sucrose; 50% of its activity was lost within 30 min at 37 C (Fig. 3).

Enzymic activity of the cultures maintained at 36 C.—To ascertain whether or not continuous cultivation at the elevated temperature affected G6PD, PGD and ICD from the organisms, the activities of these enzymes obtained from the cultures after 10 passages at 36 C were assayed at three different temperatures. The results are presented in Table 2. It is evident from this Table that the activity of PGD from the organisms after 10 serial passages at 36 C was higher than that of this enzyme from the organisms after one passage at 36 C. After 10 transfers at 36 C, the activities of G6PD and ICD are similar to those from the cultures after one passage; the activity levels are maintained for at least

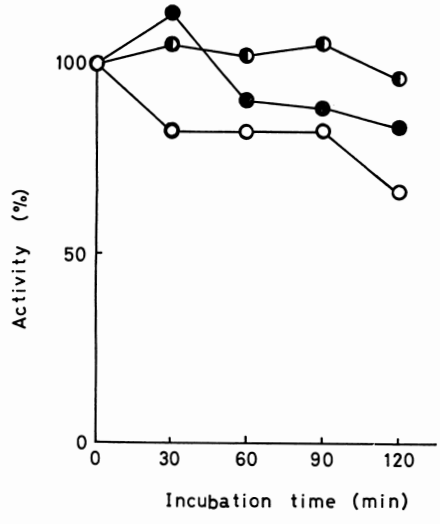

Fig. 2 Stabilities of ICD obtained from the cultures at 36 (●), 33 (◐) and 29 C (○).

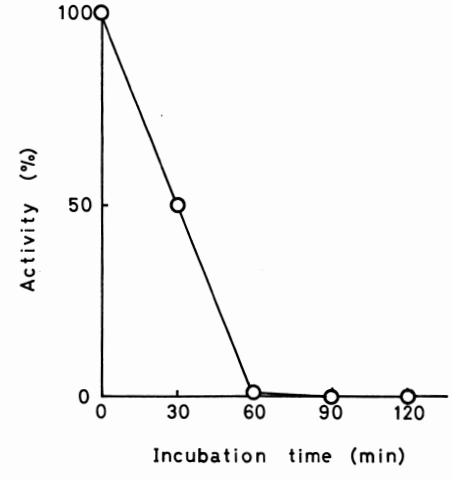

Fig. 3 Stability of PGD obtained from the cultures at 29 C.

Table 2 Temperature coefficient (Q_{10}) values for glucose-6-phosphate, isocitrate and 6-phosphogluconate dehydrogenases from cultured *T. cruzi* after transfers at 36 C

Enzyme	Assayed at		Q_{10} (25/15 C)	Assayed at	Q_{10} (35/25 C)
	15 C*	25 C*		35 C	
G6PD	2.98±0†	5.39±0.08	1.81	7.96±0.09	1.48
ICD	3.36±0.25	8.06±0.05	2.40	13.33±0.12	1.65
PGD	0.38±0.12	1.59±0.41	4.18	3.11±0.06	1.96

* Number of individual determination was 4.

† Activity expressed in nmoles of product formed/min/mg protein ±S.D.

Table 3 Morphological distribution of *Trypanosoma cruzi* cultivated in LIT medium

Temperature of cultivation	No. of passage	Distribution by morphologic types (%)			
		Trypomastigote	Epimastigote	Promastigote	Amastigote
29 C	22	3.4	96.6	0	0
33 C	1	1.0	98.8	0.2	0
36 C	1	0	99.0	1.0	0
36 C	10	0	72.2	27.8	0

10 serial passages at 36 C.

Morphological distribution in the cultures after 10 serial passage at 36 C was observed. The results are shown in Table 3.

Discussion

In order to determine whether or not changes in the temperature of cultivation of *T. cruzi* reflect in alterations of enzymic patterns, activities and temperature coefficient of these enzymes (G6PD, PGD and ICD) obtained from the organisms have been studied by changing the culture temperature or assay temperature. It was observed that the activities of these enzymes from 36 C cultures were higher than those from 29 C cultures, irrespective of the assay temperature. The increase in activities of both G6PD and PGD may be related to the enhancement of the pentose phosphate pathways at a higher temperature of cultivation. Especially, the most distinguished enhancing rate of the activity of G6PD was observed, as is shown in Table 1. The changing rate of G6PD activity when the culture temperature was elevated from 29 C to 36 C exceeded the activity increment which was estimated by changing assay temperature from 25 C to 35 C. On the other hand, the enhancing rate of PGD was quite different from that of G6PD. The increasing rate of PGD activity by elevating the culture temperature were corresponded to the rate estimated by changing the assay temperature. Further experiments are needed to elucidate the differences in the enhancing rate of activity between G6PD and PGD.

As far as the rise in G6PD activity is concerned, 3 alternative explanations can be adduced, (a) increase in the population of enzyme molecules in the organisms; (b) changes in the catalytic properties of the enzymes; and (c) decrease of total proteins with the enzyme level remaining unchanged.

The activity of ICD was higher than that of the other two enzymes but, the rise of the activity of ICD by elevating culture temperature was lower than that of the other two enzymes. ICD obtained from the organisms was thermostable under the experimental conditions. The results suggest that the physiological role of the enzyme in the living organisms was unaffected by the elevated temperature of cultivation.

