SUBUNIT INTERACTIONS IN ABNORMAL HEMOGLOBINS (HB ZÜRICH AND HB ABRUZZO)

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1. Introduction

The dissociation behaviour of normal human hemoglobin, under conditions of neutral pH and moderate ionic strength, has been compared by differential gel filtration with that of two abnormal hemoglobins, namely Hb Zürich (HbZH) [1] and Hb Abruzzo (HbAbr) [2]. In both variants the structural abnormality involves substitution in the beta chains of one histidine with an arginine residue, although the substitution occurs in very different regions of the molecule, i.e. in the heme pocket in HbZH and in the C-terminal region in HbAbr. Measurement of the dimer-tetramer equilibrium constant allows a determination of the energy of interaction among the subunits in the various proteins, under quasi-physiological conditions. The results obtained show that in both abnormal Hbs the stability of tetramer is decreased by a comparable amount. The free energy of interaction among the subunits differs from that of normal Hb by 1.5 Kcal/mole.

2. Materials and methods

Human hemoglobin A (HbA) [3] was prepared from fresh blood by the ammonium sulfate method and freed from organic ions by passage through a mixed bed ion exchange column. Hb Zürich [1] and Hb Abruzzo [2] were prepared as previously described.

Gel filtration experiments on Bio-Gel P 100 (Bio-Rad Laboratories) were performed following Chiancone et al. [4], employing for some experiments a smaller column (0.8 × 51 cm) and for others one (1.6 × 50 cm). Flow rates were maintained constant, at about 6 and 20 ml/h respectively, by means of a peristaltic pump (LKB 4812 A). The columns were thermostated at 7–10°C. The elution volume of monomer (V_1) was obtained with sperm whale myoglobin (Seravac Laboratories, Ltd., Maidenhead, England). The value of the elution volume of oxyhemoglobin A at high protein concentration (> 10 mg/ml) was taken as the elution of the tetramer (V_4) after proper correction for the extent of dissociation at this concentration. The elution volume of the hemoglobin dimer (V_2) was then calculated assuming a linear relationship between the elution volume and the logarithm of molecular weight.

3. Results and discussion

Gel filtration experiments were all performed at pH 7 and moderate ionic strength (~ 0.05–0.2 M) in various buffer systems. Under these conditions the increase in elution volume at low protein concentration observed in normal oxyhemoglobin (Hb A) reflects the reversible dissociation of $\alpha_2\beta_2$ tetramers into $\alpha\beta$ dimers [4]. In comparing the dissociation behaviour of the abnormal hemoglobins with that of Hb A a differential technique has been employed [5]. In these experiments, which involve layering the abnormal hemoglobin over Hb A at the same total protein concentration, small differences in dissociation behaviour can be detected.

The experiments performed with the oxygenated derivative, all indicate an increase in the extent of dissociation of the abnormal protein with respect to

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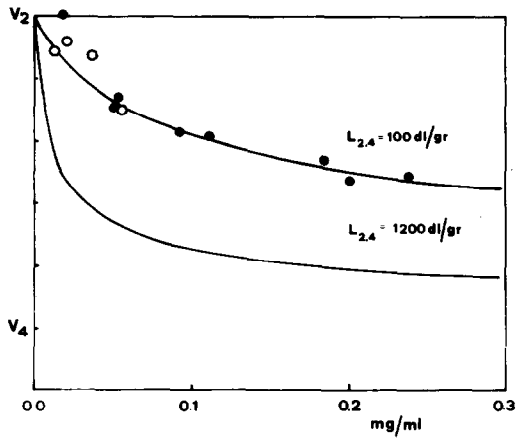

Fig.1. Relationship between elution volume and protein concentration for the oxygenated derivative of HbZH and HbAbr: (•) Hb Zürich in phosphate buffer 0.1 M pH 7. (○) Hb Abruzzo in Bis-Tris buffer 0.05 M pH 7. (——) Theoretical curves which correspond to the dimer–tetramer association constants given in the figure. The value of $L_{2,4}$ = 1200 dl/g refers to HbA in 0.1 M phosphate buffer, and 0.05 M Bis-Tris, pH 7 (see table 1). V_2 and V_4 are the elution volumes for the hemoglobin dimer and tetramer, respectively.

Hb A. A few experiments carried out with the deoxygenated derivative of Hb Abruzzo indicated that the association constant $L_{2,4}$ is larger than 10^5 dl/g. The results on the oxy derivative are reported in fig.1 in terms of normalized elution volume against protein concentration, and in table 1 in terms of dimer–tetramer association constants ($L_{2,4}$). In the same table are also included, for comparison, results on HbA. It can be seen that for both Hb Zürich and Hb Abruzzo the association constant of the oxygenated derivative is decreased about tenfold in all buffer systems. Therefore the difference in the free energy of interaction among the subunits, as between each abnormal Hb and Hb A, can be calculated from the relationship:

$$\Delta G^{\circ}_{\text{Hb abn}} - \Delta G^{\circ}_{\text{Hb A}} = RT \ln (L_{\text{Hb abn}}/L_{\text{Hb A}})$$

This amounts to about 1.5 kcal/mol for both abnormal hemoglobins.

