Characterizing the Structures of Proteins Involved in Lipolysis: G0S2 - a Novel Inhibitor of ATGL

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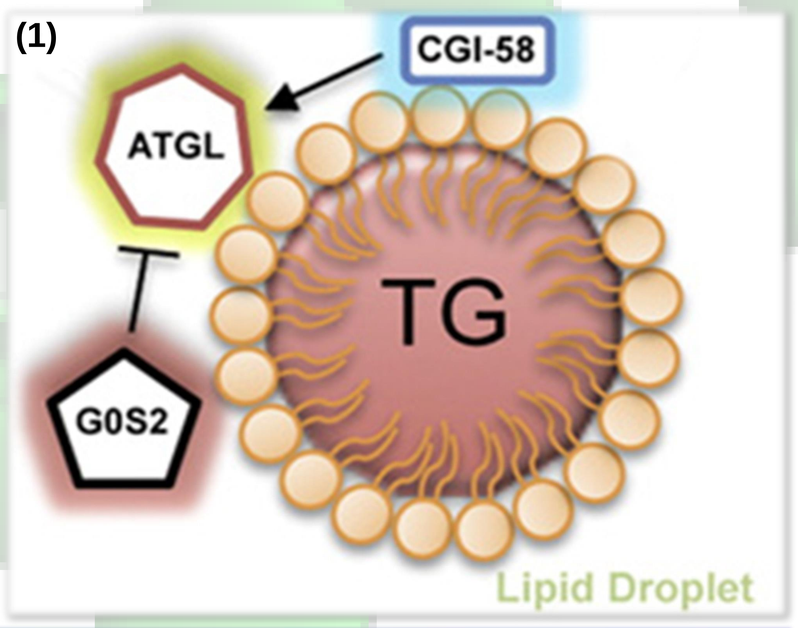
G0S2 - a Inhibitor of Lipolysis

In mammals, energy is stored predominantly in the form of triacylglycerol (TG) within lipid droplets. Lipolysis is the breakdown of lipids and involves the hydrolysis of TG into free fatty acids (FA). Adipose triglyceride lipase (ATGL) is the rate limiting enzyme of lipolysis. The proteins comparative gene identification-58 (CGI-58) and G0/G1 switch gene 2 (G0S2) represent key regulatory proteins of lipolysis and were identified as activator and inhibitor of ATGL, respectively.

(1)

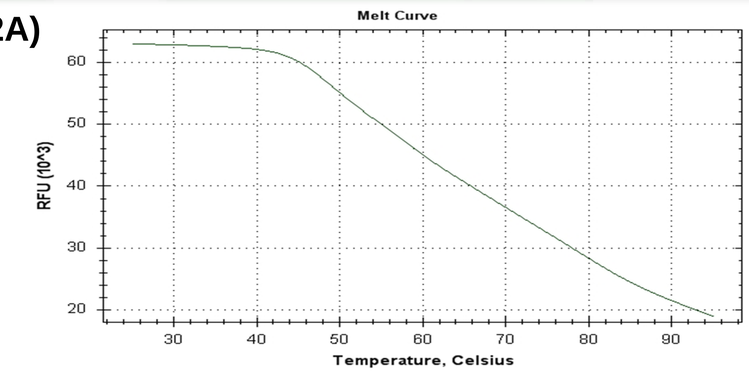


G0S2 is a small basic protein with a molecular weight of 11 kDa. The protein is expressed mainly in adipose tissue, liver and heart. Based on secondary structure prediction it consists of four potential alpha helical regions. We aim at structural characterization of this protein to unravel its mode of action at molecular detail.

Figure 1: hG0S2 inhibits ATGL at the lipid droplet (Heckmann, et al. 2012).

