

## Structure-activity relationship of channel binding affinity of quaternary ammonium ions<sup>1</sup>

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**KEY WORDS** potassium channels; ammonium compounds; structure-activity relationship; solvation energy; least-squares analysis; computer-aided design

**AIM:** To explore the structure-activity relationship of quaternary ammonium (QA) ions at the external binding site of K<sup>+</sup> channel. **METHODS:** InsightII and MOPAC 6.0 molecular modeling package were used to calculate the free energy of hydration ( $\Delta G_{\text{hydration}}$ ), the energy of the highest occupied orbital ( $E_{\text{HOMO}}$ ), and the energy of the lowest unoccupied orbital ( $E_{\text{LUMO}}$ ) for each QA ion, respectively. The partial least square method was used to analyze the relationship between the binding free energy and these descriptive parameters. **RESULTS:** Generally, the higher the  $E_{\text{LUMO}}$  of a QA ion was, the weaker its solvation was and accordingly the stronger binding affinity. For a QA ion larger than tetraethylammonium (TEA), its large size was unfavorable to its channel binding affinity. **CONCLUSION:** The binding affinity of all QA ions correlated well with  $\Delta G_{\text{hydration}}$  and  $E_{\text{LUMO}}$ .

TEA ion and other quaternary ammonium (QA) ions inhibited K<sup>+</sup> channel by blocking the ion conduction pore<sup>[1]</sup>. The blockade was modulated by multiple sites in the pore region<sup>[2,3]</sup>. Of these residues, one specific amino acid located in the pore-forming region of a K<sup>+</sup> channel (position 449 of the *Shaker B*) was critical in determining the channel

sensitivity to the external TEA<sup>[3,4]</sup>. After mutation T449F or T449Y in each of the 4 subunits, the resultant channel affinity for TEA increased about 100 fold<sup>[4]</sup>. TEA was thought to be bound within a cage of 4 aromatic residues by cation  $\pi$ -electron interaction<sup>[5]</sup>. But this interaction mechanism failed to explain the structure-activity relationship of QA ions at the external binding site. Reduction in the binding free energy corresponded well with an increase in the computed free binding energy of hydration of the TEA derivatives<sup>[6,7]</sup>. In this paper, quantum chemical study was made to deepen our understanding the mechanism of TEA selectivity.

### MATERIALS AND METHODS

Calculation and analyses were carried out on a Silicon Graphics Indigo workstation. The partial least square (PLS) method was employed to analyze the relationship between the binding free energy and explanatory variables.

**Binding free energy computation** Since the dissociation equilibrium constant ( $K_D$ ) of each QA ion had been experimentally determined, the corresponding binding free energy was calculated as  $\Delta G_{\text{binding}} = -R \times T \times \ln(1M/K_D)$ , where  $R$  was the gas constant and  $T = 298$  K.

**Calculation of solvation energy** The free energy of hydration ( $\Delta G_{\text{hydration}}$ ) of each QA ion was calculated by a continuum solvation model<sup>[7]</sup>, using the solvation module of InsightII molecular modeling package (Biosym Technologies, San Diego CA). Parameter set was set to CFF91 force field and the accuracy level was regular.

**Semi-empirical quantum chemical computation** MOPAC 6.0 package and the AM1 Hamiltonian were used to calculate the energy of the highest occupied orbital ( $E_{\text{HOMO}}$ ) and the energy of the lowest unoccupied orbital ( $E_{\text{LUMO}}$ ) for each QA ion.

