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ENTHALPY OF DENATURATION FOR HUMAN HEMOGLOBIN IN THE OXYGENATED AND DEOXYGENATED STATE

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Abstract

This paper deals with the enhanced thermostability of hemoglobin in the deoxygenated state. Calorimetric measurements show that the temperature of denaturation is increased for Deoxy-Hb by about 13 K. The specific enthalpy of denaturation changes from about 14 J/g for Oxy-Hb to 32 J/g for Deoxy-Hb.

This experimental results may have consequences for the physiological transfer of Oxy-Hb to Deoxy-Hb and vice-versa. The equivalent of the experimental results on the molecular level however is not yet understood.

Introduction

Calorimetric investigation of the specific heat capacity of hemoglobin-water-salt mixtures (12) showed, as a secondary result, two distinct denaturation peaks for Hb. Object of this paper is the verification and quantification of this effect.

Method

The experiments were done with an adiabatic calorimeter (11) and a differential scanning calorimeter (DSC 2 Perkin Elmer, Überlingen, F.R.G.). The heating rates were 10 $\frac{K}{h}$ and 10 $\frac{K}{min}$

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respectively over the temperature interval from 280 K to about 380 K. The adiabatic calorimeter needs no calibration, neither for enthalpy nor for temperature. The DSC was calibrated with iridium, n-oktadecane, and cyclohexane.

All Hb samples were prepared from fresh human blood. Erythroc suspension was obtained by centrifugation of fresh blood with 1/ citrate in addition. Erythrocytehemolysate was prepared by addit of destilled water. The hemoglobin stock solution was produced following the recipes of different authors (2,6,7,9). The stock solution was completely free of salt.

The transfer of Hb into the deoxy state was obtained by bubbl the sample with CO_2 under a CO_2 atmosphere.

Results

Ampoule sealed samples of erythrocyte suspension showed in the adiabatic calorimeter quite different areas of denaturation (fig. 1a, 1b, 1c). The only difference thereby was the time dela between sealing and measurement (1a: 12 h; 1b: 24 h; 1c:40:) The DSC investigation showed that the early measurement (fig. 1a) wa done with Oxy-Hb and the late one with Deoxy-Hb (fig. 1c).

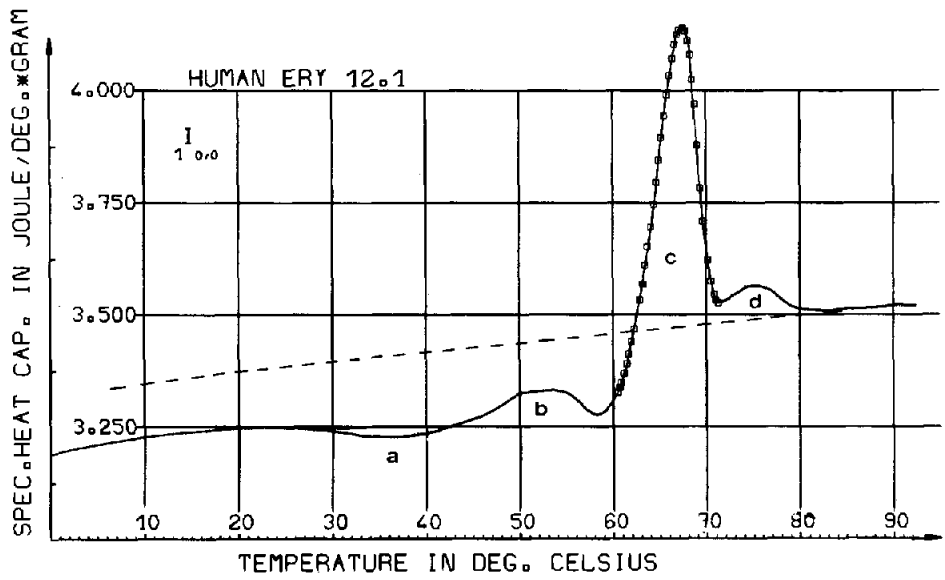

fig. 1a: "Specific heat capacity" of human erythrocytes in completely oxygenated state.
Region a: heat production of the living cells
b: melting of the membrane (8)
c: denaturation of Oxy-Hb
d: denaturation of Deoxy-Hb.
The broken line shows the c_p of the thermally denaturated sample.

