CORRELATIONS BETWEEN THERMAL STABILITY AND CIRCULAR DICHROISM OF HEMOGLOBIN DERIVATIVES OF DIFFERENT SPECIES

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1. Introduction

Studies on the thermal stability of hemoglobin compounds (CO-, O_2- and met-Hb) of various species showed ligand- and species dependent differences in denaturation [1]. These differences could not be explained by varying amounts of hydrophobic amino acid residues.

In this study, circular dichroism (CD) measurements of the same hemoglobins in the 260 nm region were carried out to prove if the intensity of ellipticity is in some way related with thermal stability.

2. Materials and methods

Cells from freshly drawn blood were washed 3–4 times with saline and hemolyzed by adding 3 volumes of distilled H_2O to 1 volume of cells. Met-Hb was prepared by intracellular oxidation adding $NaNO_2$ to washed cells and removing the excess of $NaNO_2$ by washing with saline. Fetal hemoglobin was isolated from venous cord blood by the method of Zade-Oppen [2].

The hemoglobin solution was incubated in 0.033 M phosphate buffer, pH 7.0, to a final concentration of 5×10^{-5} mole heme and warmed up to the required temperature in a thermostatically controlled water bath. At different times, the denatured portion of the samples was separated by centrifugation and the concentration of the supernatant determined at 540 nm as Hb(3)CN. From these supernatants k values were calculated assuming a first order reaction. For characterization of the different hemoglobins, temperatures for a constant half time for denaturation (37.9 min $\rightarrow -\ln k = 4$) were taken from an Arrhenius diagram.

For CD-measurements (Dichrograph type CD 185, Jouan, Paris) hemoglobin solutions ($c = 2 \times 10^{-3}$ mole heme) in 0.067 phosphate buffer were used in a 0.1 mm cuvette.

3. Results and discussion

Column 3 of table 1 shows the temperatures for half denaturation of the hemoglobins so far investi-

Table 1
Temperature for constant half-time of thermal denaturation and θ-values of different hemoglobin derivatives of various species.

Hemoglobin	Complex	$t(^\circ C)$ (τ_C)	$\theta \times 10^{-4}$ (260 nm)
Human, adult	CO	67.4	7.0
	O_2	64.2	6.3
	met-Hb	58.5	3.1
Human, fetal	CO	66.1	6.7
	O_2	61.5	6.0
	met-Hb	52.2	2.1
Carp	CO	61.1	4.1
	O_2	57.4	3.0
	met-Hb	52.0	0.3
Chicken	CO	72.8	6.9
	O_2	66.6	6.9
	met-Hb	61.9	2.4
Bovine	CO	71.2	7.2
	O_2	65.9	7.3
	met-Hb	64.1	2.9

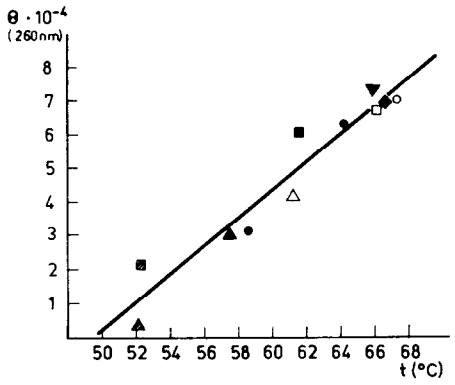

Fig. 1. Dependence of thermal stability on ellipticity in the 260 nm region (for details see text). ○ = Hb-A; □ = Hb-F; △ = carp Hb; ◇ = chicken Hb; ▽ = bovine Hb; black symbols = oxy-Hb; white symbols = CO-Hb; hatched symbols = met-Hb.

gated. It can be seen that met-Hb of all species shows lower temperatures than CO- or O_2-hemoglobin, indicating that met-Hb is less stable than the hemoglobin derivatives.

In column 4 of table 1 the ellipticities of the hemoglobins are summarized. Comparing column 3 and 4, it can be stated that low ellipticities correspond to low temperatures and high ellipticities to increased temperatures of denaturation as defined above. In fig. 1 the relationship between ellipticities and temperature is presented, omitting 4 values with greater aberration: CO- and met-Hb of chicken and bovine hemoglobin. Taking the values of all 15 observations into account a correlation coefficient of 0.88 can be calculated. The theoretical value corresponds to 0.64 with a probability of 1%, indicating that the correlation has a high degree of significance.

This correlation can be confirmed further by taking into account earlier studies of Scheler et al. [3] on horse met-Hb and the N_3- and CNS-complex. Table 2

Table 2
Temperature for constant half-time of thermal denaturation and θ-values of rhodanide- and azide-complex of horse met-hemoglobin.

	$t(^\circ C)$ (τ_C)	$\theta \times 10^{-4}$ (260 nm)
Met-Hb	46.0	3.3
Met-Hb–rhodanide	48.6	4.5
Met-Hb–azide	50.1	5.2

shows that for these complexes also the ellipticity increases with increasing temperatures. The correlation can be confirmed by a probability of 5%.

The significant correlation between thermal stability and the ellipticity in the 260 nm region supports our suggestion that the CD-band in the 260 nm region can be regarded as a measure of the interaction of aromatic amino acid residues. With increasing affinity of the ligand not only the binding distances of the heme iron are shortened [4] but also the interactions of aromatic amino acids are enhanced. This conformational change produces an increase in ellipticity [5, 6] which is related with an enhanced thermal stability.

References

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