J. Biol. Res. 2003 n. 1 - Vol. LXXIX - Rubbettino - Soveria Mannelli

Functional Effects of the Coexistence of Hb-A with High Oxygen Affinity Haemoglobins

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Abstract

In carriers of abnormal haemoglobins with increased oxygen affinity, polyglobulia is the only possible functional compensation for tissue hypoxia. However, this adaptation is reduced if some Hb-A is used to synthesize hybrid haemoglobins of the type $\alpha_2\beta^A\beta^X$ ***(heterotetramers). In fact, they display increased oxygen affinity. Four patients of this kind were studied: two carriers of Hb-Kempsey:*** β ***99Asp-Asn, one of Hb-Gàmbara:*** β ***82Lys-Glu, and one of Hb-Trento, a new variant with elongated*** β ***chains. Moreover, there are some cases, like that of Hb-Tak, also with elongated*** β ***chains, and Hb-Casper:*** β ***106Leu-Pro, in which the abnormal Hb does not interact with Hb-A to form hybrids and the patient presents good functional compensation. This is demonstrated by at least three signs: the absence of polyglobulia, the good reactivity of Hb-A towards 2,3-DPG, and the absence of left-shifting of the upper half of the dissociation curve, which is commonly considered the expression of Hb-A oxygenation.***

Introduction

Perhaps the case of Hb-Tak, a variant with elongated β chains [1], is the best introduction to this topic; in fact, the carrier was not polyglobulic and his oxygen dissociation curve from whole blood showed an evident left-shifting with a consistent biphasism. However, only the lower part of the curve was strongly left-shifted, while the upper part was almost identical to the control curve of Hb-A [1]. Moreover, the connection between the two segments of the curve, the so-called inflection point, corresponded to the percentage of Hb-Tak present (26%). Nevertheless, these findings are infrequent, since the inflection point is usually located at about half the curve, even in cases in which the abnormal Hb is much lower than 50%. The upper half of the dissociation curve, i.e. the part that usually represents the oxygenation of Hb-A, is also often left-shifted with respect to the control curve of Hb-A. This left-shifting is probably due to the presence of hybrids between Hb-X and Hb-A, i.e. heterotetramers of the type $\alpha_2\beta^A\beta^X$ [2]. In fact, these hybrids display high oxygen affinity, as demonstrated in the case of Hb-Radcliffe: $\alpha_2\beta_2$ 99 (G1)Asp-Ala [3], a variant of the Kempsey group. Therefore, the main purpose of this study was to evaluate the presence of hybrids in vitro.

Materials and methods

Haematological data were obtained using standard procedures [4], and the studies on both the abnormal haemoglobins and the patients' DNA were carried out as described elsewhere [4,5,6]. Dissociation curves for oxygen were performed at 37.5°C on freshly collected heparinized venous blood [7]. 2-ml samples were tonometered with an RNA apparatus (Bionostic Inc, Acton, USA) that allowed the blood to be equilibrated with gas mixtures of O_2, CO_2 and N_2. The gases were contained in cylinders: CO_2 was at a fixed pressure of 40 torr (5.6%), whereas oxygen had progressively increasing pO_2s and nitrogen was used to equilibrate each gaseous mixture up to atmospheric pressure. A conventional haemogasanalyser measured each pO_2 and pCO_2. The Hill's graph [7] was produced by plotting each individual log (Y/1-Y) *versus* the corresponding log pO_2, where (Y/1-Y) indicates the fractional saturation of Hb in O_2, i.e. the ratio between the saturated and non-saturated Hb. This allowed calculation of p50, the pO_2 at half saturation of Hb in oxygen, and of the n-factor, representing the "slope" of the (regression) Hill's graph, assumed as the degree of functional "cooperativity" among the four hemes of Hb [7]. The intraerythrocytic level of 2,3-diphosphoglycerate (DPG) was measured in fresh erythrocytes and expressed as a molar ratio (M/M) with the total Hb present [8]. The molar ratio between DPG and the percentage of Hb-A alone was also calculated. In fact, high oxygen affinity haemoglobins very often bind DPG with difficulty and, at least in theory, an excess of this non-diffusible intraerythrocytic anion remains available to bind the Hb-A alone. Several additional techniques were used when necessary [5,6]: electrophoresis on cellulose acetate, tris-glycine buffer at pH 9.5, and cation-exchange (CE) HPLC (Variant ™). Finally, step by step Bio-Rex-70 column chromatography was used when separation of an abnormal haemoglobin was not possible with a common preparation method [9].

