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Influence of Allosteric Effectors and Temperature on Oxygen Binding Properties and the Bohr Effect of Bovine Hemoglobin

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The O_2 binding properties of bovine Hb were examined. The increase in Cl^- and DPG concentration enhanced P_{50}. A reduction in n_{max} was observed at high Cl^- concentration, while DPG had little effect on n_{max}. An increase in Cl^- concentration enhanced the Bohr effect, the magnitude of which reached a maximum at 0.1 M Cl^- and 20°C. This concentration is nearly equal to that at the highest slope of the log P_{50} vs. log [Cl^-] plot, and also equal to the physiological Cl^- concentration (0.1 M) of bovine blood. Furthermore, the influence of Cl^- concentration on the Bohr effect is independent of temperature. On the other hand, in the absence of Cl^-, bovine Hb is sensitive to DPG; an increase in DPG concentration enhanced the Bohr effect, which reached a maximum at 3 mM DPG and 20°C. This concentration is nearly equal to that at the highest slope of the log P_{50} vs. log [DPG] plot. At low DPG concentrations, the DPG effect on the Bohr effect became small with increasing temperature, whereas at high DPG concentrations, the DPG effect was insensitive to temperature changes. At the physiological concentration of DPG (0.5 mM), increases in both Cl^- concentration and temperature diminished the DPG effect. At the physiological concentrations of Cl^- and DPG, the Bohr effect was –0.36 at 37°C. The ΔH value at the physiological concentrations of Cl^- and DPG was approximately –5.8 kcal/mol at pH 7.4. These results indicate that Cl^- and temperature are important determinants of the O_2 binding properties of bovine Hb.

Key words: hemoglobin, Bohr effect, temperature, allosteric effector, bovine, oxygen transport

INTRODUCTION

The oxygen (O_2) binding property of hemoglobin (Hb) is characterized by the position and sigmoidal shape of the oxygen equilibrium curve (OEC, O_2 saturation (S) vs. partial pressure of O_2 (P)). The position is expressed by the partial pressure of O_2 at 50% Hb saturation with O_2 (P_{50}). Mammalian Hbs can be broadly divided into two groups: low and high O_2 affinity Hbs. The Hbs of ruminant animals, such as cow, goat, and sheep, have intrinsically low O_2 affinity and are insensitive to 2,3-diphosphoglycerate (DPG) (Bunn, 1971; Perutz and Imai, 1980). The red blood cells of these animals have very low concentrations of DPG. In contrast, the Hbs of other mammalian species, such as human, horse, and dog, have intrinsically high O_2 affinity and high sensitivity to DPG (Bunn, 1971, 1980; Perutz *et al.*, 1993; Perutz and Imai, 1980). The red blood cells of these animals have high concentrations of DPG.

O_2 binding to Hb is generally an exothermic process: a decrease in temperature increases O_2 affinity, and this increased O_2 affinity suppresses O_2 release from Hb in peripheral tissues. Therefore, temperature is a critical determinant of O_2 transport by Hb. The temperature sensitivity of Hb is expressed as the overall heat of oxygenation, ΔH, including the intrinsic heat of O_2 binding to the hemes (an exothermic process) and the heats of all the processes associated with oxygenation, namely heats of proton and anion release, which are endothermic processes. The ΔH value of human Hb has been reported to vary from –9 to –14 under physiological conditions (Rossi-Fanelli *et al.*, 1964). Recently, exceptionally low ΔH values were found in arctic ruminant animals, such as reindeer and musk ox: their ΔH values are approximately 1/3 of that of human Hb (Brix *et al.*, 1989a; Condo *et al.*, 1988; di Prisco and Giardina, 2000). The physiological significance of this characteristic low sensitivity to temperature has been interpreted in terms of the animals' adaptation to low habitat temperature, *i.e.*, the low sensitivity to temperature can allow for the supply of an adequate amount of O_2 to cool peripheral tissues, such as skin and legs, which may be 10°C cooler than the lungs (Brix *et al.*, 1989a, 1989b; Giardina *et al.*, 1989).

Domesticated ruminants, such as cow, pig, and deer, also have relatively low ΔH values (approximately 2/3 of that

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of humans) (Clerbaux *et al.*, 1993; Giardina *et al.*, 1990; Willford and Hill, 1986). Therefore, these ruminant animals are cold-resistant. Investigations of the characteristic O_2 binding properties of bovine Hb have indicated that the low O_2 affinity of bovine Hb is not only an intrinsic property, but also a result of the influence of allosteric effectors (Bunn, 1971, 1980; Perutz *et al.*, 1993; Perutz and Imai, 1980). The molecular basis of the low O_2 affinity of bovine Hb was explained by Perutz and Imai (1980), who pointed out that compared with human Hb, bovine Hb has the same K_R value, but a low K_T value (*i.e.*, intrinsic low O_2 affinity of the T state) and a high L value (*i.e.*, stabilization of the T state), where K_R, K_T, and L are the MWC parameters (Monod *et al.*, 1965).

Recently, it has been suggested that the chloride (Cl^-) shift, which is the movement of Cl^- from the plasma into red blood cells, plays an important role in modulating O_2 delivery from Hb (Westen and Prange, 2003). It has also been shown that the binding of extra Cl^- to bovine Hb contributes to lowering ΔH (De Rosa *et al.*, 2004).

