CONSEQUENCES OF HEME LOSS IN UNSTABLE HEMOGLOBINS: A STUDY OF HEMOGLOBIN KÖLN

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1. Introduction

Jacob and Winterhalter [1] showed that, in unstable hemoglobins whose structural abnormality is located in the heme pocket, loss of heme is followed by an intraglobular precipitation of hemoglobin. The other physicochemical consequences of this feature have not yet been looked for.

In this work we have studied some properties of hemoglobin Köln β 98 (FG 5) Val → Met [2]. This hemoglobin spontaneously loses the heme moiety of its abnormal chain and the remaining molecule behaves like experimentally made semi-hemoglobins. Namely, its oxygen affinity is modified, the dissociation curve being hyperbolic with an interaction coefficient of 1; 2,3-diphosphoglycerate (2,3-DPG) binds to this molecule, but does not modify its oxygen affinity. Finally, the dissociation equilibrium is displaced toward the dimeric form.

2. Materials and methods

The abnormal hemoglobin was isolated by Amberlite IRC 50 chromatography as previously described [3].

Heme titration was performed by addition of ferriheme to ferrihemoglobin under spectrophotometric control according to the procedure of Rossi-Fanelli et al. [4].

Oxygen affinity was determined spectrophotometrically by the technique of Benesch et al. [5] as modified by Bellingham and Huehns [6] in whole and lysed cells.

The effect of 2,3-DPG was studied in 0.05 M tris-HCl buffer (pH 7.0) by the addition to a given amount of hemoglobin, stripped on G 25 Sephadex, of increasing amounts of 2,3-DPG.

The 2,3-DPG was estimated by the enzymatic procedure of Beutler et al. [7], measuring the disappearance of phosphoenolpyruvate in the presence of an excess of enolase and phosphoglyceromutase.

As suggested by Benesch and Benesch [8] the 2,3-DPG binding to hemoglobin was evaluated in the following way. Stripped hemoglobin was mixed with an excess of 2,3-DPG, dialysed for 24 hr against a 0.05 M tris-HCl buffer pH 7.0. The amount of 2,3-DPG still bound after dialysis was then assayed.

Dissociation equilibrium was studied by differential gel filtration on Sephadex G 100 as described by Gilbert [9] with slight modifications [3].

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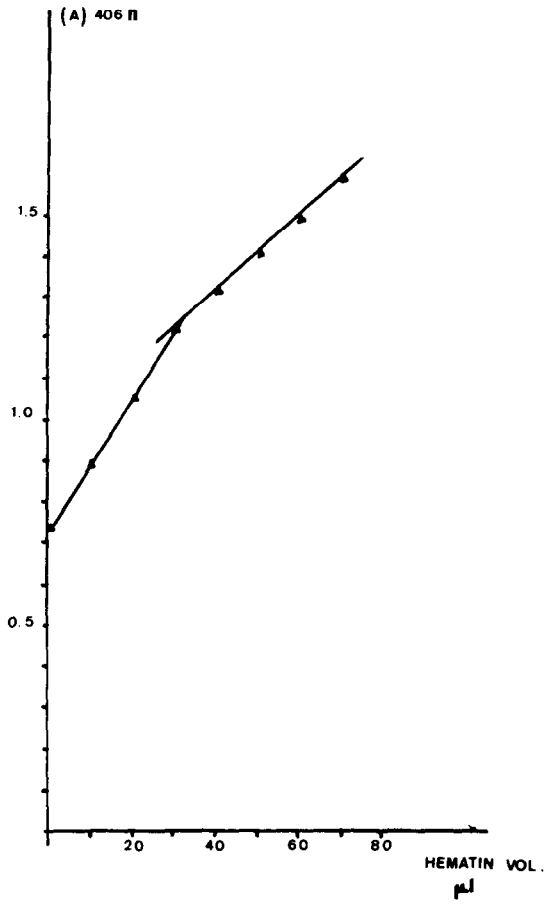

Fig. 1. Heme titration of hemoglobin Köln. The absorbance at 406 nm was followed, since the absorption coefficients of ferriheme and methemoglobin are not the same at this wavelength. The change in the slope of the curve appears at full heme saturation. About 40% of hemoglobin Köln is heme depleted.

