

ACTIVE SITES OF ENZYMES ARE CROWDED WITH CHARGE

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The chemistry of enzymes occurs at active sites that concentrate biological function into functional pockets. Functional pockets mix catalytic amino acids and substrate in tiny volumes. Here, we look for biological properties of that small space. We imagine that electric charge plays important roles, because even one charge in a small space produces large electric fields. To estimate densities of fixed charge, we measure the volume of functional pockets and count 'charged residues' (i.e., ionizable amino acids) in it. We collect locations of functional pockets from enzymes of known structure that catalyze the main six enzymatic reactions. Functional amino acids are identified by their known participation in catalysis. We measure the volume of pockets using molecular-surface model. 'Charged residues' are defined as R, K and H (positive); E and D (negative) although we have no independent knowledge of charge state. The density of ionizable amino acids is extraordinarily large (~20 Molar on average, often larger). Mobile counterions for the fixed charge are presumably nearby in high density. Active sites do not resemble the infinitely dilute ideal solutions of classical enzyme kinetics. Their enormous charge density is comparable to the charge density of solid NaCl. Different types of enzymes have different charge densities. Hydrolases show the largest values of charge density. Some enzymes have extraordinarily large charge density -phosphoglycerate mutase (PDB = 1o98, density of charge 103 Molar, Molecular Surface), or RNA Triphosphatase (PDB = 1d8h, 70 Molar, Molecular Surface). Crowding of charged side-chains and ions produces enormous steric and electrostatic forces in these tiny active sites. The balance of these forces seems likely to be important to enzyme function. Other charged pockets are found on the outer surface of the protein away from active sites. These charged pockets are likely to be involved in the many significant surface interactions of proteins. The steric and electrostatic free energy stored in them may be the motive force for changes in the conformation of proteins.

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