Changes in relative concentrations of the hemoglobin components in *Paramecium* caused by cell growth and temperature of the culture

ITARU USUKI and AKIHIRO HINO*

Department of Biology, College of General Education, Niigata University, Niigata 950-21, Japan

*Present address: Central Research Institute, MECT corporation, 1780 Kitano Tokorozawa-shi, Saitama 359, Japan

**Summary**

The Hb (hemoglobin) content of *Paramecium* has been known to maintain a constant level even when the cells are exposed to different conditions. However, the relative concentrations of the Hb components in the stocks of *P. caudatum, P. multimicronucleatum*, and *P. jenningsi* were found to change during cell growth from logarithmic young phase to stationary growth phase even when the culture was maintained at a constant temperature of 18 or 27 °C. The temperature of the culture also affected the relative concentrations of the Hb components in a cell. A large part of the growth-dependent and temperature-dependent change was attributable to a variation in the amounts of two or three major Hb components, and the variation in other minor components was almost negligible. At the time of cell growth and of a shift in temperature, the major Hb components in a cell respond in opposite ways from each other. These components differed in several properties: one of them showed a faster migration by PAGE, a smaller molecular mass and a lower pi value than another.

Key words: hemoglobin, *Paramecium*, growth-dependent change, temperature-dependent change.

**Introduction**

The ciliated protozoan *Paramecium* contains a heterogeneous hemoglobin (Hb), which is resolvable electrophoretically into several definite components (Davis and Steers, 1976; Steers and Davis, 1979). The Hb components show a species-specificity, as has been pointed out for the species complex of *P. aurelia* (Irie and Usuki, 1980; Usuki and Irie, 1983a), and for *P. multimicronucleatum, P. caudatum* and *P. jenningsi* (Steers et al., 1981; Usuki and Irie, 1983b; Usuki et al., 1989). This finding may indicate that the expression of these Hb components is controlled by different loci on the genes.

*Paramecium* Hb is a monomeric heme protein with a smaller size than any other Hbs reported from a variety of organisms (Prosser, 1973; Linzen, 1986). The primary structure of the major Hb component from *P. caudatum* has been determined, indicating a unique sequence of 116 amino acid residues with a molecular mass of 12,565 Da (Iwasa et al., 1989). However, few attempts have been made to find a role for Hbs in this animal group.

On the other hand, the Hb content in some invertebrates is known to vary considerably according to some environmental factors, especially the ambient oxygen tension (Riggs and Van Holde, 1973; Weber, 1980; Kobayashi and Hoshi, 1984). Moreover, certain organisms have been reported to change their Hb components dramatically during the process of ontogenetic growth (Moen and Kondo, 1976; Schin et al., 1979). In *Paramecium*, however, it has been shown that the Hb level in a given stock does not show significant change even when the organism is subjected to different conditions, although the Hb content shows a remarkable variation among species in this organism (Usuki and Hino, 1987).

In this paper we report the finding that *Paramecium* accommodates changes in cell growth and ambient temperature by a change in the relative amounts of Hb components, like higher invertebrates, which vary their Hb according to their physiological state.

**Materials and methods**

The ciliates used were two stocks (Sh 23 and YC) of *Paramecium caudatum* syngen 3, two stocks (NE 25 and CH 312) of *Paramecium multimicronucleatum* syngen 2, and two stocks (ATCC 30997 and ATCC 30998) of *Paramecium jenningsi*. As the culture medium, a mixture of fresh lettuce infusion and pea broth was used throughout this study, and it was supplemented with 0.5 mg l⁻¹ of stigmasterol (Merck) and inoculated with *Enterobacter aerogenes* one day before use. Each stock of the paramecia was grown previously in flasks at 27 °C, to the extent of 20–30 ml by addition of an equal volume of fresh medium when the cells reached stationary phase. The grown cells were concentrated and washed with the Dryl's solution under a light centrifugal force (150 g). Then the concentrated cells were divided in two: they were cultured again with fresh media in tanks at different room temperatures maintained to 18.0 (±0.1) °C and 27.0 (±0.1) °C, respectively. These cultures were increased several times in volume (amounting to 60–120 l in total) by addition of fresh medium after the cells reached stationary phase. These cells were harvested at logarithmic phase or stationary phase. Hb from the respective cells was partially purified as described (Usuki and Irie, 1983a; Usuki et al., 1989), and then stored in a deep freeze at −80 °C until use.

