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Sommario	The catalytically active form of vitamin B6, pyridoxal 5'-phosphate (PLP), acts as a coenzyme in a variety of different enzymatic reactions. Organisms which are not able to synthesize PLP de novo acquire B6 vitamers from nutrients and interconvert them through a salvage pathway, which involves pyridoxine 5'-phosphate oxidase (PNPOx) and pyridoxal kinase (PDXK). PNPOx converts pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP) to PLP, using flavinmononucleotide (FMN) as coenzyme. Both Escherichia coli and human PNPOx are homodimers and, although these enzymes share only 39% of sequence identity, have very similar structural and functional properties. PNPOx plays a crucial role in the regulation of PLP metabolism. It has been proposed that PLP inhibits the catalytic activity of both E. coli and human PNPOx by binding at the active site and acting as a competitive inhibitor. However, PLP can also bind tightly at a secondary site. Our kinetics characterisation suggests that PLP inhibition results from binding of this vitamer at an allosteric site, in both E. coli and human enzymes. This interpretation was confirmed by the analysis of mutated forms of E. coli PNPOx, in which PLP binding at the active site is impaired. Crystallographic studies carried out by other authors on the E. coli PNPOx indicated a possible location of the secondary PLP binding site in two surface pockets of

	the protein, but site-directed mutants of amino acid residues putatively critical for this interaction showed that this hypothesis is wrong. Molecular docking analyses identified a possible alternative PLP binding site, which is a cleft on the protein surface mainly delimited by arginine residues and located near the subunit interface. Characterisation of mutant forms of this site and crystallographic studies suggested that this might be the allosteric PLP binding site. Concerning human PNPOx, it is known that missense mutations in the gene encoding this enzyme lead to the onset of a rare neurological disease, the neonatal epileptic encephalopathy (NEE); however, the molecular reason of most PNPOx mutations remains to be established. We expressed PNPOx mutants as recombinant proteins in E. coli, purified and characterised them with respect to structural and functional properties, in order to better understand the molecular basis of the disease. The other key enzyme involved in the salvage pathway is PDXK, which converts pyridoxal (PL), pyridoxamine (PM) and pyridoxine (PN) into PLP, PMP and PNP, respectively. In Drosophila, mutations in the dPdxk gene encoding PDXK cause chromosome aberrations (CABs) and increase glucose content in larval haemolymph. This observation suggests that PDXK mutations in humans may be involved in diseases such as cancer and diabetes. We analysed the effect of the expression of four PDXK human variants in Drosophila dPdxk mutants: three of them (D87H, V128I and H246Q) are listed in databases, and one (A243G) was found in a genetic screening of patients with gestational diabetes. None of the variants was able to completely rescue CABs and glucose content. Our biochemical analysis revealed reduced catalytic activity and different affinity of these variants for PLP precursors. Overall, our findings suggest that, when PLP levels are reduced by the presence of these PDXK variants, cancer and diabetes risk may be increased.
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