In practice, for each QA ion, change in the binding free energy ( $\Delta\Delta G_{\text{binding}}$ ) and change in the free energy of dehydration ( $\Delta\Delta G_{\text{dehydration}}$ ) were respectively defined and calculated as follows:

$$\Delta\Delta G_{\text{binding}} = \Delta G_{\text{binding}}(\text{QA}) - \Delta G_{\text{binding}}(\text{TEA})$$

$$\Delta\Delta G_{\text{dehydration}} = -\Delta G_{\text{hydration}}(\text{QA}) + \Delta G_{\text{hydration}}(\text{TEA})$$

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Abbreviations: BuEt<sub>3</sub>A = butyl-triethylammonium; MeEt<sub>3</sub>A = methyl-triethylammonium; Me<sub>2</sub>Et<sub>2</sub>A = dimethyl-diethylammonium; Me<sub>3</sub>EtA = trimethyl-ethylammonium; MePrEt<sub>2</sub>A = methyl-propyl-diethyl-ammonium; Me<sub>2</sub>Pr<sub>2</sub>A = dimethyl-dipropylammonium; Pr<sub>2</sub>Et<sub>2</sub>A = dipropyl-diethylammonium; PrEt<sub>3</sub>A = propyl-triethylammonium; TEA = tetraethylammonium; TMA = tetramethylammonium; TPA = tetrapropylammonium

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## RESULTS

**Correlation between  $\Delta\Delta G_{\text{binding}}$  and  $\Delta\Delta G_{\text{dehydration}}$**  TEA was the most effective QA ion blocker at the external binding site of  $K^+$  channel, and any changes in the chemical structure of TEA ion reduced binding affinity (Tab 1, Fig 1).

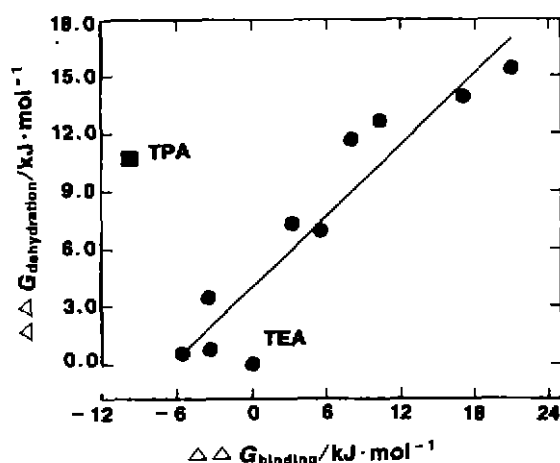


Fig 1. Correlation between change in binding free energies ( $\Delta\Delta G_{\text{binding}}$ ) and change in free energies of dehydration ( $\Delta\Delta G_{\text{dehydration}}$ ). TPA was not included in the correlation.

There existed a high correlation between  $\Delta\Delta G_{\text{binding}}$  and  $\Delta\Delta G_{\text{dehydration}}$  for most of the QA ions, except for TPA (Tab 2, Fig 1, Eq 1).

$$\Delta\Delta G_{\text{binding}} = 3.97 + 2.60 \times \Delta\Delta G_{\text{dehydration}} \quad \text{Eq (1)}$$

The stronger a QA ion hydrated, the weaker it

Tab 2. PLS analysis. TPA was excluded in the correlation.

Independent property	Explanatory variables	R squared	F values	Standard deviation of estimate
$\Delta\Delta G_{\text{binding}}$	$\Delta\Delta G_{\text{dehydration}}$	0.880	58.65	0.518
$\Delta G_{\text{hydration}}$	$E_{\text{LUMO}}$	0.985	530.89	0.276
	$E_{\text{HOMO}}, E_{\text{LUMO}}$	0.993	499.18	0.202
$\Delta\Delta G_{\text{binding}}$	$E_{\text{LUMO}}$	0.827	38.32	0.622
	$E_{\text{HOMO}}, E_{\text{LUMO}}$	0.954	72.24	0.344

interacted with the channel. This relationship was consistent with previous report and findings<sup>(6,7)</sup>.