Polyacrylamide gel electrophoresis (PAGE) was performed with a 15% mini-slab gel (1 mm × 85 mm × 85 mm) in a discontinuous...
buffer system. Isoelectric focusing (IEF) was carried out using a Pharmacia flat-bed apparatus FBE-3000 (Uppsala, Sweden) and an LKB Ampholine PAG plate (pH 3.5–9.5) (Bromma, Sweden). PAGE and IEF were performed at 2°C. Densitometric analysis was done using the Shimadzu dual-wavelength chromatocam scanner CS-910 (Kyoto, Japan), with a 420 nm sample beam and a 650 nm reference beam on unstained gels.

Results

*Paramecium* cells in logarithmic phase were harvested after about 24 h at 27°C and after 3–4 days at 18°C after the final addition of freshly prepared culture medium. At this time the culture medium retained considerable turbidity, reflecting the presence of an excess of food bacteria. The cells in stationary phase were generally obtained after 3–4 days culture at 27°C and after 7–14 days culture at 18°C; in this time the media had become less turbid, as surging crowds of paramecia came into sight.

The Hb of *P. caudatum* was resolved by IEF into six components: basic component (bHb), Hb2, Hb3, Hb10, Hb11 and a trace amount of Hb12. These Hb compositions were not modified by cell growth or culture temperature, showing a species specificity as reported (Usuki and Irie, 1983a,6; Usuki et al. 1989). The effects of cell growth from logarithmic phase to stationary phase were examined by comparison of the relative amounts of the Hb components in each of the stocks, which were cultured at a constant temperature.

In 27°C culture, the Hb of stock Sh 23 of *P. caudatum* contained 29% of bHb and 65% of Hb10 at logarithmic phase, while at stationary phase it contained 10% of bHb and 81% of Hb10, resulting in a 19% decrease in the former and a 16% increase in the latter during cell growth (Fig. 1B). Similar changes were observed for both of the Hbs from stocks Sh 23 and YC at 18°C in culture, although the effects were slightly less at this temperature (Fig. 1A). The variation in other minor Hb components was negligible in this species. In the same way, the Hb of stock NE 25 of *P. multimicronucleatum* at logarithmic phase in 27°C culture was composed of 63% of Hb11 and 25% of Hb2, and at stationary phase it yielded 80% of Hb11 and 12% of Hb2 (Fig. 1D): namely, the stock caused an increase in Hb11 of approximately 17% during cell growth at 27°C, accompanied by a decrease in Hb2 as well as other minor Hbs. The cells at 18°C in culture also showed a similar change, although the change in concentration was less than that at 27°C in culture (Fig. 1C).

During the transition from logarithmic phase to stationary phase, stock CH 312 of *P. multimicronucleatum* in 27°C culture showed an approx. 23% increase in Hb2 together with a comparable decrease in Hb11, opposite to the response to those of stock NE 25 of the same species (Fig. 1F). In culture at 18°C stock CH 312 underwent a drastic change in the concentration of the Hb components: Hb2 increased from 17% to 90% and Hb11 decreased from 76% to 8% (Fig. 1E). Such a change in concentration between Hb2 and Hb11 caused a dramatic alteration in the major Hb component that this stock contained before and after the cell growth.