From the viewpoint of the physiological significance of O_2 transport, the alkaline Bohr effect is also important to facilitate O_2 release in peripheral tissues. In bovine adult Hb, the magnitude of the alkaline Bohr effect in the absence of organic phosphates is –0.53 (Smith *et al.*, 1979), and the Bohr effect is enhanced by the addition of Cl^- and DPG (Fronticelli *et al.*, 1984; Marta *et al.*, 1998; Perutz *et al.*, 1993). Furthermore, Clementi *et al.* (1996) showed that there is no difference in the log P_{50} vs. pH plot between the presence and absence of DPG at physiological Cl^- concentration (0.1 M).

To date, much attention has been focused on the structural basis for the low sensitivity to temperature of bovine Hb (Baudin-Creuz *et al.*, 2002; Fronticelli *et al.*,1995; Marta *et al.*, 1998; Perutz *et al.*, 1993; De Rosa *et al.*, 2004), whereas the combined effects of temperature and allosteric effectors on the alkaline Bohr effect have received relatively little attention. It is important to examine the influence of a wide range of temperatures on the Bohr effect. Therefore, in this study, O_2 affinity, cooperativity, and the alkaline Bohr effect were investigated in detail over the temperature range of 20 to 37°C, with focus on the influence of individual allosteric effectors and the combined effect of the effectors and temperature.

MATERIALS AND METHODS

Preparation of Hb solution

Bovine adult blood samples were purchased from Nippon Biological Material Center. The red blood cells were washed four times by centrifugation at 3,000 rpm for 15 min with isotonic saline solution and lysed with distilled water. Stroma were removed by centrifugation at 15,000 rpm for 20 min. The red supernatant was stripped of organic phosphates with a 54 × 2 cm Sephadex G-25 column equilibrated with 0.05 M Tris buffer, pH 7.4. All preparative procedures were carried out at 0–5°C.

Oxygen equilibrium curve measurement and data analysis

The oxygen equilibrium was measured with an automatic oxygenation apparatus developed by Imai and Yonetani (1977) and Imai (1981) at an Hb concentration of 60 μM on a heme basis, at four different temperatures in the range of 20 to 37°C. O_2 saturation of Hb was calculated from the change in absorbance at 576 nm, which was measured with a Shimadzu dual-wavelength spectrophotometer (model UV-3000). The concentration of O_2 in the sample cell was decreased by replacing air with pure N_2 gas, and its change was monitored with a Clark-type O_2 electrode. Hepes buffer solutions (over the pH range of 6.8 to 8.1) and Mes buffer solutions (over the pH range of 5.9 to 6.4) were used for OEC measurements. The pH value was measured for each air-equilibrated oxygenated sample, and was adjusted at the same temperature as that for OEC measurement. Met-hemoglobin (Met-Hb) formed by autooxidation was reduced with an enzymatic reducing system as described by Hayashi *et al.* (1973). The Met-Hb concentration at the end of OEC measurement, as described by Evelyn and Malloy (1938), was less than 5% of the total Hb concentration. The concentration of DPG was measured according to the enzymatic procedure of Ericson and de Verdier (1972).

The experimentally obtained oxygen equilibrium data were analyzed by the curve-fitting method described by Imai (1981), to estimate the four Adair constants. Using the Adair constants, an OEC was generated. The sigmoid shape of the OEC is expressed by Hill's coefficient (n_{max}) as the highest slope of the Hill plot (log [S/(1-S)] vs. log P plot) (Hill, 1910), and enables the unloading of large quantities of O_2 in tissues upon small decreases in P.

The log P_{50} vs. log [Eff] plot was described by fitting experimental data to the Wyman equation (Wyman, 1964), where [Eff] is the concentration of allosteric effectors, and the slope of the log P_{50} vs. log [Eff] plot was calculated.

The magnitude of the Bohr effect was calculated from the slope of the log P_{50} vs. pH plot (dlog P_{50}/dpH, the Bohr coefficient) in the pH range of 7.2 to 7.6.

The ΔH value was calculated from the slope of the van't Hoff plot (log P_{50} vs. 1/T plot) (Wyman, 1964), that is, $\Delta H/R = d\ln P_{50}/d(1/T)$. Here, R is the gas constant and T is the absolute temperature. The ΔH values obtained were corrected for the heat of O_2 solubilization (–3.0 kcal/mol).

RESULTS AND DISCUSSION

Effect of allosteric effectors on O_2 affinity

Fig. 1A shows the change in log P_{50} and n_{max} of bovine Hb induced by increasing Cl^- concentration at 20°C and 37°C. The increases in Cl^- concentration and temperature decreased the O_2 affinity, and the total Cl^--induced changes in log P_{50} values were 0.56 and 0.42 at 20°C and 37°C, respectively. The P_{50} value of stripped Hb (in the absence of both Cl^- and DPG) was slightly higher compared with a previous study (log P_{50} = 0.62 torr; De Rosa *et al.*, 2004). As judged from the position of the slope of log P_{50} vs. log [Cl^-] plot, the log P_{50} vs. log [Cl^-] plot at 37°C was slightly shifted to the right along the log [Cl^-] axis compared with that at 20°C, indicating a reduction in sensitivity to Cl^- with increasing temperature.

In the case of DPG (Fig. 1B), the total changes in log P_{50} values were 0.52 and 0.32 at 20°C and 37°C, respectively. A marked reduction in sensitivity to DPG was observed with increasing temperature. At 20°C, Cl^- and DPG produced nearly equal total changes in the log P_{50} value.

In the case of Cl^-, n_{max} decreased with increasing Cl^- concentration and temperature. In contrast, the shape of the OEC was invariant with change in DPG concentration.

