HAEMOGLOBIN STRASBOURG $\alpha_2\beta_2$ 23 (B5) Val → Asp

Revised structure and functional properties

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1. Introduction

Haemoglobin Strasbourg, found in a Portuguese woman, was described as having the amino acid substitution β20 (B2) Val → Asp [1]. However it was hard to give a pertinent explanation for its functional properties: high oxygen affinity, reduced cooperativity, normal Bohr effect and normal reactivity to 2,3-diphosphoglycerate. In addition, genetic implications made the substitution highly improbable [2]. Reinvestigation of the structure showed that the substitution was misplaced and that the actual structure is β23 (B5) Val → Asp, indicating that mutation of the second base of codon β23 GUU gives rise to the new codon GAU, for aspartic acid.

2. Materials and methods

For structural studies the β-globin variant chain was isolated and then amino-ethylated as in [1]. The intact chain was then degraded in a protein-peptide sequenator Beckman model 890 B updated with the addition of an undercut cup and a nitrogen flush. The program used included a single coupling in a 0.8 M *N,N*-dimethyl benzylamine (DMBA) buffer and a single cleavage as in [3]. PTH amino acids were identified by chromatography on miniature silica gel thin-layer plates [4] and by gas–liquid chromatography on 10% SP 400 using a Beckman GC 45 apparatus [3]. PTH-Arg and PTH-His were identified by specific staining, respectively, with phenanthrenequinone and Pauly reagent [4].

For functional studies, Hb Strasbourg was isolated by preparative isoelectrofocusing according to [5]. Oxygen dissociation equilibria of whole blood were measured at 37°C as in [6]. The solutions of haemoglobin were freed of 2,3-diphosphoglycerate [7] and their oxygen equilibria determined by the spectrophotometric method in [8]. Measurements were made using bi–Tris 0.05 M, NaCl 0.1 M buffers in the pH range 6.45–7.45. Temperature was 25°C. The alkaline Bohr effect was calculated from the formula $\Delta\log P_{50}$ / ΔpH 6.45–7.45. The rate of spontaneous oxidation at atmospheric pressure was studied by incubation at 37°C for varying periods of time up to 1 h in phosphate buffer (pH 6.5). The % ferri Hb was then determined as in [9].

3. Results

3.1. *Structural studies*

The amino ethylated β chain from Hb Strasbourg was degraded twice by automatic Edman degradation in the sequenator. The amount of material used in the 2 experiments was 8 mg and 7.5 mg, and the repetitive yields were 97.2% and 97.0%, respectively. In both experiments the sequence of the protein up to position 32 was determined and all the residues were assigned without any ambiguity. This sequence corresponds to residues of the tryptic peptides βT1, βT2, βT3 (where the amino acid substitution is

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located) and the first 2 residues of peptide βT4. Three valine residues occupy positions β18, 20 and 23 in normal βT3 peptide. Valine was found in positions 18 and 20, the later associated with minute amounts of asparagine and no aspartic acid. In position 23 no valine was found, but aspartic acid as a large spot on thin layer chromatography and as a prominent peak by gas chromatography, was found.

Thus the actual amino acid sequence of the N-terminal portion of the tryptic peptide βT3 in Hb Strasbourg is:

18 19 20 21 22 23 24 25 26
–Val–Asn–Val–Asp–Glu–Asp–Gly–Gly–Glu–

and its amino acid substitution is β23 (B5) Val → Asp.

3.2. *Functional studies*

The oxygen equilibrium curve of the proband's red blood cells studied at pH 7.15 was at the lower range of the normal values with a P_{50} at 29 mm Hg (normal 30 ± 1 mm Hg). The 2,3-diphosphoglycerate concentration was slightly increased: 20 μmol/g Hb (normal: 15 ± 2 μmol/g Hb).

Hb Strasbourg could not be separated from Hb A using standard chromatographic methods. Consequently we separated Hb Strasbourg by isoelectrofocusing and the oxygen equilibria were obtained directly after elution from the gel. Haemoglobin was freed of phosphate as described in methods. Haemoglobin A prepared in this way had a normal P_{50} value of 6.3 mm Hg, at pH 7.15, and 25°C. Under the same conditions Hb Strasbourg showed a high oxygen affinity with a P_{50} at 2.7 mm Hg (fig.1).

The cooperativity was reduced with a Hill coefficient of 2.0 (normal 2.6 ± 0.1) while the Bohr effect and the right shift of the oxygen dissociation curve occurring in the presence of 2,3-diphosphoglycerate were normal.

The rate of auto-oxidation was identical to that of Hb A isolated in the same conditions.

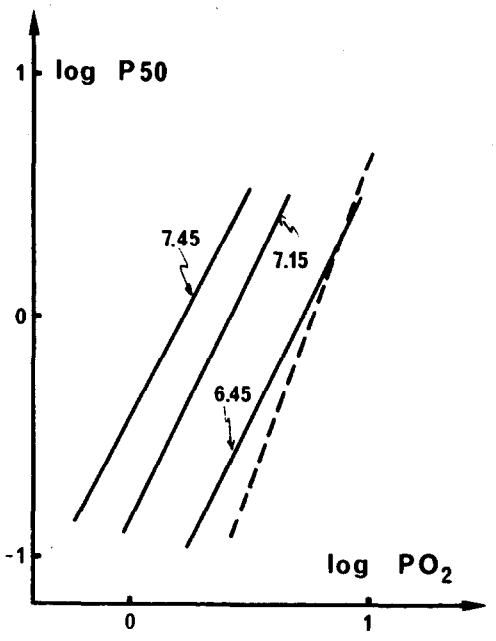

Fig.1. Hill plot of oxygen dissociation curves of phosphate free haemoglobins A at pH 7.15 (– – –) and Strasbourg (——) at indicated pH values; bis–Tris 0.05 M, NaCl 0.1 M buffers; temp. 25°C.

