Eur Biophys J (2004) 33: 490–496
DOI 10.1007/s00249-004-0401-8

ARTICLE

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Circular dichroism spectra of human hemoglobin reveal a reversible structural transition at body temperature

Received: 16 December 2003 / Revised: 9 March 2004 / Accepted: 10 March 2004 / Published online: 26 March 2004

Previously we have shown that human red blood cells (RBCs) undergo a sudden change from blocking to passing through a 1.3 ± 0.2-μm micropipette when applying an aspiration pressure of 2.3 kPa at a critical transition temperature ($T_c = 36.4 \pm 0.3$ °C). Low-shear viscosity measurements suggested that changes in the molecular properties of hemoglobin might be responsible for this effect. To evaluate structural changes in hemoglobin at the critical temperature, we have used circular dichroism (CD) spectroscopy. The thermal denaturation curves of human hemoglobin A (HbA) and hemoglobin S (HbS) upon heating between 25 and 60 °C were non-linear and showed accelerated denaturation between 35 and 39 °C with a midpoint at 37.2 ± 0.6 °C. The transition was reversible below 39 °C and independent of solution pH (pH 6.8–7.8). It was also independent of the oxygenation state of hemoglobin, since a sample that was extensively deoxygenated with N_2 showed a similar transition by CD. These findings suggest that a structural change in hemoglobin may enable the cellular passage phenomenon as well as the temperature-dependent decrease in viscosity of RBC solutions.

Keywords Circular dichroism · Heat denaturation · Hemoglobin oxygenation state · Red blood cells

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Introduction

Previously we have shown that human red blood cells (RBCs) undergo a sudden change from blocking to passing through 1.3 ± 0.2-μm micropipettes at a critical transition temperature of $T_c = 36.4 \pm 0.3$ °C when applying a −2.3 kPa aspiration pressure (Fig. 1). Low-shear viscosity measurements suggested that molecular changes in hemoglobin were initiating this cellular phenomenon (Artmann et al. 1998; Kelemen et al. 2001). From these findings we hypothesized that structural changes in hemoglobin at the critical transition temperature may account for these phenomena.

A well-known structural change of hemoglobin is related to oxygen binding. Crystallographic studies have shown that human hemoglobin A (HbA) can adopt two stable quaternary structures, deoxyhemoglobin (T-state) and oxyhemoglobin (R-state). The structural transition resulting from oxygen binding has been well characterized (Bettati et al. 1998). The two states differ from each other in their affinity for ligands and in the interactions between subunits. Binding of oxygen to the deoxygenated T-state occurs with low affinity. However, the binding of the ligand destabilizes the T-state and makes it more likely to switch to the high-affinity oxygenated R-state. Thus, as ligation proceeds, the gradual enrichment in HbA molecules in the R-state accounts for cooperativity in ligand binding (Silva et al. 1992; Li et al. 2000; Mihailescu and Russu 2001).

Sickle cell hemoglobin (HbS) differs from normal HbA only in the $\beta 6$ position, where a valyl replaces the normal glutamyl residue. Nevertheless, it is of high pathological relevance (Perutz 1990; Srinivasan and Rose 1994; Perutz et al. 1998; Walters et al. 2002).

The thermal denaturation of hemoglobin has been of great interest during the last decades as well. It has been shown that a number of variants of human hemoglobins are thermally denatured more readily than human HbA (Ruckpaul et al. 1971; Clementi et al. 1994). Half-lives of hemoglobins showing lowered thermal stability were

decreased. Kinderlerer et al. (1970, 1973) studied human oxyhemoglobins A, A2, C and S in detail. The denaturation of these hemoglobins was studied as a function of temperature between 20 and 60 °C, by using optical rotatory dispersion. HbS was of particular interest. In these studies, two plateaus of the fractional change upon heating in the observed optical rotation at 233 nm were seen, one above 55 °C and one below 25 °C. The changes observed for all hemoglobins were reversible, unless temperatures were maintained above 50 °C for a considerable period of time. No particular temperature transition was seen around 37 °C (Kinderlerer et al. 1973). Further thermal denaturation studies of hemoglobins using different methods also did not show any transition at this particular temperature (Yang and Olsen 1988, 1990).

