The structural revelation of the smallest functional pyruvate kinase from Entamoeba histolytica.

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Pyruvate kinase (Pyk) structure was determined many years ago, where its three domain architecture was unravelled. The catalytic site rests between the interface of Tim barrel A and ‘Lid’ B domains, while the C domain contains the allosteric effector site. Our group reports the smallest fully functional pyruvate kinase from Entamoeba histolytica (EhPyk) that lacks the C domain in its entirety. This domain architecture is unique to the amoebic Pyks. We have solved a 1.7Å apo crystal structure, which reveals the difference in the active site residues. Arg72, Arg119, Lys269, and Thr327 are the four critical active site residues (nomenclature with respect to 1PKN, the first pyruvate kinase structure from rabbit muscle) [1]. EhPyk contains Gly instead of Thr in its active site (Figure 1). Despite the change, the kinetic parameters are still comparable with other Pyks [2]. Further, upon in depth phylogenetic analysis, we found that EhPyk shares greater similarity with the prokaryotic homologues, rather than the eukaryotic ones. Hence, this reveals the evolution of Pyk from prokaryotes to earlier eukaryotes, and later the C domain was added to the protein in higher eukaryotes for greater regulation strategies. Our data structurally displays that the active site Thr327 is probably dispensable and also adds information regarding Pyk evolution from lower to higher eukaryotes.

![Figure 1](image_url)

**Figure 1.** The figure displays the structural superimposition of EhPyk (Purple), 4KRZ: T. cruzi (Salmon), 6K0K: E. coli PykII (Yellow), 6NN8: Human liver Pyk (Magenta), 5WRP: M. tuberculosis (Cyan). All the structures are unbound or apo structures with the active site residues shown in sticks (numbered according to EhPyk residue numbers) for comparison.


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