PGD and G6PD obtained from the organisms are found to be quite unstable at 37 C in homogenates. The thermolability of these enzymes may account for the lack of these apparent activity increases under this experimental conditions. Even though studies using homogenates of the organisms do not always accurately reflect the physiological role of the enzyme *in vivo*, it is likely that in the living organisms cultured at 36 C these enzymic functions are protected to some extent.

After the cultures were transferred from 29 C to 36 C, the enhancement of the activities of the three enzymes was maintained during 10 serial passages. It was assumed that such enzymic changes were rapidly

readjusted by metabolic regulation mechanisms of the cells.

Differences in Q_{10} values for PGD, G6PD and ICD between both ranges above and below 25 C were found in all of the cultures grown at three different temperatures. The fact that a enzyme could show such a biphasic temperature dependence was originally demonstrated by Sizer (1943), since then, Massey (1953) and Levy *et al.* (1959) have reported for various enzymes. Of the possible explanations of such observations, changes in the configuration of the protein would appear to be the most fitting one (Sizer, 1943; Levy *et al.*, 1959).

Wood and Schiller (1975) have reported differences in fatty acid metabolism between trypomastigotes and epimastigotes *in vitro*. The results from the present experiment indicated that the proportion of the morphological types changed in the cultures grown at elevated temperature. However, the changes in morphological distribution could not be correlated with the rise in ICD activity. It seems reasonable to assume that the morphological changes from epimastigotes to promastigotes did not reflect in alterations in the fatty acid metabolism.

Summary

The activities of glucose-6-phosphate dehydrogenase (G6PD) (EC No. 1.1.1.49), 6-phosphogluconate dehydrogenase (PGD) (EC. No. 1.1.1.44) and isocitrate dehydrogenase (ICD) (EC. No. 1.1.1.42) from *Trypanosoma cruzi* cultivated at 29, 33 and 36 C in LIT medium were assayed at three different temperatures. Irrespective of the assay temperature, the activities of G6PD, PGD and ICD from the organisms grown at 36 C were higher than those of these enzymes from the organisms grown at 29 or 33 C. When the culture temperature was elevated the rate of increase in the activity of G6PD was the highest, and ICD, the lowest; the increasing rate of PGD was intermediate. G6PD and PGD obtained from the cultures grown at three different temperatures were unstable at 37 C in the extracts but ICD was stable in such extracts. These stabilities of these enzymes were demonstrated at each temperature of the cultivation. The Q_{10} values of G6PD and ICD from either 36 C cultures or 29 C cultures in the temperature range below 25 C were higher than those in the range above 25 C, while the Q_{10} values of PGD were low in the range below 25 C. Such differences in Q_{10} values for the respective enzyme were observed in all of the cultures grown at the three different temperatures. The results suggest that the elevation of culture temperature was accompanied by alterations in the nature of enzymic activity.

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培養 *Trypanosoma cruzi* の glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase 活性に及ぼす温度の影響について

金田良雅　三浦左千夫
（慶応義塾大学医学部寄生虫学教室）

環境温度の変化が *Trypanosoma cruzi* の代謝系に影響を及ぼす可能性について観察が行われた．三段階の培養温度（29, 33, 36C）で培養された *T. cruzi* から抽出された glucose-6-phosphate dehydrogenase (G6PD) (EC No. 1.1.1.49), 6-phosphogluconate dehydrogenase (PGD) (EC No. 1.1.1.44), isocitrate dehydrogenase (ICD) (EC No. 1.1.1.42) の酵素活性を3種類の測定温度（15, 25, 35C）でそれぞれ測定した．その結果，36Cで培養した虫体から得られた酵素の活性が，測定温度に関係なくいずれの酵素も高い活性を示し，33Cで培養した虫体からの酵素の活性が最も低かった．培養温度を変化させた場合の酵素活性の上昇率は G6PD が測定した3種の酵素の中では最も高かった．また，測定温度を変化させて得た活性の上昇率と比較すると，培養温度の上昇による活性の上昇が高いことが観察された．PGD 及び ICD は培養温度の上昇により活性が高まることが観察されたが，その上昇率は測定温度の上昇から推察される上昇率より低いものであった．抽出された酵素の37Cに対する安定性は，0.25M sucrose 液中で ICD が非常に安定であったが，他の G6PD 及び PGD は不安定ですぐに活性を失うことが観察された．この不安定性は *in vitro* における性質であり，*in vivo* における酵素の安定性は異なっていると考えられる．すなわち，培養温度を上昇させた虫体から得た酵素の活性が温度上昇前より高まっていることから，生体内では温度に対する何らかの補償機構が存在すると思われる．観察された酵素の温度係数 (Q_{10}) のうち G6PD と ICD に関しては25C以下の場合の係数が 25C 以上の場合の係数より高い値を示していた．一方，PGD の温度係数はこの逆で，25C以下の場合の係数が低い値を示していた．このように温度係数が測定域によって違いを生ずることは酵素の立体配位が温度によって変化していることを示唆するものであろう．ここで測定された酵素が示す温度に対する性質は，培養温度36C で10代継代された虫体から得た酵素においても同様に観察された．したがって，温度に対する酵素活性の変化は非常に早く起ることが推察された．