Unravelling the pathway of regulation of photosynthetic AB-GAPDH

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Photosynthetic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a tightly regulated key enzyme of the Calvin-Benson cycle, the dark part of the photosynthesis. In higher plants two isoforms of GAPDH co-exist in the chloroplast stroma: a homo-isoform exclusively made of A subunits, and a hetero-isoform containing both A and B subunits (A-GAPDH = 36 kDa; B-GAPDH = 39 kDa). In its fully active form the quaternary structure of AB-GAPDH is represented by a heterotetramer composed by two A and B subunits. The fully inactive form of AB-GAPDH is instead considered an exadecamer, generated by the union of four tetramers of A_2B_2-GAPDH [1]. Recently SAXS analyses suggested that the AB-GAPDH isoforms exist in several state of oligomerization independently from the conditions imposed to stabilize the active or inactive state of the enzyme. To describe the dynamism of the AB-GAPDH system we purified the inactive oligomers [2] and imaged the protein using negative stain electron microscopy. In agreement with the SAXS analyses, we observed particles of 9 nm, 18 nm and 22 nm in diameter compatible with several different GAPDH oligomers (Fig.1A). The images reveal unsupervised classes averages views with two-fold, three-fold, four-fold and five-fold symmetry, compatible with A_4B_4, A_6B_6, A_8B_8 and $A_{10}B_{10}$ (Fig. 1A). We focus on A_4B_4 and A_8B_8 oligomers and we determined their low resolution negative stain EM structures by imposing C2 and C4 symmetry, respectively (Fig. 1B and C). Although preliminary, the row EM maps suggest that in the inactive conformation A_2B_2 heterotetramers can interact forming dimers and tetramers, these last having a tetragonal structure delimiting a central cavity (Fig. 1C). Further investigation of the observed compositional heterogeneity will be performed by using GraFix gradients [3] and by image analyses dealing specifically with structurally heterogeneous datasets. We will acquire in parallel cryoEM data to strengthen the structural analysis and understand how inactivation is acquired *via* oligomerization.

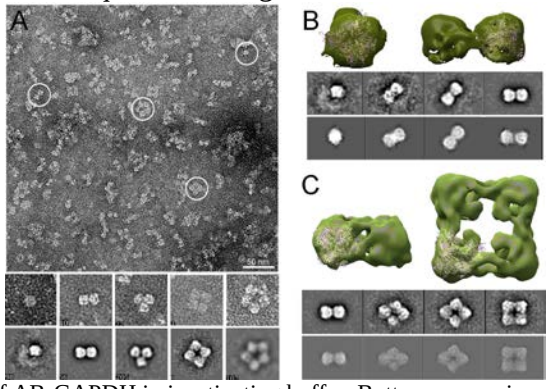

Figure 1: A, Negative staining of AB-GAPDH in inactivating buffer. Bottom rows: views of different oligomers (top) and their corresponding class averages (bottom). B, A_4B_4 3D model in top - and side - view. Below some of its corresponding class averages (top row) and projections (bottom row). C, A_8B_8 3D model in top - and side - view. Below some of its corresponding class averages (top row) and projections (bottom row). The atomic structure of A_2B_2 GAPDH was docked into the A_4B_4 and A_8B_8 3D reconstructions.

References

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