Although other authors have seen no temperature transition of human hemoglobin, we felt encouraged to reevaluate the issue. A temperature transition, as shown at the cellular level in the micropipette studies mentioned above, must have a molecular basis. We were interested in a temperature transition of the hemoglobin molecule that might occur at about human body temperature (Artmann et al. 1998; Kelemen et al. 2001). Circular dichroism (CD) is a valuable tool to study the overall structural organization of protein molecules (Chang et al. 1978; Bierzynski 2001). In hemoglobin, all chromophores of the peptide groups, the amino acid side chains, and the planar heme are intrinsically symmetrical. Thus, the optical activity of hemoglobin depends on the relative positions of the different chromophores in the three-dimensional organization of the molecule. That is why CD spectroscopy is very sensitive to conformational transitions (Geraci and Parkhurst 1981).

In this paper, we will show for human HbA and HbS that in the temperature range of 25–60 °C the thermal denaturation is non-linear. There occurs an accelerated thermal denaturation around body temperature with a turning point at $T_c = 37.2 \pm 0.6$ °C. This transition may enable the micropipette passage phenomenon (Fig. 1) and it may have further, so far unknown, physiological implications.

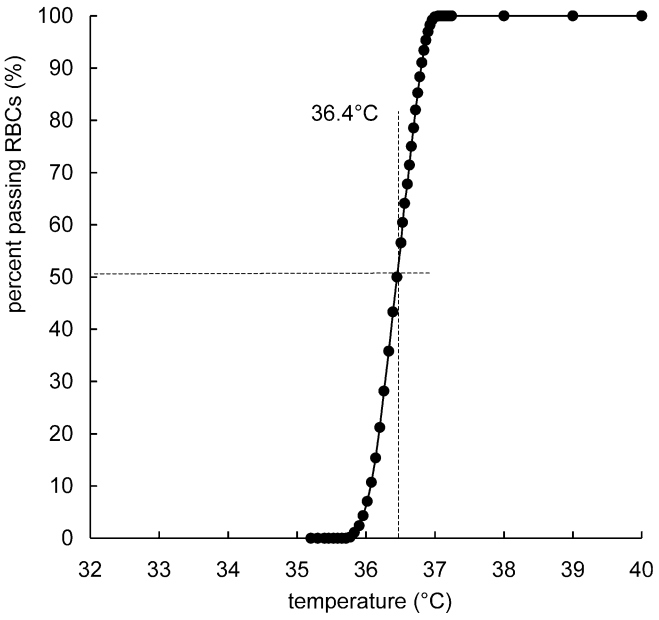

Fig. 1 Percent RBC passages through 1.3-μm micropipettes as a function of temperature, showing a critical temperature at $T_c = 36.4 \pm 0.3$ °C. To obtain these data at each temperature step, about 20–40 individual RBCs were aspirated into a pipette at an aspiration pressure of −2.3 kPa and the number of passing RBCs was counted. There occurred a "phase transition like" change from cells blocking the pipette's entrance below T_c to passing above (Artmann et al. 1998)

Materials and methods

Sample preparation

HbA was prepared from erythrocytes obtained from various healthy individuals. HbS was obtained from one homozygous sickle cell disease patient. Approximately 2 mL of heparinized blood was obtained from each individual donor. RBCs were harvested by centrifugation at 4200×*g* for 10 min. RBCs (0.5 mL) of the pellet were added to 9.5 mL buffer solution (0.1 M KCl, 61.3 mM Na_2HPO_4, 5.33 mM KH_2PO_4) (Cameron et al. 1988) and washed three times at 4200×*g* for 10 min. The appropriate pH (range 6.8–7.8) was adjusted with KH_2PO_4. RBCs (0.4 mL) were then hemolyzed in 3.6 mL distilled water and 1 mL of this solution was added to 9 mL KCl buffer, filtered, separated by column electrophoresis, and diluted further to the final concentrations used for CD measurements. The HbA solution was analyzed by SDS-PAGE electrophoresis and mass spectrometry for purity. The molecular masses of the hemoglobin subunits were determined by liquid chromatography-mass spectrometry (QSTAR, Applied Biosystems): human Hbβ at 15.9 kDa, heme at 616.2 Da, and Hbα at 15.13 kDa.