The growth-dependent changes in the Hb in stock ATCC 30997 of *P. jenningsii* in culture at 18°C resemble those for stock CH 312. Growth of the cell caused a noticeable increase in Hb2, from 22% to 60%, in association with a decrease from 54% to 26% in Hb10 and from 21% to 5% in bHb (Fig. 1G). As a consequence, the major Hb component in this stock varied between Hb11 and Hb2 before and after cell growth. In 27°C culture, however, cell growth caused a 20% increase in Hb11 from

![Fig. 1. Growth-dependent change in relative concentrations of the Hb components during transition from logarithmic phase to stationary phase. Each of the stocks was cultured at a constant temperature of 18 or 27°C, respectively. Hb levels represent an average of 6–10 measurements with standard error. 2, Hb2; 10, Hb10; 11, Hb11; b, bHb; m, minor Hb components.](image-url)
The effects of environmental temperature were investigated in P. caudatum cultured at this temperature. 25 of P. multimicronucleatum and the stocks of P. jenningsi were similar to those for stock NE 25 of P. multimicronucleatum and the stocks of P. caudatum cultured at this temperature.

Changes caused by the temperature of the culture

The effects of environmental temperature were investigated by comparison of the relative amounts of the Hb components in each of the stocks that were cultured to a logarithmic growth phase at different temperatures.

At logarithmic phase, stock Sh 23 of P. caudatum produced Hb containing 79% of Hb11 and 18% of HbHb in culture at 18°C, while at 27°C Hb10 decreased to 65% and HbHb increased to 29% (Fig. 2A). At stationary phase the effect of temperature was rather small, showing a small percentage of variation with a similarity to those at logarithmic phase (Fig. 2B). Stock YC at stationary phase also showed essentially the same variation between 18 and 27°C.

The Hb of stock NE 25 of P. multimicronucleatum contained 40% of Hb11 and 50% of Hb2 at logarithmic phase in culture at 18°C. Cells at 27°C in culture contained 63% of Hb11 and 25% of Hb2, resulting in a 23% increase in Hb11 and a 25% decrease in Hb2 by increasing the temperature (Fig. 2C). At stationary phase, the Hb in culture at 18°C contained 49% of Hb11 and 44% of Hb2. At 27°C the cells changed, to contain 80% of Hb11 and 12% of Hb2 (Fig. 2D). Thus, the shift of temperature from 18 to 27°C causes a noticeable increase in Hb11 and a decrease by a comparable amount of Hb2, especially when the cells have become full grown.

Temperature-dependent change in stock CH 312 was abrupt at stationary phase. The cells grown in culture at 18°C produced Hb containing 90% of Hb2 and 8% of Hb11, whereas the cells in culture at 27°C generated 30% of Hb2 and 59% of Hb11, reversing the quantitative relation between the two components (Fig. 2F). At logarithmic phase, the Hb composition in the cell was scarcely affected by temperature (Fig. 2E). The effects of temperature on P. jenningsi were similar to those on stock CH 312. When the cultivation temperature was shifted from 18 to 27°C, the cells in stationary phase increased their concentration of Hb11 from 26% to 69% and decreased their Hb2 from 60% to 16%, resulting in an inverse relation of the quantities of the two components (Fig. 2H). At logarithmic phase, however, the effects of temperature remained only small, as in stock CH 312 (Fig. 2G).

The data obtained from the four stocks of different species of Paramecium are compiled three-dimensionally in Fig. 3. As seen in this figure, cell growth and lowering the temperature bring about an increase in Hb11 in association with a decrease in bHb in P. caudatum, although the concentration change in this species is
moderate and smaller than in other species. Stock NE 25 of *P. multimicronucleatum* also exhibits a consistent change, giving an obvious increase in Hb1 and a compensatory decrease in Hb2 on increasing the temperature and on cell growth. On the other hand, the changes in stocks CH 312 and ATCC 30997 are curious, reflecting the result of an anomalous reduction in Hb1 at the time of growth phase at 18°C.