Two phosphate buffers at different pH (6.4 and 6.8) and molarity were used. Each eluted abnormal fraction was re-chromatographied by (CE) HPLC. Only the data for the oldest patients could not be examined by this technique (Hb-Ke_1 and Hb-Ke_2: 1992) [10].

Results

Table 1 shows the main haematological features of the four patients, while Figures 1 and 2 show their dissociation curves. Figure 3 illustrates the elution profile obtained by re-chromatographying (by (CE) HPLC) the single abnormal fraction obtained by Bio-Rex-70 chromatography from the haemolysate of a carrier of Hb-Trento (Hb-Tr) [11]. Two peaks, instead of one, were visible on this occasion. Their ratio was rather equimolar, and it is very likely that the left peak belongs to Hb-X/Hb-A hybrids. In fact, the same figure shows that the (CE) HPLC position of the main Hb-Tr peak from haemolysate (lower graph) has the same elution position as the second peak of the upper (CE) HPLC graph. Moreover, the HPLC pre-peak of haemolysate coincides with the first peak of the upper chromatographic profile. Thus, this area could represent the specific elution site of the molecular hybrids of Hb. Moreover, their presence, quantity or absence could be related to the different analytical chemical conditions. Unfortunately, the very low concentration of these eluates excluded any structural or functional analyses. Finally, the abnormal fraction of Hb-Gà was also found to be duplicated in the alkaline electrophoresis [5], very likely due to hybrids.

Discussion

Although polyglobulia represents the main functional compensation for carriers of haemoglobins with increased oxygen affinity, the heterozygote for Hb-Tak was not polyglobulic. The lower part of his left-shifted dissociation curve corresponded almost exactly to the saturation of the abnormal Hb in oxygen, whereas the upper part reflected the oxygenation of Hb-A alone. In fact, these two segments of the graph were separated by an inflection point at about 25% of the oxygen saturation, which corresponded to the percentage of abnormal Hb. This means that the two haemoglobins did not interact to form $\alpha_2\beta^{Tak}\beta^A$ hybrids and the Hb-A present was completely utilized for good compensation of the relative tissue hypoxia caused by the component that released oxygen with difficulty. Subsequently, the case of Hb-Casper: β 106 (G8) Leu-Pro was identified [12] and its behaviour was found to be similar to that of Hb-Tak. Moreover, the Hb-A of the patient was responsive to DPG. This is peculiar to Hb-A alone and not to high affinity haemoglobins. In effect, hybridisation requires the contemporary presence of $\alpha\beta^A$ and $\alpha\beta^X$ dimers during oxygenation [7]. These aggregate in tetramers only if the de-oxygenation of Hb-A and Hb-X are more or less synchronous. It was later observed that biphasism is mainly characterized by an inflection point at about half the dissociation curve, and that the upper part of this curve, considered the expression of Hb-A oxygenation, is more or less left-shifted even when biphasism is absent and/or when the percentage of abnormal Hb is more or less lower than 50%.
All these characteristics were present in our cases, including two sisters carrying Hb Kempsey (Hb-Ke_1 and K_2): β 99 (G1) Asp-Asn [10], one patient with Hb-Gàmbara (Hb-Gà): β 82 (EF 6) Lys-Glu [5], and one case of Hb-Trento (Hb-Tr), a new elongated β chain [11] belonging to the same group as Hb-Tak, Cranston and Saverne [1,13,14]. The dissociation curve of the carrier of Hb-Tr (Fig. 2), a patient without polyglobulia, differed from that of Hb-Tak, since the inflection point was at about 50-60% of Hb-O_2 saturation even though the abnormal Hb was no more than 29% and the dissociation curve of Hb-A was left-shifted. The low percentage of Hb-Tr and the absence of polyglobulia were probably due to mild haemolysis caused by some instability of Hb-Tr whereas the total Hb-A reached about 8.8 g/dl. Thus, some hybrids might have been present in this case, but they did not decrease the patient's quality of life. In contrast, in the carrier of Hb-Ke_2, with 13.3 g/dl of total Hb, only 26% of abnormal Hb was present because of chronic sideropenia due to prolonged bleeding therapy. Although the total Hb-A amounted to about 9.2 g/dl, this patient was totally invalid with regard to physical exercise. In contrast, her Ke_1 sister was in good health and had 40-45% of abnormal Hb, i.e. at least 11.7 g/dl of Hb-A. Thus, the evidence suggests that hybrids were present in both these patients, with the consequent loss of some Hb-A; yet this was deleterious only in the patient without compensatory erythrocytosis. Her Hb-A level was not low, but most of it was probably used to synthesize hybrids with increased oxygen affinity (see Hb Radcliffe: ref. 3). Another important point is the strong similarity between the dissociation curves of these two sisters, with similar p50s but very different values of total Hb. This could depend on the greater formation of hybrids in the non-polyglobulic patient, since their synthesis could be favoured by the relative dilution of the two main haemoglobins present. However, another hypothesis is possible: according to Bunn and McDonough [2], the assemblage of hybrids can follow the "binomial" formula $(X+A)^2$ in some cases. In the Hb-Ke_2 patient, this formula would produce 6.76% Hb-X, 53.38% Hb XA (hybrids) and 47.6% Hb-A. X+XA gives a total amount of about 60% high affinity haemoglobins, which corresponds almost exactly to the position of the inflection point in terms of oxy-Hb saturation. Moreover, in the case of Hb-Tr, the same formula would produce 8.41% Hb X, 38.3% AX and 43.56% A, for a total of about 50% high affinity components. Both these results cannot be merely occasional; however, it is likely that the inflection point at 50-60% of oxygen saturation represents only a change of slope of the dissociation curve, a sort of limit between two zones in which hybrids are synthesized at different concentrations or speeds. Yet the inflection point is not necessarily present, as in the case of Hb-Gà, although a duplication of the abnormal fraction was observed in the course of the alkaline electrophoresis of the haemolysate of this patient [5]. This finding suggests that the formation of hybrids could take place not only during the usual

