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Identification of the Putrescine Recognition Site on Polyamine Transport Protein PotE

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The PotE protein can catalyze both uptake and excretion of putrescine. The K_m values of putrescine for uptake and excretion are 1.8 and 73μM, respectively. Uptake of putrescine is dependent on the membrane potential, whereas excretion involves putrescine-ornithine antiporter activity. Amino acids involved in both activities were identified using mutated PotE proteins. It was found that Cys^{62}, Trp^{201}, Trp^{292}, and Tyr^{425} were strongly involved in both activities, and that Tyr^{92}, Cys^{210}, Cys^{285}, and Cys^{286} were moderately involved in the activities. Mutations of Tyr^{78}, Trp^{90}, and Trp^{422} mainly affected uptake activity, and the Km values for putrescine uptake by these PotE mutants increased greatly, indicating that these amino acids are involved in the high affinity uptake of putrescine by PotE. Mutations of Lys^{301} and Tyr^{308} mainly affected excretion activity (putrescine-ornithine antiporter activity), and excretion by these mutants was not stimulated by ornithine, indicating that these amino acids are involved in the recognition of ornithine. It was found that the putrescine and ornithine recognition site on PotE is located at the cytoplasmic surface and the vestibule of the pore consisting of 12 transmembrane segments. Based on the results of competition experiments with various putrescine analogues and the disulfide cross-linking of PotE between cytoplasmic loops and the COOH terminus, a model of the putrescine recognition site on PotE consisting of the identified amino acids is presented.