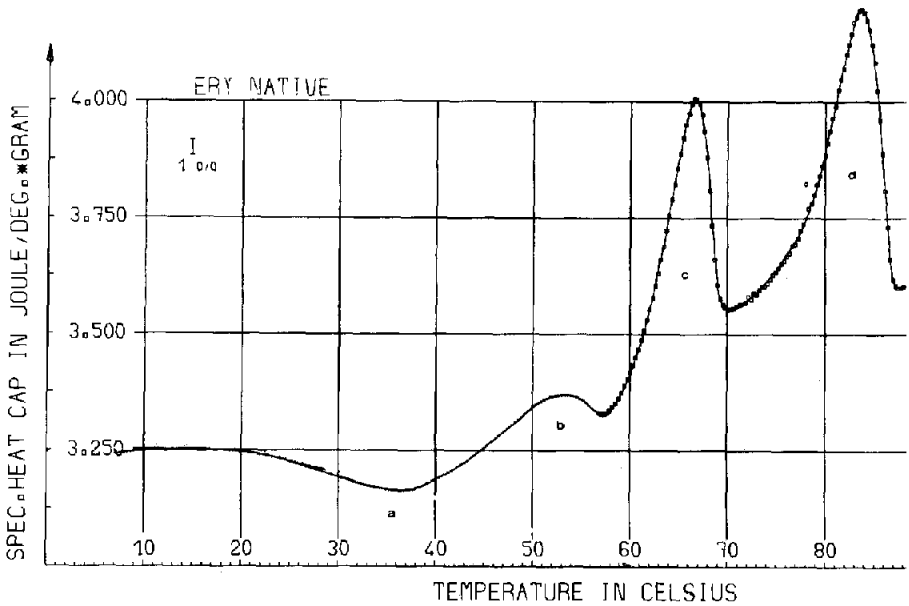

fig. 1b: The same as fig. 1a only half oxygenated.

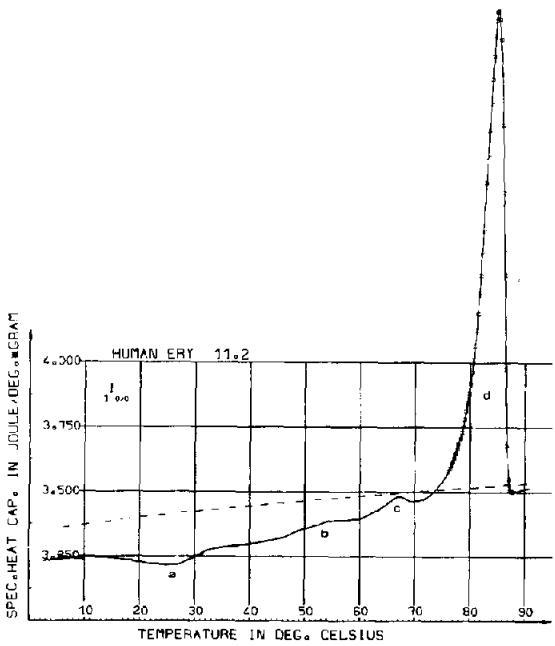

fig. 1c: The same as fig. 1a nearly fully deoxygenated.

Differential scanning calorimetry is not so precise as the adiabatic one but it was used to get results in a shorter time. The relation between the specific heat capacity of the samples and the denaturation enthalpy is a little bit unfavourable (fig. 2). Therefore it was sometimes difficult to define the baseline and the physical quantities as enthalpy and temperature of denaturation (fig. 3).

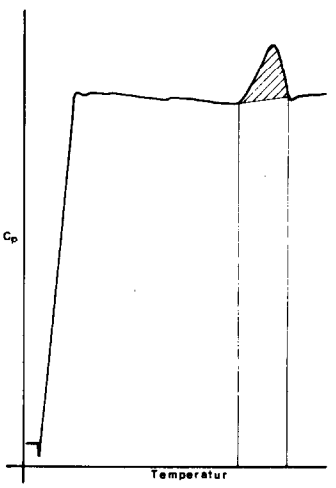

fig. 2: Complete DSC thermogram of an ery sample, where the relation of c_p and denaturation is to be seen.

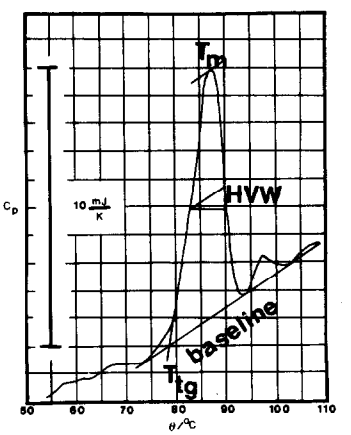

fig. 3: DSC thermogram of an oxygenated ery sample. The best estimate of baseline and the parallels for half value width and temperature of the maximum are shown. The denaturation temperature T_{tg} is the intersect of the tangent of the ascending branch with the baseline.