Circular dichroism measurements

The far-UV CD spectra were measured with a CD spectrometer (Aviv model 202, Aviv Instruments, Lakewood, USA). Temperature regulation was carried out using the built-in temperature control device. Comparison of the actual temperature inside the cuvette with the temperature set by the Peltier element showed that the deviation was < 0.1 °C. Thermal denaturation of hemoglobin was studied between 25 and 60 °C. The hemoglobin solution was first adjusted to 25 °C and then the temperature was stepwise increased. Each temperature was allowed to equilibrate for 1 min. Afterwards a complete wavelength scan (wavelength steps 1 nm, average time 4 s) was carried out in the far-UV region between 190 and 260 nm. This procedure was repeated two to three times, always using a new sample. At identical conditions to those used with hemoglobin solutions, solvent ("blank") wavelength scans of pure buffer solutions were carried out. Blank spectra were subtracted from the Hb spectra at each temperature point. From these wavelength scans the absolute ellipticity at 222 nm was derived, representing a measure of the α-helical content of the proteins (Greenfield 1996). The absolute ellipticities in CD spectroscopy depend on the Hb concentration. We investigated hemoglobin concentrations between 0.10 and 0.75 mg/mL. We calculated the fractional change in the observed ellipticity at 222 nm according to Kinderlerer et al. (1973):

$$F_{obs} = [E_{obs}(T) - E_{60}]/[E_{25} - E_{60}] \quad (1)$$

where $E_{obs}(T)$ is the ellipticity at 222 nm at temperature T, E_{60} is the ellipticity at 60 °C, and E_{25} that at 25 °C. At a wavelength of

222 nm, the CD data are most sensitive to the α-chain contents in globins (Greenfield 1996).

For estimating the α-helical contents of oxyhemoglobin A molecules at temperatures between 25 and 60 °C, the CD spectra deconvolution software CDNN 2.1 was used (software courtesy by Gerald Böhm, Martin-Luther-Universität Halle-Wittenberg, Germany).

Reversibility of the temperature denaturation

To test the reversibility of the temperature denaturation, the hemoglobin sample was first equilibrated at 25 °C and the ellipticity was measured as wavelength scans at an average time interval of 4 s between 218 and 224 nm. Then the sample was heated at a rate of 10 °C/min, and wavelength scans were taken at time points as indicated in Fig. 4. The temperature of the hemoglobin sample reached a steady state after approximately 1.5 min. After 19 min the sample was cooled down to 25 °C at a rate of 10 °C/min. The relative ellipticity was calculated as:

$$E_{\mathrm{rel}} = 100 \times [E(t) - E_0]/E_0 \quad (2)$$

where $E(t)$ is the ellipticity at 222 nm at time t and E_0 is the ellipticity at $t = 0$ (25 °C).

Deoxy/oxyhemglobin CD wavelength scans

Samples obtained from the same donor on the same day were studied under both oxy and deoxy conditions. Wavelength scans were carried out as described above. To deoxygenate hemoglobin, the sample was incubated with 100% nitrogen for 3 h before the measurements. For hemoglobin S, only the oxygenated state was investigated. In the deoxygenated state of hemoglobin S, molecular precipitation occurs (Asakura et al. 1975).

Results

Thermal denaturation of hemoglobin monitored by CD

The thermal denaturation of hemoglobin A was monitored between 190 and 260 nm at stepwise intervals between 25 and 60 °C. As expected, the far-UV CD spectra of HbA purified from RBCs contained a typical α-helical signature, with local minima at 208 and 222 nm (Li et al. 2000). The loss in ellipticity upon heating was clearly visible (Fig. 2). The wavelength of 222 nm was selected to monitor the temperature-dependent changes in ellipticity. At 222 nm between 25 and 60 °C there was in total an approximate 28% loss in ellipticity, indicating the thermal denaturation of the HbA molecules. A typical experimental error of 0.2% at 222 nm, 25 °C, and pH 7.4 was registered.

Raising the temperature from 34 to 39 °C by equidistant temperature steps of one degree did not lead to an equidistant decrease of ellipticity (Fig. 2). In particular, at 222 nm the difference in ellipticity between 36 and 37 °C as well as between 37 and 38 °C was significantly higher than those between the other spectra shown. Thus, the thermal denaturation of hemoglobin within the temperature range observed was not a linear function of temperature. Since the observed differences in ellipticity at the temperature steps chosen were tiny, the accuracy of the CD spectrometer became a critical issue. The decrease in ellipticity between 25 and 60 °C was independent of protein concentration over the range 0.18–0.74 mg/mL, suggesting that this unfolding transition was not due to changes in the association state of hemoglobin (Guidotti 1967).