4. Discussion

In the original description of Hb Strasbourg, the amino acid sequence was determined by Edman-dansyl degradation of the mutant peptide βT3 eluted from thin-layer finger prints [1]. It was impossible to reach position 5 of the peptide (β23) during that experiment because of the low amount of material available at that time and a high level of overlapping. The existence of a repetitive sequence Val–Asp increased the difficulties. The aspartic acid found in position 3 (β20) was in fact the overlap of asparagine 2 (β19) that was converted to the parent acid during the dansylation step. In addition the presence of some valine in position 3 was misinterpreted as coming from contaminating peptides eluted from the plate as well as some left over from Val 1 (β18). The present studies using the protein sequenator overcame all the difficulties. This technique allowed us to get cleaner results without overlapping and to assign the positions of the amides without ambiguity. This is one more example of the usefulness of the technique in the description of amino acid substitution inside repetitive sequences and where amides are involved [10].

The complete nucleotide sequence of human β-globin messenger RNA was described [11–13]. Valine β20 is coded by GUG and valine β23 by GAU or GAC.

According to the genetic code the codon for aspartic acid is GAU or GAC. The initial description of Hb Strasbourg β20 Val → Asp implied changes of the second and third bases of codon 20. The occurrence of such an event is highly improbable. An alternative explanation could be the polymorphism of human populations in the respect of codon β20 [14]. Nevertheless the present study indicates that neither of these mechanisms is involved. The actual amino acid substitution in β23 suggests the interchange U → A for the second base of the codon, and implies that the new codon for aspartic acid in Hb Strasbourg is GAU. Two other abnormal haemoglobins are either double mutations or support the hypothesis of genetic polymorphism [14]. Hb Bristol β67 Val → Asp [2] implies interchanges of codon GUG to GAC or GAU, and Hb Edmonton β50 Thr → Lys [2] those of ACU to AAA or AAG. Our experience with Hb Strasbourg indicates that the amino acid substitution of these other 2 mutant haemoglobins must be checked with more accurate techniques prior to further genetic conclusions being drawn.

In the case of Hb Strasbourg, the replacement of a valyl by an aspartyl residue induced a small modification of charge, visible only by isoelectrofocusing. This indicates that the aspartyl residue is involved in a salt bridge which neutralizes its negative charge. In view of the molecular model of Perutz, this new salt bridge could exist between Asp β23 and His β117 which is located just above. The β23 residue is localized at the surface of the molecule and it is difficult to explain the modified functional properties of Hb Strasbourg accordingly. However, the stereochemical modifications induced by the new salt bond could be responsible for the high oxygen affinity and decreased cooperativity of Hb Strasbourg. It is notable that in the case of Hb Olympia (β20 (B2) Val → Met), the authors could not relate the high oxygen affinity of this mutant with its structural modification [15]. The β20 residue is also located at the surface of the chain as is the β23 residue. These facts lead us to hypothesize that this part of the molecule could be indirectly involved in the interactions between subunits. The X-ray analysis of Hb Strasbourg is currently in progress.

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References

[1] Garel, M. C., Blouquit, Y., Arous, N., Rosa, J. and North, M. L. (1976) FEBS Lett. 72, 1–4.
[2] Bunn, H. F., Forget, B. G. and Ranney, H. M. (1977) in: Hemoglobinopathies, W. H. Saunders Co., Philadelphia, London, Toronto.
[3] Hermodson, M. A., Ericsson, L. H., Titani, K., Nevrath, H. and Walsh, K. A. (1972) Biochemistry, 11, 4493–4501
[4] Cohen-Solal, M. and Bernard, J. L. (1973) J. Chromatogr. 80, 140–143.
[5] Thillet, J., Blouquit, Y., Perrone, F. and Rosa, J. (1977) Biochim. Biophys. Acta, 491, 16–22.
[6] Cohen-Solal, M., Thillet, J., Gaillardon, J. and Rosa, J. (1972). Rev. Eur. Clin. Biol. 17, 988–993.
[7] Kilmartin, J. V. (1973) Biochemistry 133, 725–733.
[8] Benesch, R., Mac Duff, G. and Benesch, R. E. (1965) Anal. Biochem. 11, 81–87.
[9] Benesch, R. E., Benesch, R. and Yung, S. (1973) Anal. Biochem. 55, 245–248.
[10] Cohen-Solal, M., Blouquit, Y. and Rosa, J. (1974) Haematologia, 8, 97–109.
[11] Marotta, C. A., Forget, B. G., Cohen-Solal, M., Wilson, J. T. and Weissman, S. M. (1977) J. Biol. Chem. 252, 5019–5031.
[12] Marotta, C. A., Wilson, J. T., Forget, B. G. and Weissman, S. M. (1977) J. Biol. Chem. 252, 5040–5053.
[13] Cohen-Solal, M., Forget, B. G., Prensky, W., Marotta, C. A. and Weissman, S. M. (1977) J. Biol. Chem. 252, 5032–5039.
[14] Wilson, J. T., Forget, B. G. and Weissman, S. M. (1977) Science 196, 200–202.
[15] Stamatoyannopoulos, G., Nute, P. E., Adamson, J. W., Bellingham, A. J. and Fink, D. (1973) J. Clin. Invest. 52, 342–349.