Results shown in Fig. 1A and B imply that the physiological concentration of Cl^- is not sufficient to saturate Hb at 37°C; therefore, Cl^- plays some role in the regulation of O_2 transport *in vivo*. On the other hand, the physiological concentration of DPG (0.5 mM) is too low to regulate the O_2

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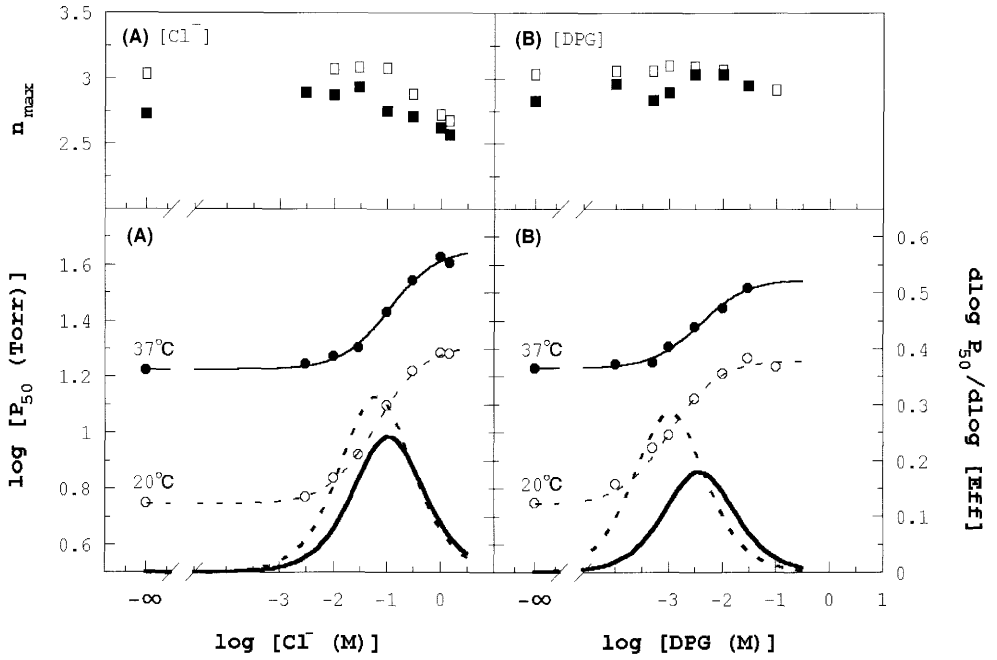

Fig. 1. Effect of **(A)** Cl^- and **(B)** DPG concentration on log P_{50} (○, ●) and n_{max} (□, ■) of bovine Hb in 0.1 M Hepes buffer at pH 7.4. Dashed and solid lines represent the slope of log P_{50} vs. log [Eff] plot. The temperature was 20°C for open symbols and dashed line and 37°C for closed symbols and solid line.

binding of bovine Hb.

Fig. 2A shows the effect of increasing Cl^- concentration on log P_{50} and n_{max} of bovine Hb in the absence of DPG, in the presence of 0.5 mM DPG, and in the presence of 5 mM DPG at pH 7.4 and 20°C. At low Cl^- concentrations, DPG strongly reduced O_2 affinity. Increase in Cl^- concentration led to an increase in P_{50}, and the highest P_{50} value was reached at 1 M Cl^-. Further increases in the Cl^- concentration instead reduced P_{50} and caused drastic decreases in cooperativity, suggesting the formation of Hb dimers at extremely high salt concentrations.

Fig. 2B shows the effect on log P_{50} and n_{max} values of increasing DPG concentrations at the physiological concentration of Cl^-. The effect in the absence of Cl^-, which was taken from Fig. 1, is also illustrated for comparison. At the physiological concentration of Cl^-, the log P_{50} value at the