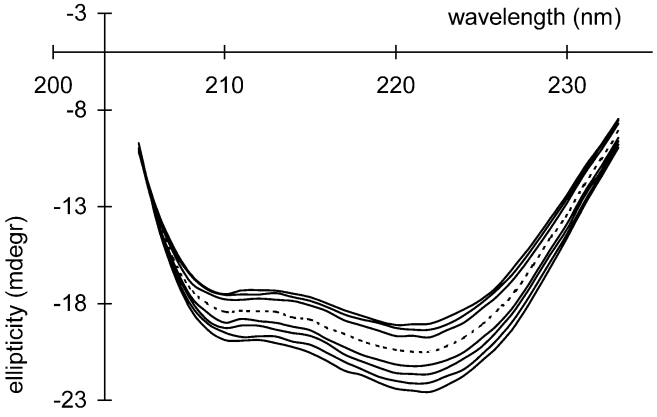

Fig. 2 Original far-UV wavelength scans of HbA in oxy form in the 205–235 nm region. Each line represents one wavelength scan obtained at constant temperature. The temperature range scanned was 25–60 °C. The figure gives an overall impression about temperature denaturation of hemoglobin A. It clearly shows that the differences in ellipticity at 222 nm between 36 and 37 °C as well as between 37 and 38 °C are significantly higher than those between scans obtained at other temperatures

Experiments have also been carried out as a function of exposure time at constant wavelengths and temperatures. Above 41 °C, the thermal denaturation became time dependent. Hence, in order to obtain comparable F_{obs} versus temperature curves, it was critical to maintain the same time pattern in all experiments, particularly for experiments conducted above 41 °C.

Effects of solution pH on the thermal denaturation of hemoglobin

We investigated pH effects on the thermal denaturation of HbA. The absolute ellipticity obtained at 222 nm was plotted against temperature. At all pH values chosen (pH 6.8, 7.0, 7.2, 7.4, and 7.8), non-linear, polynomial thermal denaturation curves were obtained, showing an average midpoint at $T_c = 37.2 \pm 0.6$ °C (Fig. 3a). The absolute ellipticities at 222 nm differ at the various pH values. The ellipticities observed within the range of temperatures studied were always lowest at pH 6.8, which represents the isoelectric point of HbA. In contrast, it was always highest at pH 7.0. At pH 6.8 as well as at 7.0 the slope of the denaturation curve above 50 °C appeared to be higher than those at other pH values. Using solutions at pH 7.2 and 7.4, respectively, the corresponding ellipticities at 222 nm did not differ significantly from each other.

To analyze and compare the CD data obtained under various conditions, we plotted the fractional change in