Further evidence that in Hb Abruzzo interactions among dimers are significantly, but not dramatically different from those existing in Hb A, is provided by hybridization experiments between these two hemoglobins (in 0.09 M NaCl plus phosphate). This type of measurement is performed by flowing through the column a solution of Hb A followed by a mixture of Hb A and the abnormal hemoglobin [6]. The observed difference in elution volume is a function of all the equilibrium constants, including that for the formation

Table 1
Dimer–tetramer association constant ($L_{2,4}$) of the oxygentaed derivative of HbA, Hb Zürich, and Hb Abruzzo, at pH 7.0 in different solvents, and 7–10°C

Protein	Solvent	$L_{2,4}$ (dl/g)
HbA	NaCl (0.09 M) plus phosphate (0.01 M as Na^+)	250
	Phosphate 0.1 M	1200
	Bis-Tris 0.1 M	1200
	Bis-Tris (0.05 M) with IHP (30 μM)	40 000
Hb Zürich	NaCl (0.09 M) plus phosphate (0.01 M as Na^+)	25
	Phosphate (0.1 M + EDTA 10^{-4} M)	100
Hb Abruzzo	NaCl (0.09 M) plus phosphate (0.01 M as Na^+)	50
	Bis-Tris (0.05 M)	100
	Bis-Tris with IHP (30 μM)	7000

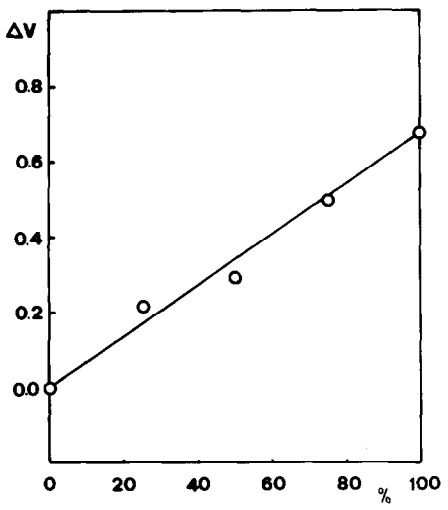

Fig.2. Difference in elution volume as a function of percentage of Hb Abruzzo in a mixture of Hb A and Hb Abruzzo. Protein concentration: 0.035 mg/ml in NaCl (0.09 M) plus phosphate (0.01 M as Na^+), pH 7.

of hybrid tetramers ($\alpha_2\beta^A\beta^{Abr}$). The experimental points obtained at different ratios of Hb A and Hb Abr, lie on a straight line, as shown in fig.2. This result indicates that formation of hybrid tetramers is a statistical process, i.e. that within the accuracy of the method there is no preferential binding across the hybrid interface.

These findings show that the same ammino acid substitution (His → Arg) occurring in the β chains has very similar effects on the stability of the tetramer, although it occurs in different regions of the molecule, i.e. the heme pocket in Hb Zürich and the DPG binding site in Hb Abruzzo. This result, in turn, indicates that the bulk of the free energy of tetramer formation comes from other molecular interactions, most likely those at, or near, the $\alpha\beta$ interfaces. In this connection the dissociation behaviour of Hb Zürich and Hb Abruzzo may be compared with that of Hb Kansas (β102 Asn → Thr) [7] where the substitution occurs at the $\alpha_1\beta_2$ contact. In the latter case the dimers are very loosely associated in the oxy form ($L_{2,4} \sim 3$ dl/g), and the difference in free energy of interaction among the subunits as compared to Hb A is 3.6 kcal/mol.

Therefore it seems conclusive that the free energy of subunit interaction is stored mainly at the molecular contacts between the chains, as also indicated by the well known dissociating effect of *p*-chloromercury benzoate [8,9]. However it seems also evident from these results that chemical modifications far removed from the interface can alter the subunit interactions, and this is presumably achieved through tertiary conformational changes induced in one type chain by the substitution.

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References

[1] Winterhalter, K. H., Anderson, N. M., Amiconi, G., Antonini, E. and Brunori, M. (1969) European J. Biochem. 11, 435–440.
[2] Bonaventura, C., Bonaventura, J., Amiconi, G., Tentori, L. and Brunori, M. (1975) J. Biol. Chem. 250, 6273–6277.
[3] Antonini, E. and Brunori, M. (1971) Hemoglobin and Myoglobin in their Reactions with Ligands, North-Holland, Amsterdam.
[4] Chiancone, E., Gilbert, L. M., Gilbert, G. A. and Kellet, G. L. (1968) J. Biol. Chem. 231, 1212–1219.
[5] Gilbert, G. A. (1966) Nature 212, 296–298.
[6] Gilbert, G. A., Gilbert, L. M., Owens, C. E. and Shawky, N. A. (1972) Nature New Biol. 235, 110–112.
[7] Bonaventura, J., Riggs, A. (1968) J. Biol. Chem. 243, 980–991.
[8] Bucci, E., Fronticelli, C. (1965) J. Biol. Chem. 240, PC 551.
[9] Rossi Fanelli, M. R., Chiancone, E., Spagnuolo, C. and Antonini, E., Wyman, J. (1969) Soc. It. Biof. Biol. Mol. Roma p. 39.