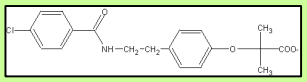
Docking studies and ligand recognition for allosteric modifiers of hemoglobin

M.Cristina De Rosa[a], Donato Mosca[b], Cristiana Carelli Alinovi[a] and Bruno Giardina[a]

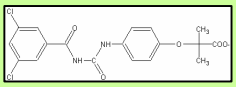
[a]Institute of Biochemistry and Clinical Biochemistry and C.N.R., Institute of Chemistry of Molecular Recognition, Catholic University, I-00168 Rome, Italy
[b]ACRAF, Angelini Ricerche, Rome, Italy
Email : mariacristina.derosa@icrm.cnr.it

INTRODUCTION

Oxygen binding affinity of hemoglobin (Hb) can be modulated by various natural and synthetic allosteric effectors. There is considerable interest in designing agents that produce low-affinity Hbs. Such agents have several potential clinical applications including radiosensitization of tumors, prolongation of stored blood half-life and treatment of ischemic diseases. Bezafibrate (BZF), an antilipidemic drug, has been shown to be a potent allosteric effector of Hb and a number of synthetic analogues were designed and evaluated for allosteric inhibition of hemoglobin. The aim of this study is to show how a molecular docking strategy can be successfully used to investigate the inhibition mechanism of these synthetic compounds. The interaction of fibrates with semiHbs has been studied. α and β semiHbs are heme-deficient dimers of the form α(heme)β(apo) and α(apo)β(heme) respectively, which exhibit the oxygen binding characteristics of non-cooperative high affinity systems. Yonetani (Tsuneshige, A. et al. *J. Biol. Chem.* 2004, *279*, 48959-48967) found that L35, a bezafibrate related compound, exerted a more pronounced effect on β semiHb rather than on α semi-Hb. Until now no evidence has been provided for the spatial location of L35 binding site in semiHbs. The aim of this work was therefore to better understand the structural basis of ligand recognition in semihemoglobins and identify potential modes of binding for L35.



BZF

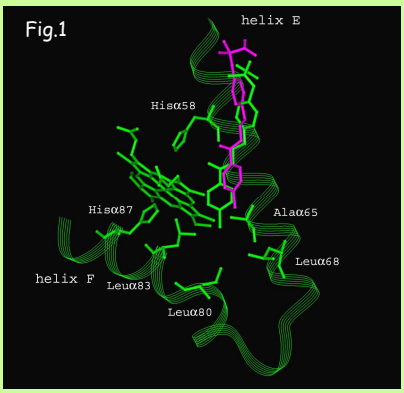


L35

METHODS

❑ **Glue calculations**. Docking calculations were performed using the program GLUE implemented in GRID v22 (Goodford, P.J. *J. Med. Chem.* 1985, 28, 849-857). GLUE finds possible interaction sites for a small rigid molecule within a target molecule by fitting the maps generated by GRID for the probes which mimic the structure of the ligand. The program then positions hydrophobic and polar atoms of the ligands over their corresponding energy maps. Favourable modes of binding are optimised within the protein cavity by successive torsions and translations driven by the protein-ligand interaction energy computed by the GRID force field. To validate the computational protocol, we performed two tests that are based on the available crystallographic structures. In the first test we attempted to reproduce the crystallographically detected mode of binding of fibrates to horse carbomonoxy. The following single atom probes were used in the calculations: H, OH2, DRY, CL, N1, O, O::, OC2 (Fig. 1). In the second test we attempted to reconstitute dimeric hemoglobin ($\alpha^h\beta^h$) from semihemoglobins (α- and β) and ferrous heme (Fig. 2). The following single atom probes were used in this second test: H, OH2, DRY, C3, O:: . The computational procudure which successfully predicted known crystallographic complexes was then applied to investigate the mode of binding of L35 to α- and β semihemoglobins (Fig. 3).

❑ **GRID analysis.** To explore the hydrophobic and hydrophilic characteristics of the α and β heme cavities , two different probes were used: the hydrophobic probe (DRY) and the water probe (OH2). The results are displayed as contour maps showing regions of the empty α and β heme pockets which favourably interact with the hydrophobic and the hydrophilic probes (Fig.4 and Fig.5). The contour maps are displayed using InsightII (Accelrys).



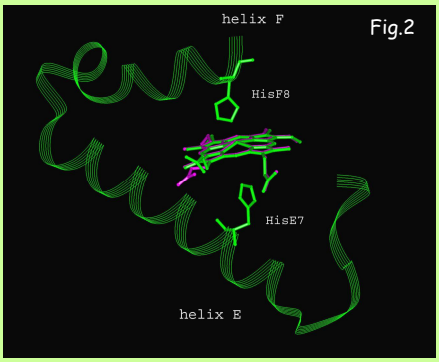
RESULTS

Figure 1 The only crystallographic structure of BZF or of an analogue co-complexed with hemoglobin that has been deposited to date in the Protein Data Bank is that with horse carbomonoxy Hb (pdb code 1IWH). Information about the bound ligand position is not used and the GRID program predicted the correct mode of binding of BZF in the crystallographic complex. A root-mean-square deviation, rmsd, of heavy atoms of ligand docking pose (magenta) from the reference x-ray structure (green) <2.0 (1.34 Å) was calculated.

Figure 2 Dimeric hemoglobin ($\alpha^h\beta^h$) from semiHbs (α- and β) and ferrous heme was reconstituted. Estimated binding energies of heme for α and β heme pockets were -32.8 kcal/mol and -27.0 kcal/mol respectively. Theoretical results are in agreement with the experimentally detected lower affinity of the β pocket for heme.

Figure 3 The same successfully computational approach was then used to investigate potential binding sites of L35 in semiHbs. GLUE calculations on α(apo)β(apo) dimer showed that L35 interacts preferentially with the a-heme pocket with respect to the β one. Estimated binding energies of L35 with α and β heme pockets were -13.3 kcal/mol and -10.5 kcal/mol respectively. The same ligand orientation was predicted in the two pockets, with L35 (magenta) hydrogen bonded to the proxymal histidine F8.

Figures 4-5 Regions of favourable hydrophobic and hydrophilic interactions within heme pockets were explored using the hydrophobic probe (DRY) and the water probe (OH2) in the absence of hemes. The GRID analysis of the α heme cavity (Fig. 4) reveals one main hydrophobic region inside the cavity (yellow) and two well defined hydrophilic areas (red). Contour maps at -1.0 kcal/mol and at -9.0 kcal/mol for DRY and OH2 probes respectively are shown in Fig 4. L35 molecule is superimposed on the contours. Same contour maps values were plotted for the β heme cavity (Fig. 5). In this case the hydrophilic contours (red) cover several areas within the pocket thus probably reducing the binding affinity for the hydrophobic L35 molecule. It is worth noticing that in β heme pocket a Glu residue (β101) replaces ValG3(α96).



CONCLUSIONS

To determine whether we could rationalize why in the presence of L35 larger effects on oxygen affinity were observed for β-semi Hb than for α-semi Hb we exploited the potential modes of binding of L35 within α- and β-semihemoglobin. In the absence of experimental evidences on the interaction we used a theoretical approach mainly based on the application of GRID program and its docking module GLUE. Computational results suggest that the larger effects on oxygen affinity observed for β-semi Hb might be explained with the evidence for different hydrophobic and hydrophilic characteristics between α and β pockets.