Biophysical Characterization

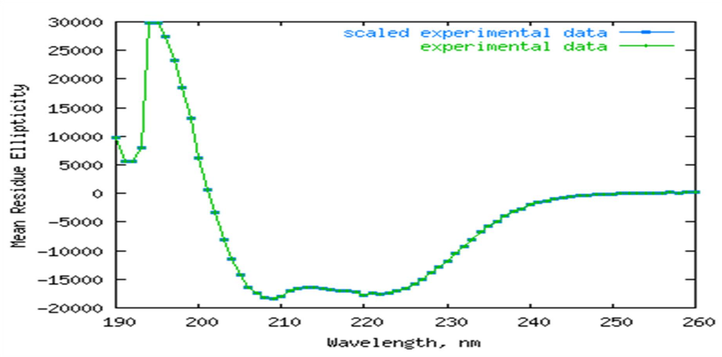
(2A)



(2B)

(2C)

Helix1	Helix2	Strand1	Strand2	Turns	Unordered	Total
0.42	0.12	0.13	0.09	0.08	0.16	1



(2D)

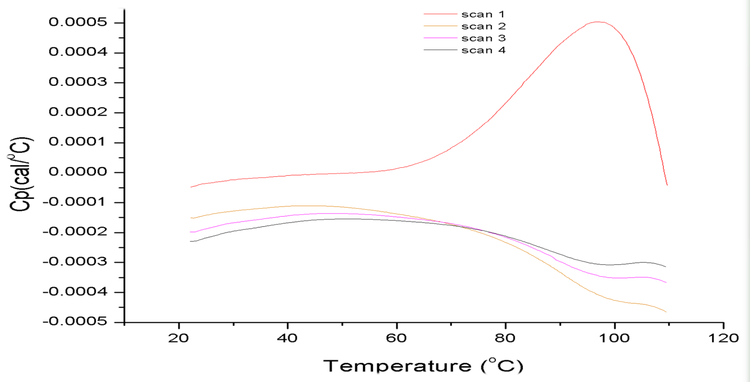


Figure 2: **(2A)** Thermofluor measurement of hG0S2 showing its hydrophobic nature. **(2B)** According to secondary structure prediction (Phyre[2]), human G0S2 consists of four α-helices and is highly disordered. **(2C)** Circular dichronism (CD) spectroscopy indicates mostly an α-helical content for hG0S2. **(2D)** Differential scanning calorimetry (DSC) measurement of hG0S2 indicates a TM of 97 ° C.

Cellular Localization

In cultivated COS-7 cells human G0S2 localizes to lipid droplets. CFP-fusion of wild type and truncated versions of G0S2 were produced in order to investigate whether truncated G0S2 variants, which fail to inhibit ATGL also fail to localize to the lipid droplet in COS-7 cells.

(3A) **(3B)**

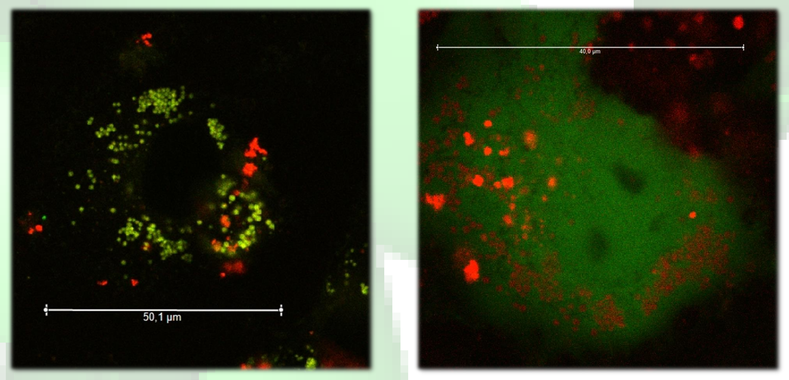
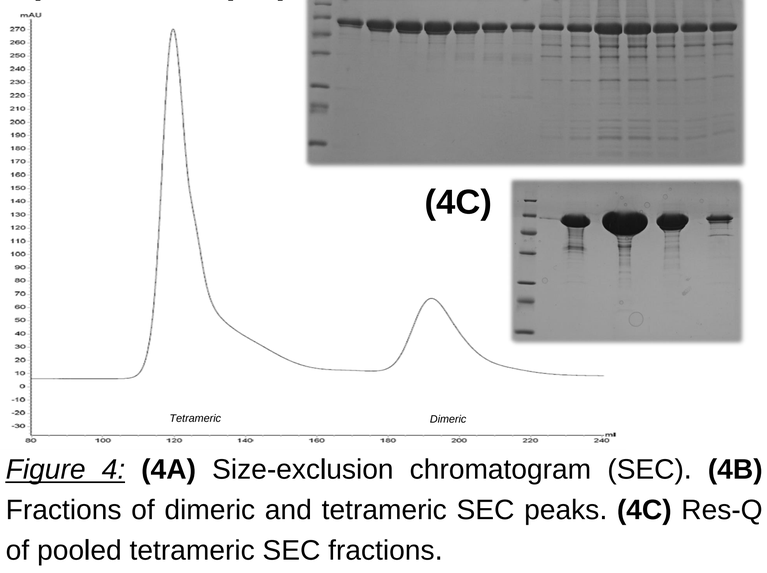


