Structural comparison between the crystal structures of muscle glycogen phosphorylase b and liver glycogen phosphorylase a complexed with indole-2-carboxamide inhibitors, potential antidiabetic drugs

N.G. Oikonomakos[1], S.E. Zographos[1], V.T. Skamnaki[1] and Georgios Archontis[2]

[1]Institute of Biological Research and Biotechnology, The National Hellenic Research Foundation, 48 Vas. Constantinou Avenue, Athens 11635, Greece. [2]Department of Physics, University of Cyprus, Box 20537, CY1678,, Nicosia, Cyprus.

CP320626, a potential antidiabetic drug, has been shown to be a potent inhibitor of human liver glycogen phosphorylase a (LGPa) (IC_{50}=0.2 μM) and to produce marked glucose lowering in diabetic *ob/ob* mice [1]. CP320626 was also demonstrated to be a potent inhibitor of muscle glycogen phosphorylase b (MGPb) (IC_{50}=0.3 μM) and to act in synergism with glucose (IC_{50}=0.2 μM) [2]. The observed synergism could be an important physiological feature of a LGPa inhibitor, because the decrease in inhibitor potency, as glucose concentrations decrease *in vivo*, should minimize the risk of hypoglycemia. We cocrystallised MGPb with both CP320626 and glucose, determined the structure of the complex by x-ray crystallographic methods at 1.76 Å resolution and refined to a crystallographic *R* value of 0.211 (R_{free} =0.235).

The 1.76 Å resolution complex structure has revealed differences in the conformation of the 4-hydroxy-piperidyl moiety of the ligand as compared to the 2.3 Å resolution structure [2]. The electron density maps, derived from the previous analysis, were interpreted with the 4-hydroxy-piperidyl moiety in $^1C_4$ rather than in $^4C_1$ conformation. The resolution of the present structure allowed us to define more accurately the conformation of the 4-hydroxy-piperidyl moiety (Fig. 1). The 4-hydroxy-piperidyl moiety adopts the most stable puckered ring $^4C_1$ conformation. The fitted to the electronic density map $^4C_1$ chair conformation of the CP320626 piperidine moiety was optimized by *ab initio* and Density Functional Theory methods with the program GAUSSIAN; the resulting chair structures were similar and superimposed very well to the X-PLOR-refined geometry [3].

Human LGPa is the most important target enzyme in terms of treatment of type 2 diabetes because of its direct influence on blood sugar level. In order to assess the validity of using MGPb as a model for the LGPa in structure-assisted drug design, we have compared the structure of CP320626 complexed with MGPb with the structure of CP403700 bound to LGPa [4]. The superposition of the LGPa-1-GlcNAc-CP403700 complex structure with MGPb-glucose-CP320626 complex structure over well defined residues 23-249, 260-313, 326-830 gave r.m.s. deviation of 0.537 Å for Cα atoms, indicating that the two structures have very similar overall conformations. Furthermore, the superposition of the CP403700 complex with the CP320626 complex over 27 residues of the new allosteric inhibitor site (37'-40', 53'-57', 60-67, 188-192, 185'-188', and 229) gave r.m.s. deviations of 0.307, 0.300, and 0.978 Å for Cα, main chain, and side chain atoms, respectively, indicating that the two structures superimpose well and they closely resemble in the vicinity of the new allosteric site (Fig. 2), indicating the accuracy of MGPb as a model in the design and optimisation of indole-2-carboxamide inhibitors.

References

[1]. D.J. Hoover, S. Lefkowitz-Snow, J.L. Burgess-Henry, W.H. Martin, S.J. Armento, I.A. Stock, R.K. McPherson, P.E. Genereux, E.M. Gibbs, and J.L. Treadway, J. Med. Chem. 41, 2934-2938 (1998).
[2]. N.G. Oikonomakos, V.T. Skamnaki, K.E. Tsitsanou, N.G. Gavalas, and L.N. Johnson, Structure 8, 575-584 (2000).
[3]. N.G. Oikonomakos, S.E. Zographos, V.T. Skamnaki, and G. Archontis, Bioorg. Med. Chem., in press (2001).
[4]. V.L. Rath, M. Ammirati, D.E. Danley, J.L. Ekstrom, E.M. Gibbs, T.R. Hynes, A.M. Mathiowetz, R.K. McPherson, T.V. Olson, J.L. Treadway, and D.J. Hoover, Chem. Biol. 7, 677-682 (2000).

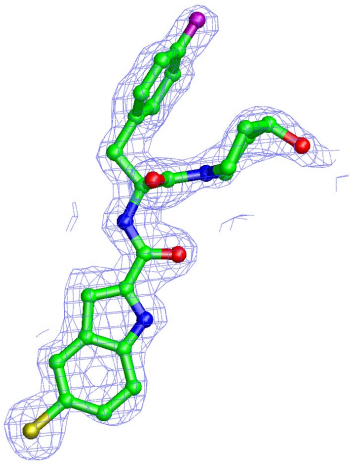

Fig. 1. Schematic diagram of the electron density of the bound CP320626 to GPb from a 1.76 Å simulated annealing (F_o-F_c) omit map (contoured at 2.5σ level).

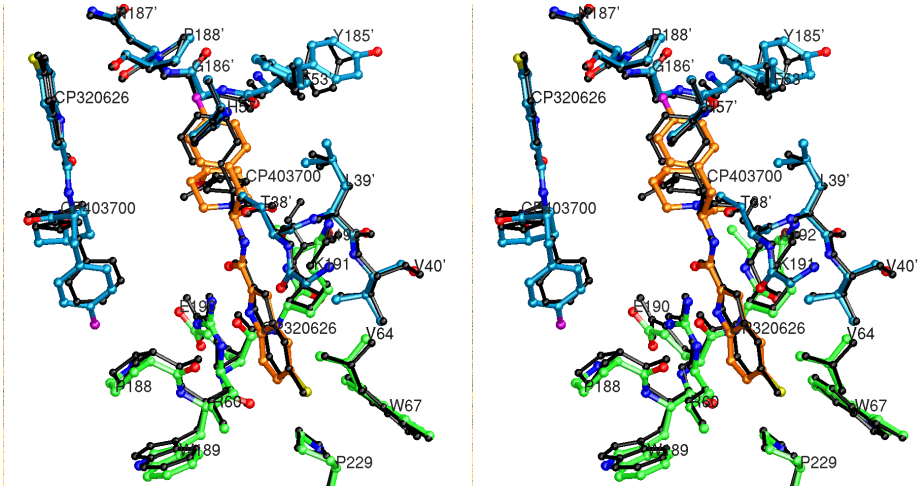

Fig. 2. Stereodiagram showing a comparison of CP320626 bound to MGPb-glucose complex with bound CP403700 to LGPa (code 1exv) in the vicinity of the new allosteric inhibitor site. Grey: MGPb-glucose-CP320626 complex; black: LGPb-1-GlcNAc-CP403700 complex.