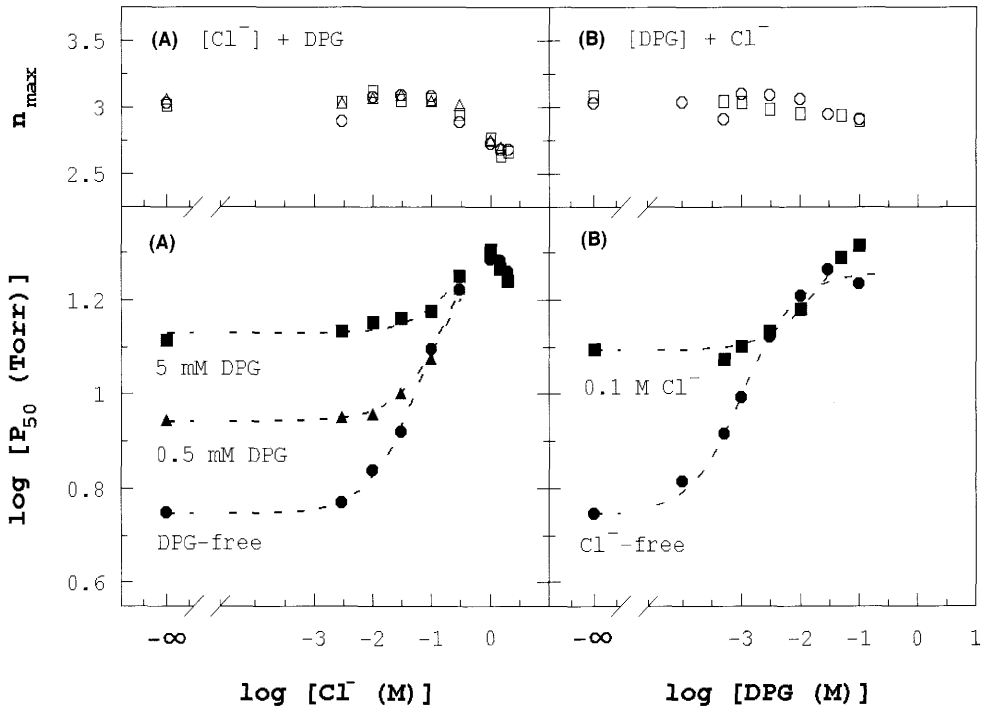

Fig. 2. Effect of **(A)** Cl^- and **(B)** DPG concentration on log P_{50} (closed symbols) and n_{max} (open symbols) of bovine Hb in 0.1 M Hepes buffer at pH 7.4 and 20°C. **(A)** ●, ▲, and ■ were in the absence of DPG and in the presence of 0.5 mM and 5 mM DPG, respectively. **(B)** ● and ■ were in the absence of Cl^- and in the presence of 0.1 M Cl^-, respectively. Data shown by ● were taken from Fig. 1.

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highest DPG concentration was higher than that attained by DPG only. In this connection, De Rosa *et al.* (2004) reported that at the physiological concentration of Cl^-, the increase in DPG concentration enhanced log P_{50}, and the total change in log P_{50} was approximately 0.12 higher than that obtained by DPG alone. The synergistic effects of Cl^- and DPG explain this characteristic phenomenon, which has been noted in bear Hb as well (Colleta *et al.*, 1994). In the case of human adult Hb, such a synergistic effect was not observed, which is explained by the loss of additional Cl^- binding sites (De Rosa *et al.*, 2004).

Influence of allosteric effector concentration on log P_{50} and n_{max} at three pH values

Fig. 3A shows the effect of increasing Cl^- concentration on log P_{50} and n_{max} of bovine Hb at 20°C in the absence of DPG and at three pH values (7.2, 7.4, and 7.6). The log P_{50} vs. log [Cl^-] plot shifted to the right with increasing pH, but the shapes of the curves at the three pH values were almost identical.

The effect on log P_{50} and n_{max} of increasing DPG concentration in the absence of Cl^-, and under the same experimental conditions as those for Cl^-, is shown in Fig. 3B. The curve shifted to the right with increasing pH, but the shapes of the three curves were almost identical.

Fig. 3C shows the effect on log P_{50} and n_{max} of increasing Cl^- concentration in the presence of the physiological concentration of DPG, and under the same experimental conditions as in Fig. 3A. At low Cl^- concentration, DPG enhanced log P_{50}. Increasing Cl^- concentration decreased the DPG effect, and at 0.1 M Cl^-, the log P_{50} value was nearly equal to that of DPG-free medium (Fig. 3A). This apparent disappearance of the DPG effect is in agreement with the results of a previous study (Clementi *et al.*, 1996). Using the results shown in Fig. 3, we estimated the magnitude of the Bohr coefficient (dlog P_{50}/dpH). A reduction in n_{max} at high Cl^- concentrations is apparent in Figs. 3A and 3C. In contrast to Cl^-, DPG had little effect on n_{max} (Fig. 3B).

Influence of temperature on magnitude of Bohr effect

Fig. 4A shows the influence of temperature on the magnitude of the alkaline Bohr effect of bovine Hb at six Cl^- concentrations in the absence of DPG. Increase in Cl^- concentration enhanced the Bohr coefficient, which reached a maximum at 0.1 M Cl^- and 20°C. However, further increases in Cl^- concentration instead decreased the Bohr coefficient. The change in temperature had little effect on the Bohr coefficient. A temperature-insensitive alkaline Bohr effect has also been reported in musk ox (Brix *et al.*, 1989a) and pig Hbs (Sinet *et al.*, 1982).

The results obtained from experiments similar to those performed above, but with seven DPG concentrations in the absence of Cl^-, are illustrated in Fig. 4B. The highest Bohr coefficient was observed at 3 mM DPG and 20°C. In contrast to Cl^-, at low DPG concentrations (0.1–3 mM), the DPG effects decreased with increasing temperature, whereas at high DPG concentrations (10–30 mM), the DPG effect was insensitive to temperature changes.

Fig. 4C shows the influence of temperature on the Bohr coefficient at six Cl^- concentrations in the presence of the physiological concentration of DPG. The highest Bohr coefficient at 0.1 M Cl^- is similar to the literature values reported by Clementi *et al.* (1996). The Bohr coefficient progressively decreased with increasing Cl^- concentration and temperature, and was drastically diminished at 37°C (–0.36).

By comparing the effect of temperature on the Bohr coefficient at the physiological concentration of Cl^-, which is shown in Fig. 4A (×), it is evident that DPG influences the Bohr coefficient at 20°C, whereas, its effect virtually disappears at high temperature (37°C). Taken together, these