The plotted thermograms were digitized with an ultrasound stick and the areas of denaturation were calculated by computer (PDP 11/ Digital Equipment). The different enthalpies of denaturation fc Oxy-Hb and Deoxy-Hb have to be calculated by iterative computati The reason is that the unknown quantities, the weight fractions (w_1, w_2) and the enthalpies of denaturation (Δh_1, Δh_2), could not be separated (equ. 1)

(1) $$\Delta h = w_1 \Delta h_1 + w_2 \Delta h_2$$

with Δh: specific enthalpy of denaturatic

w_1, w_2: weight fraction of Oxy-Hb, Deoxy-Hb respectively

Δh_1, Δh_2: specific enthalpy of denaturatic of Oxy-Hb, Deoxy-Hb respectivel

For the iterative computation equation (1) was transformed into equations (2) and (3)

(2) $$\Delta h_1 = \Delta h \left[z_1 + y_1 (1 - z_1)\right]$$

(3) $$\Delta h_2 = \Delta h \left[z_2 + y_2 (1 - z_2)\right]$$

with $z_1 = \frac{\Delta h_1}{\Delta h_2}$, $z_2 = \frac{\Delta h_2}{\Delta h_1}$

and $y_1 = \frac{w_1 \Delta h_1}{\Delta h}$, $y_2 = \frac{w_2 \Delta h_2}{\Delta h}$,

where y_1 and y_2 are experimental values calculated from the of denaturation, while $z_1 = z_2^{-1}$ is the parameter of iterat

The following table shows a survey of the experimental results.

Table 1: experimental results

sample type	Δh_1 / $J \cdot g^{-1}$	Δh_2 / $J \cdot g^{-1}$	$T_{tg,1}$ / °C	$T_{tg,2}$ / °C
1	14.2 (0.5)	32.5 (1.3)	78.9 (0.5)	92.6 (0.5)
2	16.2	33.0 (3.2)	76.4	89.8 (0.5)
3	---	27.1 (4.3)	---	90.4 (0.4)
4	15.4 (1.0)	35.6 (2.2)	79.8 (0.7)	93.1 (0.2)
5	20.0 (4.1)	45.6 (9.5)	79.5 (0.5)	88.3
6	14.4 (0.5)	32.8 (1.6)	74.1 (0.3)	91.4 (0.2)
7	14.7 (2.4)	33.6 (5.6)	76.1 (0.5)	85.1 (0.5)
8	13.6 (0.4)	31.0 (1.0)	74.7 (0.4)	91.3 (0.4)
9	13.4 (1.0)	30.7 (2.2)	72.7 (0.5)	88.0 (0.4)

Sample type (1): fresh erythrocyte suspension; (2) as (1) after 48 h sealed storage; (3) erythrocyte suspension under CO_2; (4) erythrocyte hemolysate ;(5) as (4) under CO_2; (6) Hb-solution ;(7) as (6) under CO_2; (8) Hb-H_2O-NaCl mixture; (9) as (8) under CO_2.
The values in parenthesis are the single standard deviations of five measurements at most.

Discussion

The transfer of Oxy-Hb into Deoxy-Hb is accompanied with an enhanced thermostability. The temperature as well as the enthalpy of denaturation are increased.
Inter- and intramolecular interaction may be the reason for a change

of thermostability. Azari (1) already showed in 1958 that Conalbu and Transferrin became more stable against proteolysis and denatu by binding iron ions. Donovan et al. (4) verified this finding 19 by differential scanning calorimetry. The binding of much larger molecules to proteins may induce also a change of thermostability Donovan et al. (3) described an increased thermostability for a trypsin-inhibitor complex. Thereby only the denaturation temperatu was increased while the enthalpy remained unchanged.

There was also Donovan et al. (5), who published a threefold increase in denaturation enthalpy for the system Avidin-Biotin, which can't be explained by the enthalpy of binding.

Two effects my be considered as responsible for the thermostab of Deoxy-Hb:

1) Hemoglobin is stabilized by 2,3-Diphosphoglycerate (2,3-DPG in the deoxygenated state. The enthalpy of binding 2,3-DPG to Deoxy-Hb however amounts only to 7 kcal/Mol or to about 0.43 J/g of hemoglobin. This is to small to explain an effe of 19 J/g. Moreover there could not be seen any significant difference between the salt- and 2,3-DPG-free samples and the other samples (table 1, sample type 6,7,8,9).

2) Because of the increased number of hydrophobic interactions between water and denatured Hb its specific heat capacity i increased for a Δc_p of about 0.33 J g^{-1} k^{-1} (12). An enhanc denaturation temperature with the amount of ΔT therefore sh give an increased denaturation enthalpy with

$$\Delta\Delta h = \Delta T \cdot \Delta c_p.$$

For Deoxy-Hb, $\Delta\Delta h = 13\ K \cdot 0.33\ J\ g^{-1} = 4.3\ J/g$, nearly is one fourth the experimental effect.

The surplus denaturation enthalpy of Deoxy-Hb of about 14 to 15 J/g or 235 kcal/Mol of tetramer is not yet understood. Probably an alteration of ternary structure by the transition from Oxy- to Deoxy-Hb should be considered too supplementary to the well known change of quaternary structure (10, 13). This should be done unaffected by the very established results of X-ray studies, which suggest only minimal ternary alteration in the neighbourhood of the porphyrin ring.

From the calorimetric point of view an alteration of the ternary structure of the α- and β-subunits doesn't seem unlikely, because the known alterations and the additional four salt-bridges of Deoxy-Hb (13) can't be responsible for the surplus denaturation enthalpy.

Calorimetric investigation of isolated α- and β-chains and myoglobin as well should give a little more insight in this problem.

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