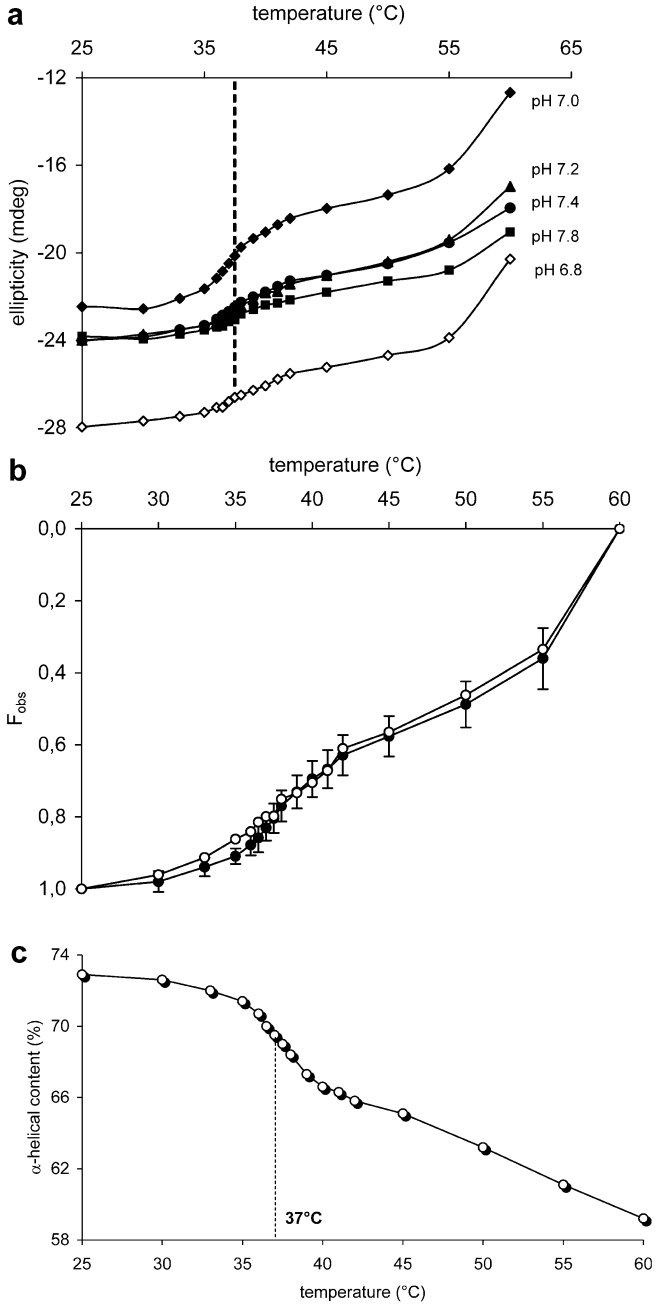

Fig. 3 a Absolute change of ellipticity of oxyhemoglobin A at 222 nm with temperature and pH (6.8–7.8). **b** Fractional change in ellipticity at 222 nm (F_{obs}) with temperature for oxyhemoglobin A. Data points (*filled circles*) were averaged from the original data obtained from hemoglobin solutions at the pH values of 6.8, 7.0, 7.2, 7.4 and 7.8. The *error bars* represent the standard deviation of the respective fractional changes. *Open circles* represent data points obtained at pH 7.4 alone. **c** Estimated α-helical content of oxyhemoglobin A molecules at temperatures between 25 and 60 °C obtained with the CD spectra deconvolution software CDNN 2.1

ellipticity (F_{obs}) at 222 nm versus temperature (Kinderlerer et al. 1973). By varying the solution pH (Fig. 3b) it was revealed that F_{obs} was highly non-linear and independent of pH. The slope of F_{obs} was increased at

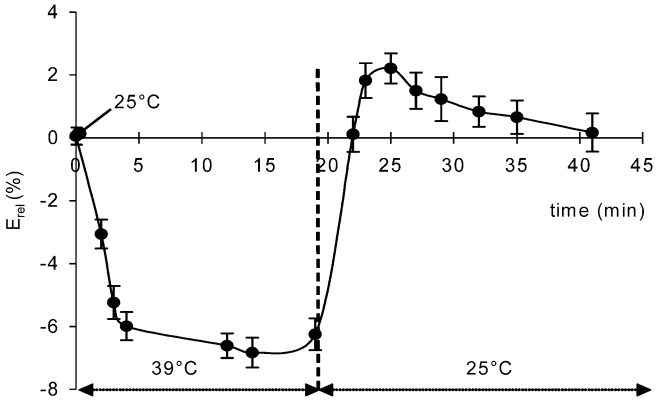

Fig. 4 Structural change of hemoglobin with temperature, tested for its reversibility. The hemoglobin sample was first equilibrated at 25 °C and the ellipticity was measured as wavelength scan at 4 s average time between 218 and 224 nm. Then, at a heating rate of 10 °C/min, the solution was heated to 39 °C and wavelength scans were taken at time points indicated in the figure. The solution temperature had reached a steady state after approximately 1.5 min. The temperature was kept at 39 °C for 19 min. At the end of this period the sample was cooled down to 25 °C. Again, wavelength scans were taken at different time intervals. The relative ellipticity at time t was calculated as $E_{rel} = 100 \times [E(t) - E_0]/E_0$, where E_0 was the ellipticity at 25 °C

temperatures around 37 °C: between 35 and 39 °C there appeared to be an accelerated fractional change with a midpoint at $T_c = 37.2 \pm 0.6$ °C. Counting F_{obs} between 25 and 60 °C at 222 nm as 100%, about 21.5% goes to the account of a temperature change from 35 to 39 °C, in other words to the account of raising the temperature by 4 °C only.

