Small Angle X-ray Scattering of human glucokinase in complex with a potent activator

E. Anastasi[1] E., S.E. Zographos[1], V. Skamnaki[1], D.D.Leonidas[1],R. Kardakaris[1], P.V. Konarev[2], D.I. Svergun[2], D. Schmoll[3], E. Defossa[3] and N.G. Oikonomakos[1]

[1]Institute of Organic and Pharmaceutical Chemistry, The National Hellenic Research Foundation, Athens, 48 Vas. Constantinou Avenue, Athens 11634, Greece, [2]EMBL Hamburg Outstation, Building 25A Notkestrasse, 85 22603 Hamburg Germany [3]Sanofi-Aventis Pharma Deutschland GmbH, a company of the sanofi-aventis group, D-65926 Frankfurt am Main, Germany

Glucokinase (GK) is an allosteric monomeric enzyme (50 kDa), catalyzing the phosphorylation of glucose in pancreatic β-cells and hepatocytes. GK exhibits low affinity (8 mM) and sigmoidal saturation (n_H=1.5) for glucose and displays a key role in blood glucose control both by stimulating the glucose uptake and glycogen synthesis in liver and insulin secretion in β-cells [1, 2]. Structural analyses of GK-activator complexes may contribute to the detailed understanding of allosteric function and is likely to form the basis for structure based rational design of more potent activators with potential pharmacological significance for type 2 diabetes.

The recently reported structures of the truncated hepatic forms give insights into the structural basis for allosteric regulation of human GK [3], and also revealed an allosteric binding site for activators which can be exploited for the development of new therapeutic agents (**Figure 1**). Kamata and co-workers [3] succeeded to crystallize the ternary complex of human truncated GK with glucose and an activator (Banyu) in the closed (*active*) form as well as the free form of GK in the super-open (*inactive*) form.

The compound RO-28-1675 (**Figure 1**) is a nonessential mixed type activator of GK that decreases the Km for the glucose 4- to 5-times and increase the maximum enzymic velocity (Vmax) from 1.5 to almost 2-times [4, 5]. SAXS experiments performed at X33 EMBL beamline (as shown in **Table 1**) indicated that glucose, ATP or RO-28-1675 alone can not promote the active form of GK, and the experimental scattering curves fit the theoretical scattering curves based on the known crystal structure (1V4T) of the super-open (*inactive*) form (**Table 1**). Interestingly, the SAXS data obtained for the ternary complex of GK with RO-28-1675 and glucose as well as for the binary complex of GK with the native product ADP showed that these complexes are more likely to exist in solution in an intermediate conformation compared with the closed (1V4S) and super-open form (1V4T) (**Table1**) [4]. Based on the scattering data, atomic models of the Cα backbone atoms of GK were produced by rigid body refinement, using the program MASSHA v2.3 (**Figure 2**) [6]. The calculated radius of gyration (Rg) for the GK-glucose-activator and GK-ADP SAXS models, found to be similar (Rg values of 24,71 Å and 24,57 Å, respectively) and the values varied between the calculated Rg values for the crystallographic models 1v4T (25,69 Å) and 1v4S (24,02 Å).

The present study supports the existence of an open form for the human enzyme and gives insights for the mechanism of the allosteric activation and the positive cooperativity of GK. SAXS data is in agreement with the mechanism previous proposed [3], where GK utilizes two catalytic cycles. According to this model a slow cycle between the super-open and the closed form and a fast one between the open and the closed form occur. Our study indicates that the product ADP alone or the RO-28-1675 in the presence of glucose can promote the open form whereas the glucose, the ATP or the activator alone cannot. Thus these structural data suggest that the enzyme in the presence of both substrates (ATP and glucose) carries out a first slow cycle between the super-open form and the

closed form. After completion of the reaction, the products stabilize the open conformation and they are released on binding of new substrate molecules for the fast catalytic cycle [4, 7]. Furthemore it can be suggested that the RO-28-1675 in the presence of glucose promotes an open active form similar to that adopted in the presence of the products (Glc-6-P and ADP). This is a new mode of action compared with the Banyu compound which in the presence of glucose seems to promote a closed conformation [3]. Future SAXS experiments of GK in the presence of various concentrations of activator, substrates (glucose and ATP), products (ADP and glucose-6-P), might lead to the mapping of GK structure fine tuning in the presence of its natural and synthetic regulators.

Table 1. Comparison of experimental scattering curves with the theoretical ones derived from the crystallographic models using the program Crysol [8]

Exp no.	Experimental conditions	Chi values	
		1V4S (Closed/active)	1V4T(Super-open/inactive)
1	Free Glucokinase (2 mg/ml)	1.602	1.336
2	Free Glucokinase (2 mg/ml) and 5% DMSO	2.077	1.813
3	Glucokinase (2mg/ml), 250 μM RO-281675 and 5% DMSO	2.076	1.581
4	Glucokinase (2mg/ml), 50 mM glucose, 250 μM RO-281675 and 5% DMSO	2.490	2.224
5	Glucokinase (2mg/ml) and 1 mM ADP	4.543	4.665
6	Glucokinase (2mg/ml) and 1 mM ATP	2.015	1.519

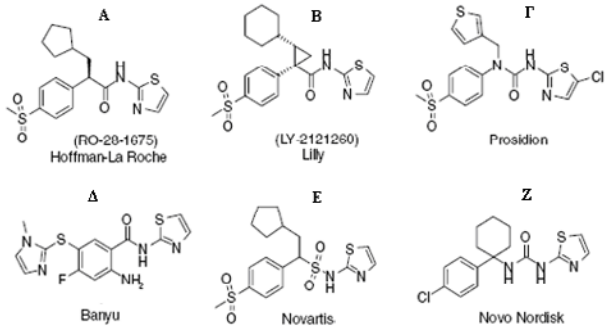

Figure 1. Synthetic activators of glucokinase

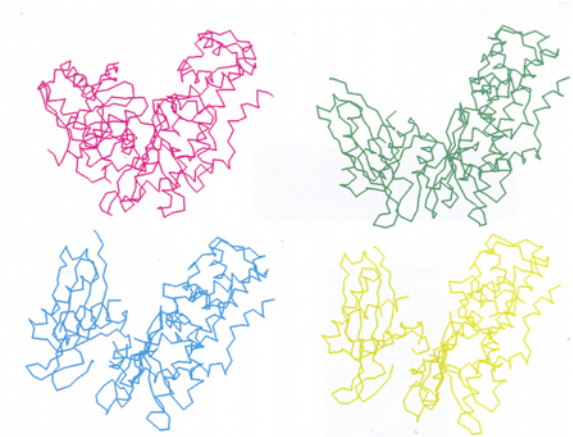

Figure 2. Comparison of the crystallographic models of Super-open (1v4T- green, Rg=25.69) and closed (1v4S-pi Rg=24.02) forms of GK with the atomic models construc based on the GK-glucose-activator (blue, Rg=24.71) a GK-ADP (yellow, Rg=24.57) scattering data.

References

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