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STRUCTURAL ASSEMBLY OF THE PHOTOSYNTHETIC AB-ISOFORM OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE

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Oxygenic photosynthetic organisms produce sugars through the Calvin–Benson cycle, a metabolism that is tightly linked to the light reactions of photosynthesis and is regulated by different mechanisms. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is responsible for the single reducing step of the cycle, thus consuming the NADPH produced by the light phase 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. The main difference between A- and AB-GAPDH concerns their regulatory mechanism. The homo-isoform achieves its dark inactivation state through the interaction with phosphoribulokinase via the scaffold protein CP12. On the contrary, the hetero-isoform turned off its activity through an auto assembly process leading to the formation of the inactive oligomer composed by eight A- and eight B-subunits.

Recently Size Exclusion Chromatography (SEC) coupled with Small Angle X-ray Scattering (SAXS) analyses highlighted the coexistence of different oligomerization states, regardless the conditions imposed to stabilize the active A_2B_2 -GAPDH isoform or the inactive A_8B_8 -GAPDH isoform. This finding suggests an intriguing dynamism of the AB-GAPDH system and let us to speculate that dynamism should be a relevant component of this biological system.

To further describe the dynamism of the AB-GAPDH system, the inactive form of the enzyme was purified and analysed by negative staining electron microscopy followed by single particle analysis. Particles with a diameter of 9 nm, 18 nm and 22 nm, and compatible with A_4B_4 , A_6B_6 , A_8B_8 and $A_{10}B_{10}$ oligomerization states, were observed, in agreement with SAXS data. Focusing the attention on A_8B_8 oligomer a low resolution 3D model was determined using particle averaging by imposing a C4 symmetry. Although preliminary, the inactive form of AB-GAPDH appears as a tetragonal structure delimiting a central cavity.