The α-helical content of oxyhemoglobin A molecules at temperatures between 25 and 60 °C was estimated. As expected, it was not a linear function of temperature. Considering the loss of α-helices between 25 and 60 °C as 100%, the thermal denaturation led to a loss of α-helices of about 18.8%. Out of this 18.8%, however, 5.6% was lost between 35 and 39 °C, a 4 °C rise only (Fig. 3c).

Unfolding reversibility of hemoglobin at pH 7.4

We studied the reversibility of the structural change for oxy-hemoglobin A solutions at pH 7.4 (Fig. 4). By ramping the temperature at a rate of 10 °C/min, a steady-state value of 39 °C was attained after approximately 1.5 min. The ellipticity reached an essentially steady state after approximately 5 min, i.e. 3.5 min after the attainment of the steady temperature at 39 °C. About 19 min after the beginning of heating, the sample was rapidly cooled down to 25 °C at a rate of 10 °C/min. The relative ellipticity rose and overshot to positive values, with a maximum at 5 min after the cooling started. It took about another 15 min for the relative ellipticity to return to the baseline value prior to a new heating step.

Effects of deoxygenation on the thermal unfolding of hemoglobin

We compared the CD spectra of oxygenated versus deoxygenated human HbA. Figure 5 (left) shows the respective fractional changes versus temperature at 222 nm for these two forms of hemoglobin. The nonlinearity as seen in previous pH studies is clearly visible, with a change in the steepness of the slope between 35 and 37 °C. There was almost no difference between the fractional changes of oxygenated and deoxygenated hemoglobin. The temperature transition for hemoglobin conformation as observed around body temperature seemed to be independent of the state of oxygenation of hemoglobin.

Thermal unfolding of sickle cell hemoglobin

Sickle cell hemoglobin (HbS) differs from normal HbA only in one amino acid. We used this natural mutation to investigate whether it affected the fractional change of ellipticity (F_{obs}) at 222 nm as a function of temperature. As can be seen in Fig. 5 (right), we observed basically the same polynomial curve as for HbA. Thus, even though this mutation is present, there was no effect to the structural transition at around body temperature, as observed at various conditions in HbA.

In summary, for the various experimental conditions and the types of hemoglobins studied, we always found an accelerated loss in structure around body temperature. These results indicate a common, structural transition of human hemoglobin at around body temperature.

Discussion

Human RBCs undergo a sudden change from blocking to passing through 1.3-μm micropipettes (Fig. 1) at a transition temperature (T_c) of 36.4 °C (Artmann et al. 1998; Kelemen et al. 2001). In the same studies, it was revealed by low-shear viscosity experiments that concentrated hemoglobin solutions exhibited a temperature transition as well at about 37 °C. From this we concluded that the temperature transition of RBCs entering small pipettes is a biophysical cellular phenomenon, which is caused by the hemoglobin molecule solely as the major cytosolic protein. Inasmuch as the experimental boundary conditions below and above T_c were the same, the hemoglobin molecule must have undergone a transition in its structure at T_c. The current study supports this model in which there is a temperature-dependent structural change in hemoglobin around body temperature.

The use of CD spectroscopy is generally of great advantage in protein structural studies (Chang et al. 1978; Geraci and Parkhurst 1981; Perutz et al. 2002). The most striking result in our experiments was a nonlinear thermal denaturation curve for human hemoglobin between 25 and 60 °C (Figs. 2, 4, 5). Out of the total thermal denaturation observed between 25 and 60 °C, 21.5% goes to the account of a temperature increase from 35 to 39 °C only. The midpoint of these curves at all hemoglobins investigated and at all experimental conditions examined was at $T_c = 37.2 \pm 0.6$ °C. The estimated α-helical content of the HbA molecule showed a similar temperature course.