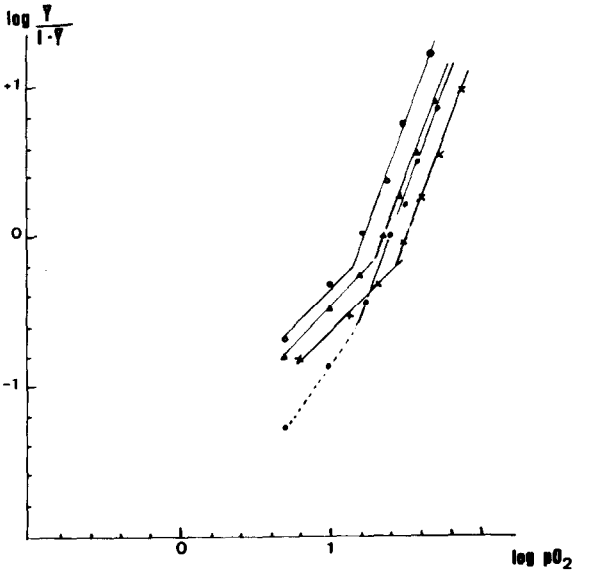

Fig. 2. Hill plot of oxygen affinity of whole cells. (●—● - - ●) Hemoglobin A pH 7.13. Heterozygote hemoglobin Köln/ hemoglobin A pH 7.47 (○—○) pH 7.13 (△—△), pH 6.46 (x—x). The shape of the curve in the case of the heterozygote shows the presence of two molecular species: in the first one $n = 1$, in the second one $n = 2.8$.

3. Results and discussion

The structural abnormality of hemoglobin Köln is located on the heme pocket of the β chain. This region is directly involved in the conformational transition from the oxygenated to the deoxygenated form [10]. The substitution of valine FG 5 by methionine gives rise to spontaneous heme depletion, as is shown by the ability of this molecule to reassociate with added ferriheme or carboxyheme. Once heme-repleted, hemoglobin Köln has the same electrophoretic mobility as normal hemoglobin. Heme-saturated hemoglobin Köln seems to be stable only when the molecule is in the oxygenated conformation. The transition to the deoxygenated form is followed by structural instability and heme loss.

In fresh whole hemolysates from the heterozygote patient, heme depletion is roughly 10%. In purified abnormal hemoglobin it is around 40% (fig. 1).

The oxygen affinity curve of whole cells is diphasic. The Hill plot

$$\log \frac{\overline{Y}}{1-\overline{Y}} = n \log p\,O_2 + K$$

shows the obvious presence of two well-defined molecular species (figs. 2–3). Between 10 and 40 percent oxygen saturation, the slope is 1, corresponding to an absence of cooperativity. At higher saturation levels the slope is 2.9, which is the same as with normal hemoglobin.

These two fractions were isolated in pure form by

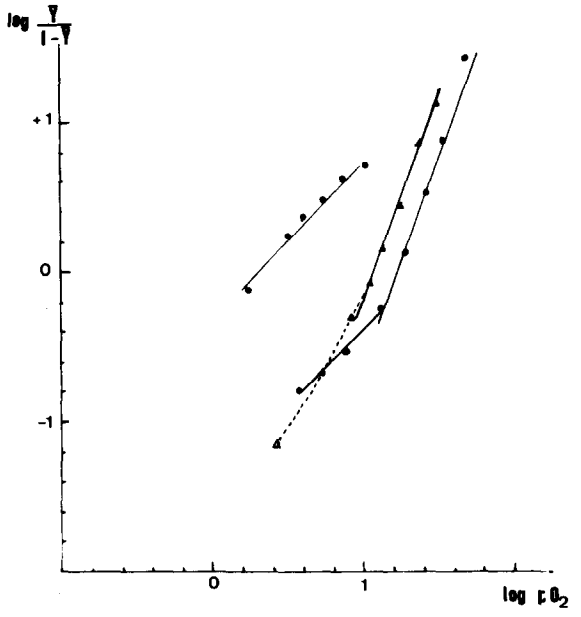

Fig. 3. Hill plot of oxygen affinity of the isolated purified fractions. (○—○) Hemoglobin Köln; (△—△) hemoglobin A; (●—●) whole hemolysate. The curve of the whole hemolysate is the superposition of the curves of the isolated fractions.

chromatography on Amberlite IRC 50. The abnormal one was identified as hemoglobin Köln by purification of hydrolysis peptides and amino acid composition study. It behaves like the semihemoglobins described by Banerjee et al. [11, 12] and Winterhalter et al. [13, 14].

This constituent binds 2,3-DPG. After a 24 hr dialysis, 13.64 μmoles 2,3-DPG remains bound per gram of this hemoglobin (vs 16 μmoles per gram of hemoglobin A). Oxygen affinity of stripped hemoglobin Köln is not modified by the addition of increasing quantities of 2,3-DPG, the curve remains hyperbolic and the $p_{1/2}$ equal to 2 mm Hg. In hemoglobin A the same quantity of 2,3-DPG shifts the $p_{1/2}$ from 11 mm Hg to 27 mm Hg. These results mean that tetramer integrity is necessary for the regulatory effect of 2,3-DPG (fig. 4).