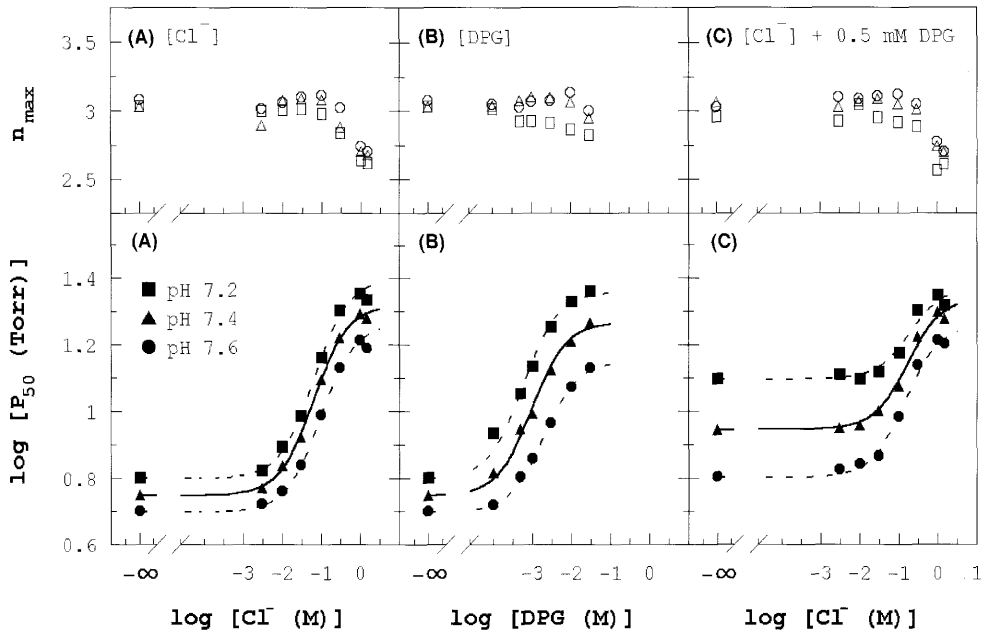

Fig. 3. Effect of **(A)** Cl^- concentration in the absence of DPG, **(B)** DPG concentration in the absence of Cl^-, and **(C)** Cl^- concentration in the presence of 0.5 mM DPG on log P_{50} (closed symbols) and n_{max} (open symbols) of bovine Hb in 0.1 M Hepes buffer at pH values of 7.2 (■), 7.4 (▲), and 7.6 (●), at 20°C.

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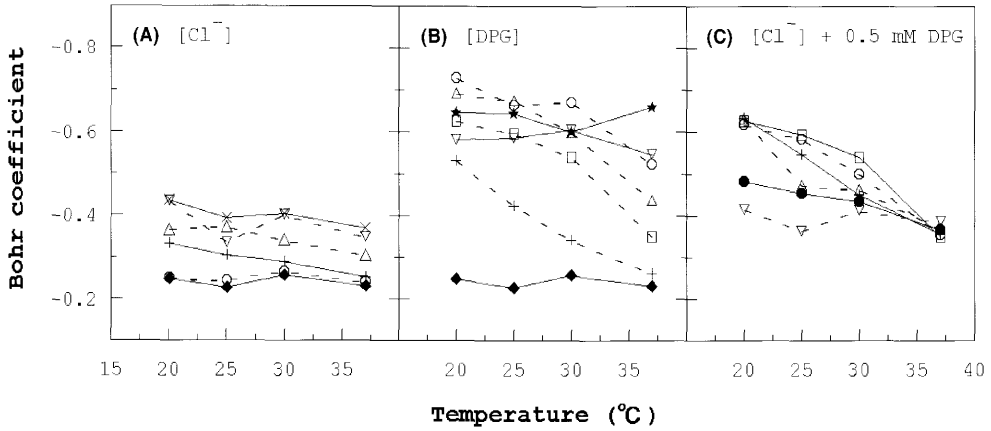

Fig. 4. Effect of temperature on the magnitude of the alkaline Bohr effect of bovine Hb in 0.1 M Hepes buffer: **(A)** at various Cl^- concentrations; **(B)** at various DPG concentrations; **(C)** at various Cl^- concentrations and in the presence of 0.5 mM DPG. **(A)** (◆) Cl^--free; (○) 0.003 M Cl^-; (+) 0.01 M Cl^-; (△) 0.03 M Cl^-; (×) 0.1 M Cl^-; (▽) 0.3 M Cl^-. **(B)** (◆) DPG-free; (+) 0.1 mM DPG; (□) 0.5 mM DPG; (△) 1 mM DPG; (○) 3 mM DPG; (★) 10 mM DPG; (▽) 30 mM DPG. **(C)** (□) Cl^--free + 0.5 mM DPG; (○) 0.003 M Cl^- + 0.5 mM DPG; (+) 0.01 M Cl^- + 0.5 mM DPG; (△) 0.03 M Cl^- + 0.5 mM DPG; (●) 0.1 M Cl^- + 0.5 mM DPG; (▽) 0.3 M Cl^- + 0.5 mM DPG.

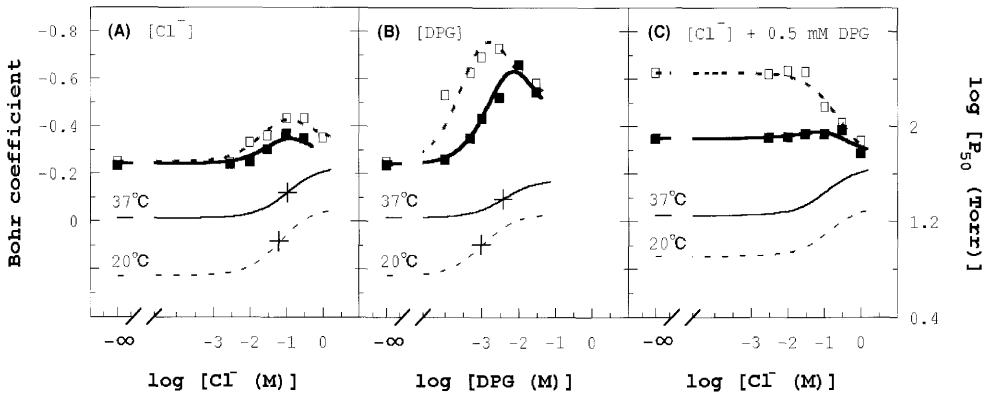

Fig. 5. Effect of **(A)** Cl^- concentration, **(B)** DPG concentration, and **(C)** Cl^- concentration in the presence of 0.5 mM DPG on log P_{50} and the magnitude of the alkaline Bohr effect of bovine Hb in 0.1 M Hepes buffer. Dashed and solid lines represent the log P_{50} vs. log [Eff] plot at pH 7.4, and 20°C and 37°C, respectively. Pluses represent the highest slope of the log P_{50} vs. log [Eff] plot. Open and closed squares represent the Bohr coefficient values at 20°C and 37°C, respectively. All the data were taken from Fig. 4.