This experimental finding led us to suggest the following mechanism underlying the temperature transitions observed earlier in experiments on RBC micropipette aspiration and hemoglobin viscosity measurements. A slight structural change of the hemoglobin molecule upon heating occurs at around T_c. This alters the physical properties of the molecule to trigger the sudden onset of the RBC passage through narrow pipettes at T_c as well as the sudden decrease in the viscosity of highly concentrated hemoglobin solutions (Artmann et al. 1998). Since the sharp drop of the viscosity of hemoglobin solutions at around 37 °C can only be seen at concentrations above 45 g/dL but not at 33 g/dL (physiological concentration), it was reasonable to conclude that the average intermolecular space and the corresponding forces are critical factors (Kelemen et al. 2001). At these high protein concentrations, below T_c a gel might form and be maintained by structured water bridges between the hemoglobin molecules. The hemoglobin molecule's structural change at T_c may have two

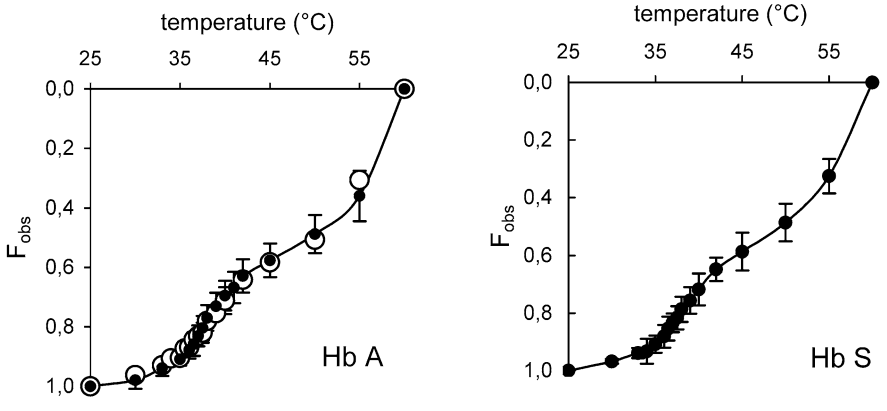

Fig. 5 Fractional changes (F_{obs}) in ellipticity at 222 nm with temperature for human hemoglobin A (*left*) and hemoglobin S (*right*). The *filled circles* represent the oxygenated state of the hemoglobins. The *error bars* represent the standard deviation of the respective fractional changes. The deoxygenated state of hemoglobin S was not studied because of the molecular precipitation occurring in the deoxygenated state (Asakura et al. 1975)

possible effects: (1) the transformation of hemoglobin-bound water into free water (Lumry and Rajender 1970; Privalov et al. 1971; Privalov 1990; Hildebrandt et al. 2002) and (2) an increase in hydrophobicity of the surface of the molecule, resulting in a partial release of bound water and/or aggregation of the hemoglobin molecules (Perutz et al. 2002). Both mechanisms would result in lower viscosity values above T_c and thus would explain the gel-to-fluid transition at T_c. Further experiments are needed to investigate whether bound water release and/or molecular aggregation occur at T_c.

Interest in the thermal stability of human hemoglobins is not new. Kinderlerer et al. (1973) studied the thermal denaturation of various human oxyhemoglobins and hemoglobin S as a function of temperature (20–60 °C) by using optical rotatory dispersion. In those studies, two plateaus of the fractional change in the observed optical rotation at 233 nm were seen, one above 55 °C and one below 25 °C. No particular temperature transition was found around human body temperature. This is in clear contradiction to the non-linear polynomial function we found with CD spectroscopy. There may have been several reasons why this thermal transition of hemoglobin with a midpoint at 37.2 ± 0.6 °C had not been observed before: (1) the modern instrumental technology and precision required was not available some decades ago, (2) the effect easily could have been missed because, although significant, it is very small, (3) Kinderlerer et al. used optical rotatory dispersion spectroscopy at 233 nm.

The structure of hemoglobin solutions is affected by pH (Monti et al. 1989). The absolute ellipticities of hemoglobins between pH 6.8 and 7.8 as a function of temperature were significantly different from each other. Between 25 and 60 °C the ellipticities were always highest at pH 7.0 and always lowest at pH 6.8, respectively. At pH 6.8 as well as at 7.0 the denaturation above 50 °C appeared to be little higher than those at other pH values. Figure 3b shows that the fractional change in ellipiticity, F_{obs}, was highly non-linear but independent of pH. The standard deviations of the fractional change as seen in Fig. 3b were significantly higher at temperatures above 42 °C than those below. This does not necessarily indicate that the thermal denaturation of hemoglobin above 42 °C is significantly stronger affected by solution pH than below 42 °C. The larger error bars at higher temperature are probably due to irreversible unfolding, since the ellipticity is highly dependent on the heating time at the higher temperatures.