Differential gel filtration on Sephadex G-100 using $\alpha_2\ \beta_2$ hemoglobin as tetrameric marker and myoglobin as monomeric marker shows a shift of the dissociation equilibrium toward the dimeric forms (fig. 5). The elution volumes were 44 ml for hemoglobin A, 48 ml for myoglobin and 45 ml for hemoglobin Köln;

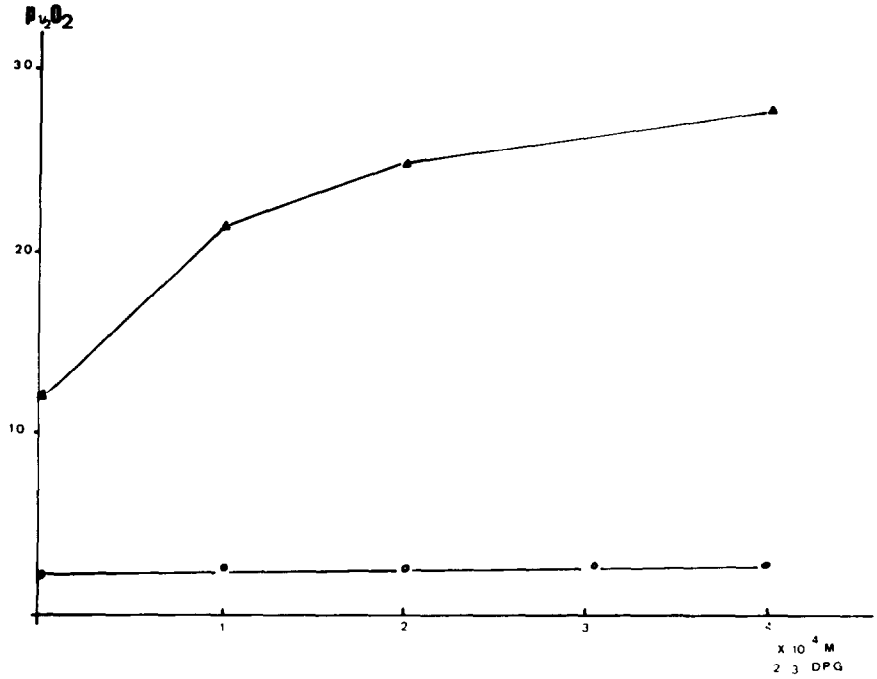

Fig. 4. 2,3-DPG effect on oxygen affinity. (△—△) Hemoglobin A; (○—○) hemoglobin Köln. Addition of 2,3-DPG reduces the oxygen affinity of hemoglobin A but remains without any effect on hemoglobin Köln.

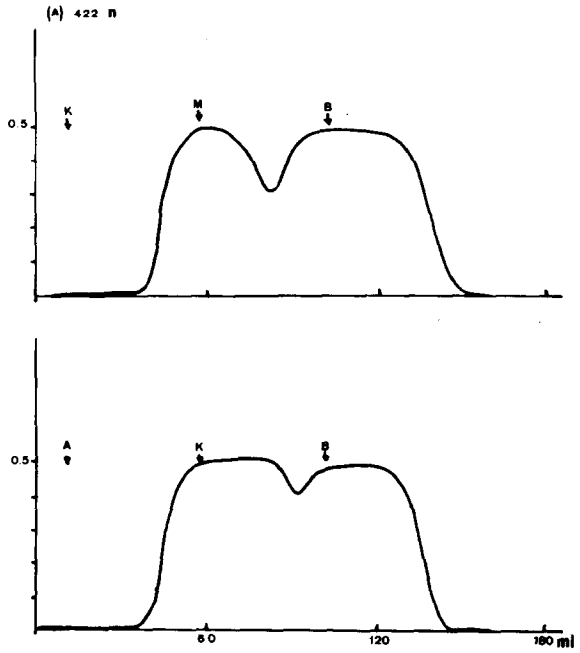

Fig. 5. Dissociation equilibrium study by differential gel filtration. The letters indicate the different markers of hemoglobins studied (K = hemoglobin Köln, M = myoglobin, A = hemoglobin A, B - buffer). From the comparison of the different elution volumes the mean molecular weight of hemoglobin Köln can be estimated to be about 50,000.

from this data, using the relation

$$V_e = A + B \log \text{mol. wt.}$$

an average molecular weight of roughly 50,000 can be assumed for hemoglobin Köln.

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