results confirm that Cl^- plays an important role in regulating the *in vivo* function of Hb.

Log P_{50} vs. log [Eff] plot and Bohr effect

Figs. 5A and B show the relationship between the log P_{50} vs. log [Eff] plots at pH 7.4, and the magnitude of the Bohr coefficient. The highest slope of the log P_{50} vs. log [Eff] plot is shown by a "plus" in the figure. Since in Fig. 3 the log P_{50} vs. log [Eff] plots had nearly the same sigmoidal shape, it was expected that the maximum difference in log P_{50} (*i.e.*, the highest Bohr coefficient) would occur at the highest slope of the curve. As expected, the data in the figure showed that the Cl^- concentration that gave the highest slope was nearly equal to the Cl^- concentration at which the Bohr coefficient is maximal (Fig. 5A). Interestingly, at 37°C, this Cl^- concentration was nearly equal to the physiological Cl^- concentration.

In the case of DPG (Fig. 5B), the highest Bohr coefficient was also observed at the highest slope of the log P_{50} vs. log [DPG] plot. The DPG concentration that gave the highest Bohr coefficient at 37°C (10 mM) was higher than the physiological DPG concentration (0.5 mM).

In the presence of 0.5 mM DPG (Fig. 5C), the Bohr coefficient was the largest at low Cl^- concentration, and decreased strongly with increasing Cl^- concentration. Therefore, the Bohr coefficient vs. log [Cl^-] plot (Fig. 5C) was different from those involving a change in the concentration of Cl^- or DPG alone (Fig. 5A, 5B).

Correlation between P_{50} and magnitude of Bohr effect

The Bohr coefficient is useful for comparing interspecies difference in the magnitude of the Bohr coefficient. However, it does not directly indicate the Bohr effect-induced additional amount of O_2 released from Hb at the peripheral tissues and loading to Hb at the respiratory organs. The additional amount of O_2 released due to the Bohr shift at the O_2 release site depends on not only the magnitude of the Bohr shift but also the position and shape of the OEC and the O_2 pressure at the O_2 release site. In a previous study, we estimated the effectiveness of the Bohr shift in terms of the change in S at O_2 pressure per unit change in P_{50} ($dS_{(PO2)}/dP_{50}$), and it has been pointed out that the physiological P_{50} of human and horse Hbs is nearly optimized in order to receive the maximum benefits from the Bohr shift at

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the O_2 release site (Itoh *et al.*, 2001; Zhang *et al.*, 2003a, b). In this respect, it is also important to consider the magnitude of the Bohr effect in relation to P_{50}. Using the data shown in Fig. 4, we plotted the magnitude of the Bohr coefficient against log P_{50} (Fig. 6). This result showed that the influence of 0.1 M Cl^- alone and the combined influence of the physiological concentrations of Cl^- and DPG on the Bohr coefficient at 37°C were almost the same, meaning that Cl^- is a major determinant of the magnitude of the Bohr coefficient and P_{50}. However, it is important to note that the relatively low O_2 affinity (the physiological P_{50} of bovine Hb = 31.0 torr) is achieved at high temperature. In the case of the physiological concentrations of Cl^- and DPG, the decrease in P_{50} due to lowering the temperature slightly enhanced the Bohr coefficient. This may have been due to the presence of 0.5 mM DPG.

Van't Hoff plot at three pH values

Fig. 7 shows the van't Hoff plots of bovine Hb at three pH values. In the absence of both Cl^- and DPG (Fig. 7A), there was no difference in slope among the three plots at pH 7.2, 7.4, and 7.6. This means that the Bohr coefficient is independent of temperature, and that the ΔH values are independent of pH. The same trend was observed in the presence of physiological concentrations of Cl^- (Fig. 7B).

In the presence of the physiological concentration of DPG (Fig. 7C), the slope of the plot at pH 7.6 was higher