The cytosolic pH of RBCs in the microcirculation varies between pH 7.2 and 7.4 (Monti et al. 1989). We found that the corresponding ellipticities at 222 nm did not differ much from each other (Fig. 3a). This means that physiological changes of cytosolic pH values hardly alter the non-linear nature of the fractional ellipticity changes as seen in Fig. 3b. This non-linearity does not depend on solution pH. The curve characteristic is caused solely by heat denaturation. This result supports the hypothesis that the temperature transition is a so-called predenaturing transition. Predenaturing processes usually are weakly dependent on pH (Lumry 1974).

The structural change upon heating of human hemoglobin A at pH 7.4 is reversible, as shown in Fig. 4. At 25 °C the molecules assume their native structure; at 39 °C the molecules have passed the structural transition at T_c. Kinderlerer et al. (1970, 1973) also found that the temperature denaturation was reversible over the same temperature range, unless the temperatures were maintained above 50 °C for a considerable period of time. Furthermore, they reported that the speed of denaturation was faster than the speed of renaturation, which we found as well. Our new finding, however, was the non-linear heat denaturation curve showing a midpoint at 37.2 ± 0.6 °C. This transition was reversible at least up to 39 °C. Above 41 °C the thermal denaturation became time dependent, which may indicate the onset of an irreversible denaturation of HbA.

We carried out CD measurements with oxygenated as well as with deoxygenated HbA. The fractional change at 222 nm in our study did not show any significant difference between oxygenated and deoxygenated hemoglobin (Fig. 5, left). This leads to the conclusion that the structural temperature transition at T_c is independent of the state of oxygenation of hemoglobin.

It was also of interest to see whether the single amino acid replacement encountered in HbS would change the heat denaturation pattern found in HbA. In fact the temperature course of the fractional change of oxygenated HbS was almost identical to those observed with HbA (Fig. 5, right).

The temperature transition as observed in the CD experiments with $T_c = 37.2$ °C and and $\Delta T = \pm 0.6$ °C is not as narrow as the transition observed at the cellular level in pipette experiments with $T_c = 36.4$ °C and $\Delta T = \pm 0.3$ °C (Artmann et al. 1998). Thus, it is possible that the thermally induced structural changes of the hemoglobin molecule has to reach a certain threshold until the hemoglobin solution becomes dynamically unstable and the gel-to-sol viscosity breakdown sets in (Makarevic et al. 2002).

In summary, the denaturation of human HbA and HbS upon heating is highly non-linear and shows a midpoint at $T_c = 37.2 \pm 0.6$ °C. It is reversible below 39 °C and is independent of the pH level (6.8–7.8) or the state of oxygenation.

Regarding the clinical and physiological significance of these findings, there can only be speculations at the moment. However, some interesting research might emerge in the future. If accelerated structural changes of hemoglobin around 37 °C would involve changes in the proportion of molecular bound water and/or cause a molecular aggregation, then this would affect the osmotic pressure balance between cytosol and blood plasma. This then might play a role in homeostasis at fever conditions (Ryan and Levy 2003). In terms of RBC fluidity and disease, exposure to cold may be relevant in some cases of Raynaud disease (Charkoudian 2003).

Improved RBC fluidity at moderate hyperthermia may be relevant to exercise physiology (Temiz et al. 2000). Finally, above 41 °C, a temperature found in most severe hyperthermic diseases (e.g. severe sepsis, heat stroke, etc.), the irreversibility of the structural transition may have pathophysiological relevance (Hildebrandt et al. 2002).

Acknowledgements This work was supported by grants to G.M.A. from the Ministry of Science and Education in North Rhine Westfalia, Germany. Experiments have in part been performed in the Department of Bioengineering, University of California, San Diego and at the Department of Chemistry and Biochemistry (Prof. Susan Taylor). We thank Dipl. Ing. Carsten Meixner for his patience in carrying out a significant part of the CD experiments presented here. We also thank Prof. Y.C. Fung who followed these experiments with great interest, enthusiasm and always with a fabulous smile.

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