**Relationship between  $\Delta G_{\text{hydration}}$ ,  $E_{\text{LUMO}}$ , and  $E_{\text{HOMO}}$**  It was clear that  $\Delta G_{\text{hydration}}$  correlated highly with  $E_{\text{LUMO}}$  (Tab 2, Eq 2).

$$\Delta G_{\text{hydration}} = 16.01 + 57.6 \times E_{\text{LUMO}} \quad \text{Eq (2)}$$

The higher the  $E_{\text{LUMO}}$  was, the weaker the solvation of a QA ion was. This linear relationship improved slightly with respect to both  $E_{\text{LUMO}}$  and  $E_{\text{HOMO}}$  (Tab 2, Eq 3).

$$\Delta G_{\text{hydration}} = 26.96 - 2.05 \times E_{\text{HOMO}} + 68.8 \times E_{\text{LUMO}} \quad \text{Eq (3)}$$

In contrast to  $E_{\text{LUMO}}$ , an increase in  $E_{\text{HOMO}}$  favored hydration. The relative contribution of  $E_{\text{LUMO}}$  and  $E_{\text{HOMO}}$  were 80 % and 20 % respectively. According to Eq 3,  $\Delta G_{\text{hydration}}$  of a QA ion could be successfully predicted, which, in turn, verified the reliability of our regression model (Fig 2).

Tab 1. Calculated dimensions, frontier orbital energies, free energies for hydration and binding free energies for QA ions.

	Volume/ $\text{nm}^3$	SAS/ $\text{nm}^2$	$E_{\text{HOMO}}$ / eV	$E_{\text{LUMO}}$ / eV	$\Delta G_{\text{hydration}}$ / $\text{kJ} \cdot \text{mol}^{-1}$	$\Delta\Delta G_{\text{dehydration}}$ / $\text{kJ} \cdot \text{mol}^{-1}$	$\Delta\Delta G_{\text{binding}}$ / $\text{kJ} \cdot \text{mol}^{-1}$
TMA	0.091	2.57	-17.97	-4.18	-223.24	20.99	15.35
Me <sub>3</sub> EtA	0.107	2.80	-16.55	-4.07	-219.30	17.06	13.88
Me <sub>2</sub> Et <sub>2</sub> A	0.123	3.09	-16.31	-3.97	-212.57	10.33	12.63
Me <sub>2</sub> Pr <sub>2</sub> A	0.156	3.70	-15.42	-3.89	-210.19	7.95	11.67
TPA	0.222	4.59	-18.18	-3.64	-192.46	-9.79	10.71
MePrEt <sub>2</sub> A	0.156	3.58	-15.40	-3.83	-205.46	3.22	7.32
MeEt <sub>3</sub> A	0.139	3.23	-16.11	-3.88	-207.72	5.48	6.94
BuEt <sub>3</sub> A	0.188	3.97	-14.63	-3.73	-198.81	-3.43	3.43
PrEt <sub>3</sub> A	0.172	3.69	-15.34	-3.75	-198.90	-3.35	0.71
Pr <sub>2</sub> Et <sub>2</sub> A	0.188	4.01	-15.27	-3.71	-196.68	-5.56	0.54
TEA	0.155	3.39	-15.96	-3.79	-202.24	0.00	0.00

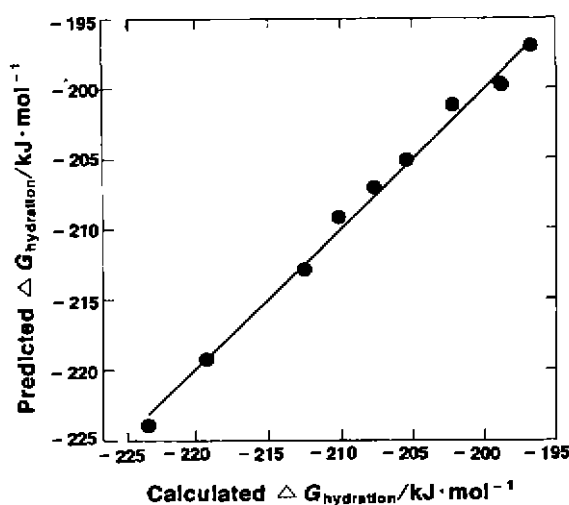


Fig 2. Plot of predicted free energies of hydration ( $\Delta G_{\text{hydration}}$ ) versus actually calculated  $\Delta G_{\text{hydration}}$ .

