Poster

Regulation pathway of photosynthetic glyceraldehyde 3-phosphate dehydrogenase

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Photosynthetic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a key enzyme of the Calvin–Benson cycle through which oxygenic phototrophs perform carbon fixation. The cycle as well as the light-harvesting reactions of photosynthesis are modulated by different mechanisms to adjust them to rapid environmental changes.

Different GAPDH isoforms exist in higher plants: the most abundant is a hetero-tetramer of A and B-subunits (AB-GAPDH) while the least abundant is formed by A_4 homotetramers [1]. Being the major consumer of photosynthetic NADPH, the activity of GAPDH is strictly regulated. In the case of the AB-isoform, the oxidation of a pair of cysteines located at the C-terminal extension (CTE) of B- subunits and the substitution of NADP(H) preferred coenzyme, with NAD(H) in the cofactor binding domain, change the oligomerization state of the enzyme strongly inhibiting its activity.

By combining small angle x-ray scattering coupled with size exclusion chromatography (SEC-SAXS) and cryo-electron microscopy (cryo-EM), we revealed the presence of several AB-GAPDH oligomers [$(A_2B_2)_n$-GAPDH with n=1, 2, 4 and 5] co-existing in a dynamic system [2] (Fig. 1). Moreover, the most abundant oligomer A_8B_8-GAPDH was present in two different conformers.

Despite the sample's significant compositional and conformational heterogeneity, we solved the two A_8B_8-GAPDH hexadecameric conformers at a resolution around 3 Å, while the other GAPDH oligomers were instead solved at intermediate resolution. In all GAPDH oligomers, we observed the same oligomerization/inhibition mechanism based on the mutual exchange between adjacent B- subunits of their CTEs, effectively preventing the binding of the substrate 1,3-bisphosphate-glycerate in the B subunits. The nearly atomic resolution allowed us to detail the molecular interactions of CTE with the active site residues of the B-subunit, the cofactor and the cofactor binding domain residues of the adjacent A-subunit as well as the interactions at the A_2B_2-tetramer interfaces stabilizing the different oligomeric assemblies.

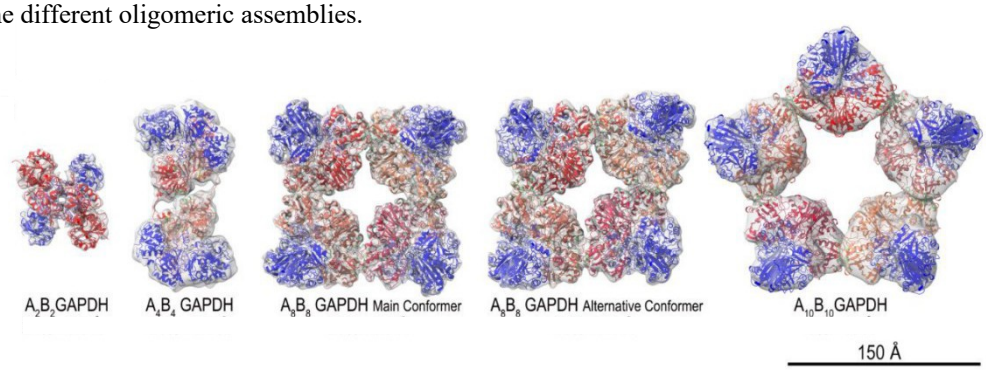

Figure 1. AB-GAPDH oligomers cryo-EM density maps co-existing in the chloroplast stroma. The cryo-EM electron density maps were fitted by models derived from the crystal structure of oxidized A_2B_2-GAPDH complexed with $NADP^+$ [3].

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[2] Marotta R., Del Giudice A., Gurrieri L., Fanti S., Swuec P., Galantini L., Falini G., Trost P., Fermani S., Sparla F. (2022). *Acta Crystallogr D Struct Biol*, **78**, 1399.

[3] Fermani, S., Sparla, F., Falini, G., Martelli, P. L., Casadio, R., Pupillo, P., Ripamonti, A., Trost, P. (2007). *Proc. Natl Acad. Sci.* USA, **104**, 11109.