respiratory cycle of Hb, but also during the chemical handling related to the various laboratory procedures. The last observation concerns the molecular defect of Hb-Tr [11], which belongs to the group of elongated β chains. This depends on a deletion of one of the two A nucleotides of codon 144, (AAG=Lys), which triggers a frame-shift reading of the genetic code similar, but not identical, to those of Hb-Tak, Cranston and Saverne [13,14]. Thus, the elongation of the β^{Tr}-chains continues until a new termination signal (TAA) is encountered. This occurs at position 157 and then the chain stops at position 156 (see Fig. 1-2).

Key Words

haemoglobins; high oxygen affinity; Hb-A; $\alpha_2\beta^A\beta^X$ hybrids.

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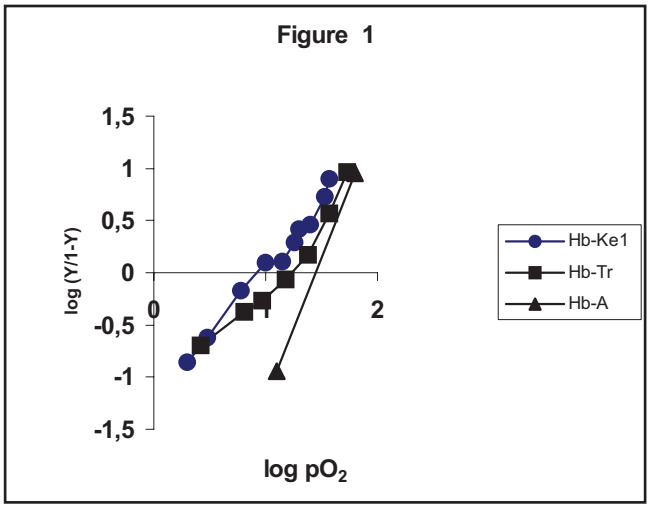