**Discussion**

In *Paramecium* a method for reliable synchronous culture has not yet been established. In this study, therefore, the organism was cultured by the usual method and the cells were harvested at logarithmic phase or stationary phase: the former phase was expected to be full of growing and proliferating young cells and the latter should contain mature cells. The difference in growth phase may affect intracellular conditions greatly. For example, many authors have remarked that the susceptibility of respiration to cyanide varies considerably during the growth of a variety of eukaryotic cells (Pace, 1945; Edwards and Lloyd, 1977; Lloyd et al. 1980; Palmer, 1981). These findings have been studied in connection with the function of an alternative respiratory system that produces a large excess of electrons at the time of active biosynthesis (Doussiére et al. 1979, Young, 1983). In addition to this, paramecia in this study were cultured at two different temperatures, 18 and 27°C. In ciliates temperature-dependent changes have been reported in ciliary activity or swimming behaviour (Tawada and Oosawa, 1972; Connolly et al. 1985a), in lipid composition and membrane fluidity (Nozawa et al. 1974; Connolly et al. 1985b), and in surface antigens (Preer, 1986; Bannon et al. 1986; Love et al. 1986), etc. We ascertained that each of the paramecia stocks that are transplanted into fresh culture medium proliferates at a rate of 0.3–0.8 division every day at 18°C and 2.5–3 divisions at 27°C.

This study made it clear that the cell growth of *Paramecium* from logarithmic, young phase to stationary growth phase is accompanied by an obvious change in Hb1 and Hb2 in *P. caudatum*, Hb1 and Hb2 in *P. multimicronucleatum*, and Hb1 and Hb2 or bHb in *P. jenningsi*, respectively. These components increase in opposite directions to each other according to the growth of the cell and the ambient temperature, although the physiological meaning of these changes remains obscure; whereas, other Hb components in these *Paramecium* species are too small in quantity and are generally less effective, even if their amounts vary.

On the other hand, the Hb components from various stocks in different species of *Paramecium* have been divided into two groups by their molecular masses: estimation by SDS–PAGE shows 1.1 kDa for Hb0 and Hb11, and 1.3 kDa for Hb2 and bHb (Usuki and Irie, 1983a,b; Usuki et al. 1989). However, the isoelectric point measured in our laboratory varied greatly among the *Paramecium* species: the pi value was 3.9 for Hb0 and 9.8–10.5 for bHbs from *P. caudatum*, 4.0–4.2 for Hb11 and 6.2–6.5 for Hb2 from *P. multimicronucleatum* and *P. jenningsi*, and 8.8 for bHb from *P. jenningsi* (Usuki et al. 1989; unpublished data). Therefore, we can state that both the growth-dependent and temperature-dependent changes observed in this study are realized by participation of two different types of Hb components: one of them shows faster migration by PAGE, has a smaller molecular mass and a significantly lower pi value than those of the other. This may permit a reverse control for the two Hb components in a cell.

These results were unexpected and showed that stocks CH 312 of *P. multimicronucleatum* and ATCC 30997 of *P. jenningsi* exhibited an abnormal decrease in Hb1 at stationary phase, which was reached after a prolonged period at 18°C. *P. jenningsi* is known to be a species of tropical origin (Diller and Earl, 1958). According to Dr Y. Takagi, stock CH 312 is a descendant of artificially induced conjugants, which have been gained through chemical treatments by Dr A. Miyake (personal communication). We have little information about the physiological, biochemical and ecological characteristics of these two stocks. However, we have found that the isoelectric points of Hb1 from these stocks are lower by approximately 0.2 pH unit than that of stock NE 25 (unpublished data). This finding may indicate that Hb1 possesses some difference in its molecular structure. So, we put forward the hypothesis that, during a prolonged cultivation period at 18°C, the stability of the Hb1 molecule in these stocks breaks down and/or some process necessary for Hb1 synthesis in these cells is susceptible to temperature. In connection with this, an interesting finding has been reported that the expression of a temperature-dependent surface antigen of *Tetrahymena* is directly correlated with mRNA abundance, which is controlled by a dramatic temperature-dependent change in mRNA stability (Love et al. 1988).

We thank Dr T. Watanabe, College of General Education, Tohoku University, Sendai, and Dr K. Mikami, Miyagi College of Education, Sendai, for the supply of stocks of *P. caudatum*. The stocks of *P. multimicronucleatum* were provided by Dr Y. Takagi, Faculty of Science, Nara Women’s University, Nara. We are also indebted to Dr M. Fujishima, Faculty of Science, Yamaguchi University, Yamaguchi, for supply of the seed cells of *P. jenningsi*. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

**References**