**$\Delta\Delta G_{\text{binding}}$  correlation with  $E_{\text{LUMO}}$  and  $E_{\text{HOMO}}$**  Since  $\Delta\Delta G_{\text{binding}}$  correlated with  $\Delta\Delta G_{\text{dehydration}}$ , and the latter related well with the frontier orbital energies, the former was expected to correlate with  $E_{\text{LUMO}}$  and  $E_{\text{HOMO}}$  as well (Tab 2, Eq 4, Eq 5).

$$\Delta\Delta G_{\text{binding}} = -127.91 - 34.86 \times E_{\text{LUMO}} \quad \text{Eq (4)}$$

$$\Delta\Delta G_{\text{binding}} = -156.87 + 5.43 \times E_{\text{HOMO}} - 64.54 \times E_{\text{LUMO}} \quad \text{Eq (5)}$$

Electronic descriptors  $E_{\text{LUMO}}$  and  $E_{\text{HOMO}}$  were essentially responsible for the binding affinity of a QA ion. Compared with  $E_{\text{HOMO}}$ ,  $E_{\text{LUMO}}$  term made the dominant contribution to the binding free energy. The higher  $E_{\text{LUMO}}$  and the lower  $E_{\text{HOMO}}$  resulted in the stronger binding of a QA ion (Eq 5). Plot of actual  $\Delta\Delta G_{\text{binding}}$  versus predicted values reconfirmed the good correlation between the binding free energy and the energies of frontier orbitals (Fig 3).

## DISCUSSION

The finding that  $\Delta\Delta G_{\text{binding}}$  correlated with  $\Delta\Delta G_{\text{dehydration}}$  suggested a general mechanism in which water that was bound to the QA ions must be partially removed to establish cation  $\pi$ -electron interaction<sup>[8]</sup> (Fig 4). The dehydration energy requirement was different for each QA ion, whereas the subsequent energy provided by the binding site might be similar for all QA ions. Thus the dehydration process was critical for the binding affinity of a QA ion. Our study showed that

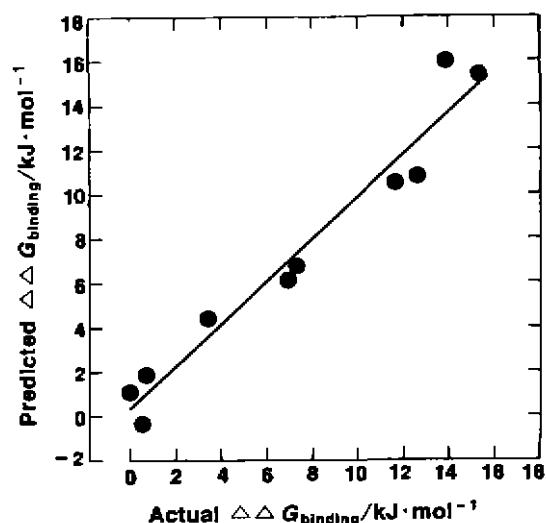


Fig 3. Plot of predicted change in binding free energy ( $\Delta\Delta G_{\text{binding}}$ ) versus actual  $\Delta\Delta G_{\text{binding}}$ .

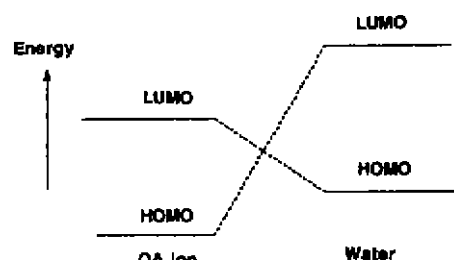


Fig 4. Frontier orbital interaction between solute (QA) and solvent (water).

the solvent effects played a pivotal role in the binding of the QA ions.