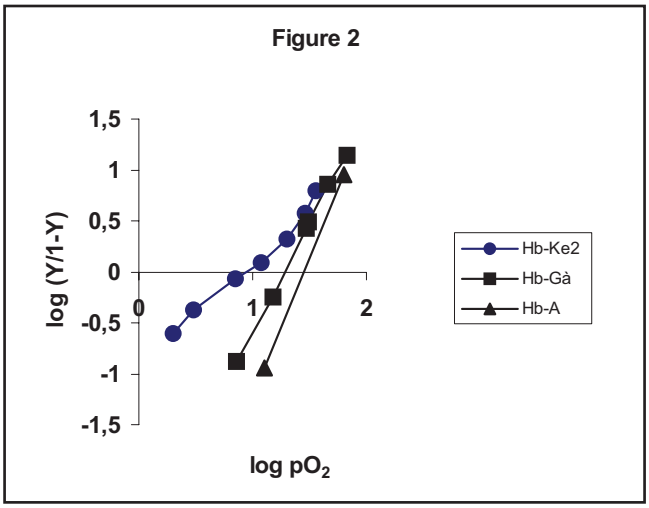

Fig. 1 e 2. Hill's graphs from whole blood of four carriers of high oxygen affinity. From left: Hb-Ke1, Hb-Gà, Hb-A; and Hb-Ke2, Hb-Tr, Hb-A.
Note: the nucleotide pattern and aminoacid sequences of the abnormal β tail of Hb-Tr are as follows:
5'.....144(AGT=Ser)-145(ATC=Ileu)-146(ACT=Thr)-147(AAG=Lys)-148(CTC=Leu)-149(GCT=Ala)-150(TTC=Phe)-151(TTG=Leu)-152(CTG=Leu)-153(TCC=Ser)-154(AAT=Asn)-155(TTC=Phe)-156(TAT=Tyr)-157(TAA=stop).....'3.

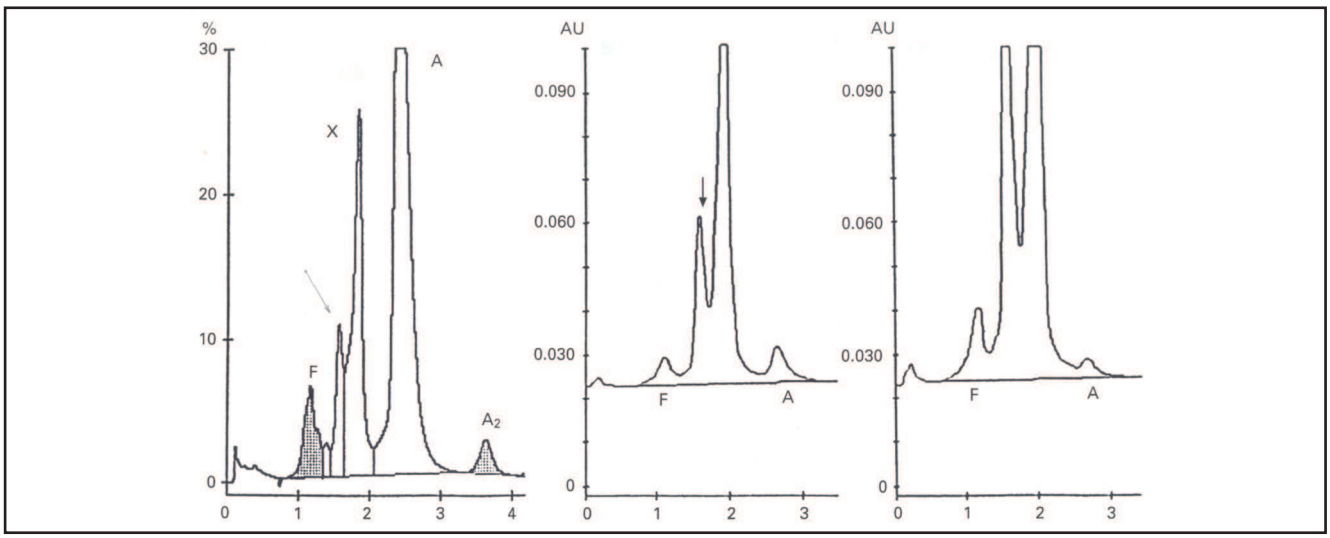

Fig. 3. Left: direct HPLC of the hemolysate containing Hb-Tr. The main abnormal Hb(X) elutes after a consistent pre-peak (arrow). Centre: HPLC of the isolated Hb-Tr: the pre-peak is increased. Right: final equilibrium between the two HPLC components. Since the former peak grows in the course of the chromatography, and its elution position differs from that of Hb-Tr, this suggests that the additional component is due to the formation of Hb-X/A hybrids

	Hb (g/dl)	Torr p 50	n-value	DPG/Hb ratio	DPG/Hb-A ratio	HbX:%	Hb A%
Hb-K1	21.2	9.5	1.26	0.85	1.55	40	55
Hb-K2	13.3	9.8	1.3	1.52	2.2	26	69
Hb-Gà	17.8	19.3	2.39	0.68	1.58	52	43
Hb-Tr	13.4	14.3	1.42	0.9	1.364	29	66
Hb-A	15	26	2.7	0.9	/	/	/

Table 1. Hematological, functional and hemoglobin data from four patients.