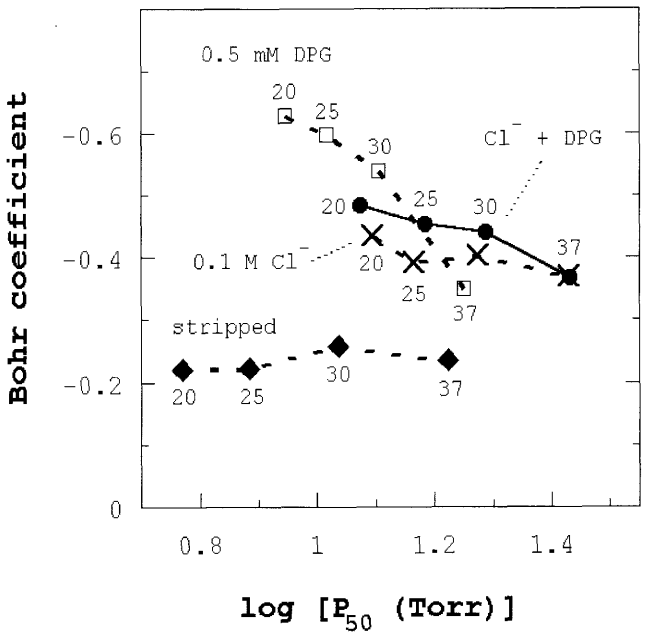

Fig. 6. Relationship between the magnitude of the alkaline Bohr effect and log P_{50} at pH 7.4 of bovine Hb. Conditions: 0.1 M Hepes buffer at four temperature (20, 25, 30, and 37°C) in the absence of both Cl^- and DPG (stripped Hb) (◆), in the presence of 0.1 M Cl^- (×), in the presence of 0.5 mM DPG (□), and in the presence of both 0.1 M Cl^- and 0.5 mM DPG (●). Numbers in the figure represent temperature values. All data were taken from Fig. 4.

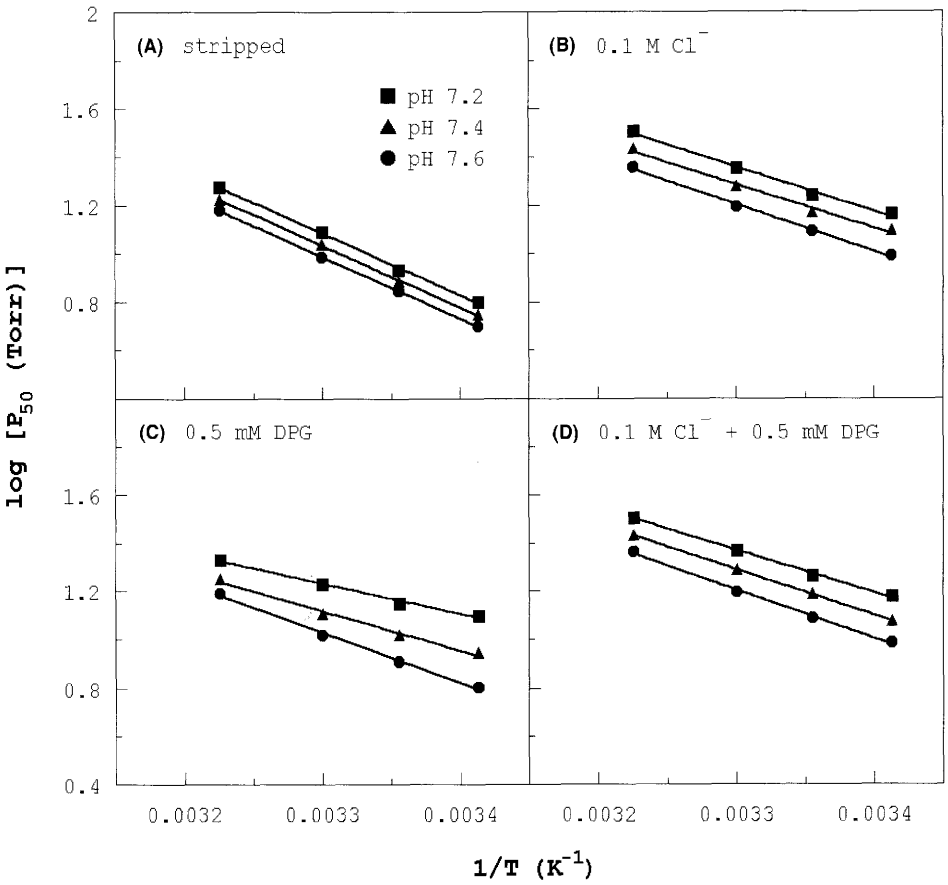

Fig. 7. Van't Hoff plots of the temperature dependence of log P_{50} values for bovine Hb in 0.1 M Hepes buffer at pH 7.2 (■), 7.4 (▲), and 7.6 (●): **(A)** in the absence of Cl^- and DPG (stripped Hb); **(B)** in the presence of 0.1 M Cl^-; **(C)** in the presence of 0.5 mM DPG; **(D)** in the presence of 0.1 M Cl^- and 0.5 mM DPG.

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than that at pH 7.2. This indicates that the Bohr coefficient decreases upon temperature increases, and that ΔH increases at high pH values. Almost the same trend was observed in the presence of physiological concentrations of Cl^- and DPG (Fig. 7D).

Correlation between ΔH and pH

For the same experimental conditions as in Fig. 6, Fig. 8 shows the ΔH of bovine Hb as a function of pH. The ΔH value depends markedly on pH. The concomitant presence of Cl^- and DPG reduced the temperature dependence of O_2 binding, because binding of O_2 is an exothermic process, whereas the accompanying release of allosteric effecters is an endothermic process. In the presence of 0.1 M Cl^- and in the absence and presence of 0.5 mM DPG, the ΔH values were nearly equal. The lowest value of –3.5 kcal/mol (absolute value) was observed in the presence of 0.5 mM DPG at pH 6.8. At the physiological concentration of Cl^- and DPG and at pH 7.4, the ΔH value was –5.8 kcal/mol.