Figure 3: Confocal Fluorescence Microscopy. Overlay of hG0S2-CFP (green) and LD-stain LD540 (red). **(3A)** Wildtype hG0S2 localizes to the lipid droplet. **(3B)** No co-localization with LD is observed for an inactive variant Met1-Gly41.

Purification

For structural characterization, purified and active G0S2 in the mg/ml range is required. Isoforms of human and mouse G0S2 with different fusion partners were tested for overexpression, activity and purification. The most promising results were obtained fused to *E. coli* trigger factor.

(4A) **(4B)**



(4C)

Figure 4: **(4A)** Size-exclusion chromatogram (SEC). **(4B)** Fractions of dimeric and tetrameric SEC peaks. **(4C)** Res-Q of pooled tetrameric SEC fractions.

Structural Characterization

Figure 5: NMR-Spectroscopy, ^{1}H-^{15}N-HSQC spectrum of hG0S2_1-103 (blue). Due to highly unordered or α-helical regions, the central part of ^{1}H-^{15}N-HSQC spectrum is collapsed. The overlay with a ^{1}H-^{15}N-HSQC spectrum of hG0S2_1-52 (red) simplified the data and helped to identify four N-terminal glycines and the C-terminus of hG0S2_52. This is in line with disorder prediction of hG0S2 showing a highly unstructured C-terminus.

(6A) **(6B)**

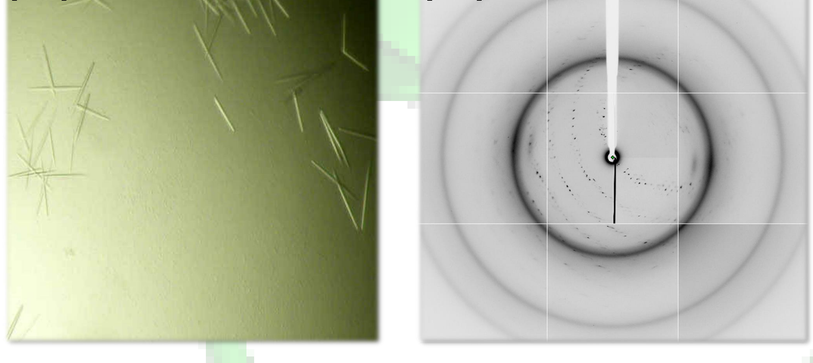


Figure 6: Protein crystallization, **(6A)** Crystallization screens with the tetrameric fraction of TF-mG0S2 led to initial crystals in form of thin needles. **(6B)** Optimization of the initial condition using vapor diffusion technique and additives produced anisotropic diffracting crystals up to 2.8Å .

(5)