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RESEARCH NEWS

How a tyrosine primes the pump

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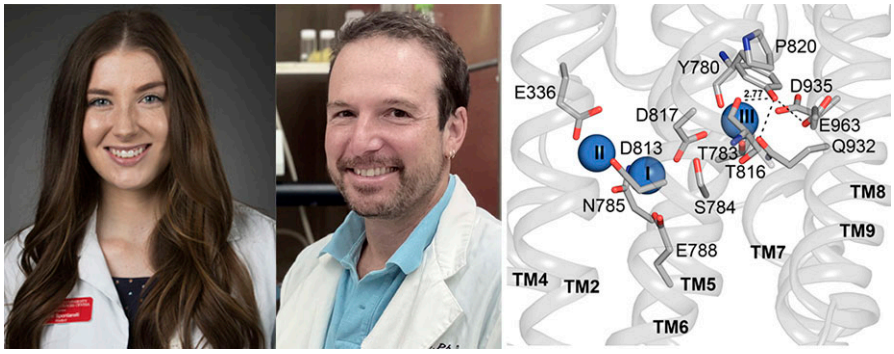
***JGP* study uses both natural and unnatural amino acid substitutions to examine how a key tyrosine residue controls the selectivity of the Na^+/K^+ pump.**

Numerous cellular processes, including nutrient uptake and the transmission of nerve impulses, depend on the electrochemical gradients of Na^+ and K^+ ions across the plasma membrane. These gradients are established and maintained by the Na^+/K^+ pump, a P-type ATPase that expels three Na^+ ions and imports two K^+ ions with every round of ATP hydrolysis. In this issue of *JGP*, Spontarelli et al. (1) probe the function of a critical tyrosine residue in the Na^+/K^+ pump's ion selectivity.

The Na^+/K^+ pump has three cation-binding sites. Sites I and II somehow change their selectivity depending on the pump's conformation, binding Na^+ at the intracellular side of the membrane and K^+ at the extracellular side. Site III, in contrast, specifically binds Na^+ and contains a critical tyrosine residue that is well conserved across all P-type ATPases, including the neuron-specific Na^+/K^+ pump ATP1A3, where its mutation is linked to the neurological disorder alternating hemiplegia of childhood (2).

The crystal structure of the Na^+/K^+ pump shows that the side chain of this tyrosine residue sits immediately above the bound Na^+ ion at site III, suggesting that the phenol ring's π electrons could directly contribute to cation binding (3). However, the tyrosine residue could also contribute indirectly by participating in a hydrogen-bond network with other amino acids that coordinate the Na^+ ion. "We wanted to investigate how this conserved tyrosine residue contributes to ion binding," explains Pablo Artigas from Texas Tech University Health Sciences Center.

Artigas and colleagues, led by first author Kerri Spontarelli, generated versions of the *Xenopus laevis* Na^+/K^+ pump in which this critical tyrosine residue—Y780—was mutated to other amino acids (1). They expressed these proteins in *Xenopus* oocytes or COS-1 cells and determined their affinities for cations using electrophysiology and, with the help of Bente Vilsen's group at Aarhus University, biochemical assays.



Kerri Spontarelli (left), Pablo Artigas (center), and colleagues investigate the role of a conserved tyrosine residue in the ion selectivity of the Na^+/K^+ pump, using non-sense suppression to substitute this residue with unnatural, fluorinated analogs. The study suggests that the tyrosine (Y780 in the *Xenopus* pump) mainly contributes to Na^+ binding at site III by forming H-bonds with neighboring residues, rather than through a cation–π interaction.

Replacing Y780 with phenylalanine—maintaining the aromatic ring and its π electrons but removing the hydroxyl group capable of forming H-bonds—dramatically reduced the pump's affinity for Na^+ at both the internal and external sides of the membrane. Other substitutions that disrupt the H-bond network also reduced the apparent affinity for Na^+, whereas mutating Y780 to glutamine increased the pump's affinity for Na^+, probably by enabling the formation of additional H-bonds between site III residues.

This suggests that the H-bonds formed by Y780 are more important than the cation–π interaction for Na^+ binding. To address this question more precisely, Spontarelli et al. worked with Christopher Ahern's lab at the University of Iowa to introduce unnatural, fluorinated derivatives of tyrosine or phenylalanine at position 780 using non-sense suppression. The fluoro group withdraws electrons from the aromatic ring, weakening any potential cation–π interaction. The technique has previously been used to study the roles of aromatic residues in ion channels (4), but is more challenging for pumps, whose lower transport rates necessitate much higher levels of protein expression.

Nevertheless, by repeatedly injecting cells with tRNAs carrying the fluorinated amino acids, Spontarelli et al. were able to express sufficient amounts of the mutant pumps to estimate the change in affinity for

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Na^+ induced by the substitutions. "We observed that fluorination has very little effect on Na^+ binding," Artigas says, indicating a minimal contribution of cation–π interaction to Na^+ coordination.

Instead, it appears that the H-bond network formed by Y780 is critical for Na^+ binding at site III. Intriguingly, Spontarelli et al. found that mutating Y780 also reduced the Na^+/K^+ pump's apparent affinity for K^+, suggesting that it participates in a complex H-bond network proposed to control the cation selectivity switch at sites I and II as the pump changes its conformation (5). Artigas and colleagues now plan to extend their use of unnatural amino acids to understand this selectivity switch in more detail.

References

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