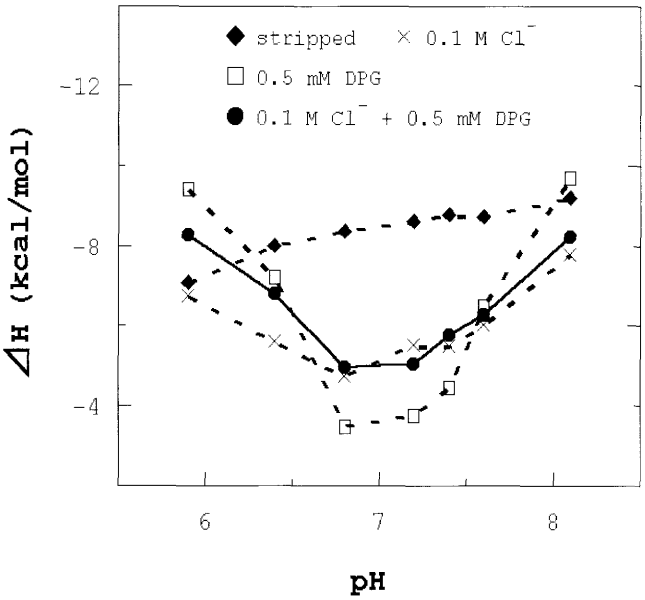

Fig. 8. Relationship between the ΔH of bovine Hb and pH. The ΔH values were calculated from the integrated van't Hoff equation by using the OEC: for 0.1 M Hepes buffer and 0.1 M Mes buffer in the absence of both Cl^- and DPG (stripped Hb) (◆); in the presence of 0.1 M Cl^- (×); in the presence of 0.5 mM DPG (□); and in the presence of 0.1 M Cl^- and 0.5 mM DPG (●). ΔH values were corrected for the heat contribution of O_2 in solution (–3.0 kcal/mol).

Physiological significance of low ΔH of bovine Hb

Fig. 9 illustrates a theoretical OEC that clarifies the physiological significance of the low ΔH of bovine Hb on O_2 delivery to peripheral tissues, where the temperature is assumed to be 5°C lower than that of the lungs (37°C). The OEC in (a) (P_{50} = 31.0 torr; Faber and Thornburg, 1983) is the standard OEC of bovine blood measured under physiological concentrations of Cl^- and DPG and at pH 7.4 and 37°C. The OECs in (b) (P_{50} = 24.5 torr) and (c) (P_{50} = 22.1 torr), respectively, were calculated to be those of bovine Hb (ΔH = –5.8 kcal/mol; cold-resistant Hb) and human Hb (ΔH = –9.6 kcal/mol; Imai and Yonetani, 1975; non-cold-resistant Hb) under physiological solvent conditions and at 32°C. The

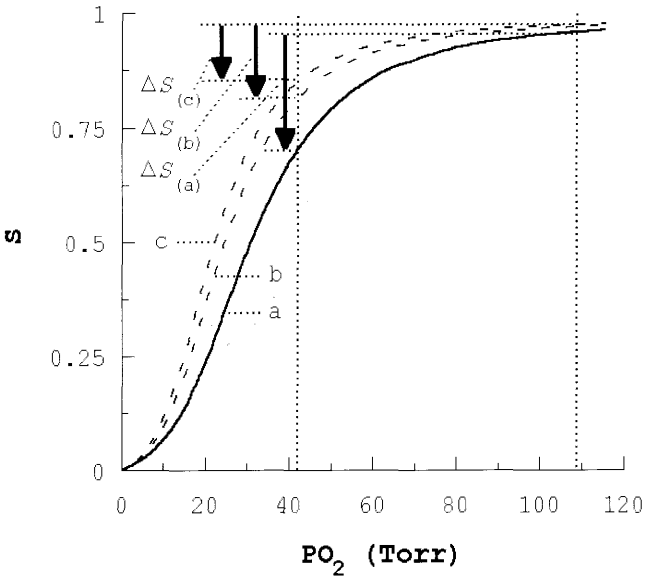

Fig. 9. Example data showing the amount of O_2 delivered to the tissues by Hb with ΔH values of –5.8 and –9.6 kcal/mol at peripheral tissues at 32 and 37°C. The OEC data with a P_{50} of 31 torr and an n_{max} of 2.8 of adult bovine blood measured under standard conditions (Faber and Thornburg, 1983) was used to generate OECs with various P_{50} values. O_2 delivered to the tissues was estimated using the theoretical OEC with a P_{50} value of 31.0 (a, 37°C), 24.5 (b, 32°C), and 22.1 torr (c, 32°C). The arrows, ΔS(a), ΔS(b), and ΔS(c), represent the arterio-venous O_2 saturation difference, O_2 delivered to the tissues, calculated using the OEC of (a) , (b) and (c), respectively. O_2 pressures of arterial and venous blood were 108 and 42 torr, respectively (Taylor *et al.*, 1987).

arrows (ΔS(a), (b), and (c)) indicate the arterio-venous saturation difference, which expresses the amount of O_2 delivered to the peripheral tissues by OEC (a), (b, cold-resistant Hb) and (c, non-cold-resistant Hb), respectively. The O_2 pressures (PO_2) of arterial (108 torr) and venous (42 torr) blood were taken from Taylor *et al.* (1987). When there was no decrease in the temperature of the peripheral tissues, the amount of O_2 delivered to the tissues was given by ΔS(a). On the other hand, when the temperature was 5°C lower, bovine Hb (ΔS(b)) could deliver about 4/3 of the O_2 delivered by human Hb (ΔS(c)). This difference seems to show the contribution of low temperature sensitivity of Hb to O_2 delivery to cool peripheral tissues. On the contrary, human and horse have non-cold-resistant Hbs. At body temperatures increased by hard exercise, these Hbs seem to have a lower O_2 affinity than cold-resistant Hb, which is advantageous for O_2 release.

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