The calculated  $E_{\text{LUMO}}$  and  $E_{\text{HOMO}}$  of water molecule was  $-12.46$  eV and  $4.42$  eV, respectively. When a QA ion was immersed in water, there was a strong orbital interaction between LUMO (QA) and HOMO ( $\text{H}_2\text{O}$ ) (Fig 4). The smaller the gap between  $E_{\text{LUMO}}(\text{QA})$  and  $E_{\text{HOMO}}(\text{H}_2\text{O})$  was, the stronger the interaction and solvation were. The interaction between LUMO ( $\text{H}_2\text{O}$ ) and HOMO (QA) is relatively weak. Thus for QA ions, interaction between LUMO (QA) and HOMO ( $\text{H}_2\text{O}$ ) contributed substantially to  $\Delta G_{\text{hydration}}$ . The lower  $E_{\text{LUMO}}(\text{QA})$  resulted in the stronger hydration and accordingly the weaker binding affinity of a QA ion. On the basis of these discussions, the relationships and physical meanings of Eq 2, Eq 3, Eq 4, and Eq 5 could be easily understood and explained.

For charged QA ions, the electrostatic contribution

to  $\Delta G_{\text{hydration}}$  was by far the dominant term and outweighed the non-electrostatic contribution arising from van der Waals interaction between the solute (QA) and the solvent (water) and the entropy penalty for creating a cavity for the solute in the solvent. As a result, though the non-electrostatic contribution of a QA ion was supposed to be proportional to its solvent accessible area (SAS), the total  $\Delta G_{\text{hydration}}$  lacked of correlation with its volume or SAS. This did not mean that the volume or SAS of a QA ion had nothing to do with its binding affinity. A careful insight into the relationship between  $\Delta\Delta G_{\text{binding}}$  and  $\Delta\Delta G_{\text{dehydration}}$  found that TPA, BuEt<sub>3</sub>A, PrEt<sub>3</sub>, and Pr<sub>2</sub>Et<sub>2</sub>A dehydrated more easily than TEA did, but they all bound more weakly than TEA. It was difficult to explain this inconsistency in terms of  $\Delta G_{\text{dehydration}}$  or  $E_{\text{LUMO}}$ .

By comparing chemico-physical characteristics of the related QA compounds, we noticed that TPA, BuEt<sub>3</sub>A, PrEt<sub>3</sub>A, and Pr<sub>2</sub>Et<sub>2</sub>A were more bulky than TEA, which was probably at least the partial reason for this observed discrepancy. By cation  $\pi$ -electron interaction, a QA ion bound at the cage site formed by four aromatic residues<sup>(8)</sup> (Fig 5), the size of which was assumed to accommodate QA ions not larger than TEA. For TPA, most probably, it was too large to bind at the TEA binding site, and this was probably not the case for BuEt<sub>3</sub>A, PrEt<sub>3</sub>A, and Pr<sub>2</sub>Et<sub>2</sub>A. These three ions were only slightly larger than TEA, they could bind the same locus as TEA by changing their conformation and adapting themselves to the dimension of the binding site. And thus, additional energy was required to compensate for conformational change, and the corresponding affinity was reduced.

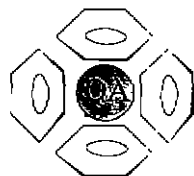


Fig 5. TEA binding site formed by a cage of aromatic residues.

Here, we concluded that the binding affinity of each QA ion generally correlated well with its free energy of hydration or  $E_{\text{LUMO}}$ . The lower  $E_{\text{LUMO}}$  (QA) was, the stronger it hydrated, and consequently the weaker was its binding affinity. For a QA ion smaller than TEA, its size did not influence affinity.

On the other hand, for a QA ion larger than TEA, its large size was unfavorable to its binding to the channel. Taking the size effect into account, the structure-activity relationship of QA ions and the mechanism of TEA selectivity were more easily understood.

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季铵离子通道亲和力的结构-活性关系<sup>1</sup>

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关键词 钾通道; 铵化合物; 结构-活性关系; 溶剂化能; 最小二乘法分析; 计算机辅助设计

亲和力

目的: 探讨季铵离子在钾通道外部结合位点的结构-活性关系. 方法: 用 InsightIII 分子建模软件包和 MOPAC 6.0 计算每个季铵离子的水化自由能 ( $\Delta G_{\text{hydration}}$ )、最高占有轨道能量 ( $E_{\text{HOMO}}$ ) 和最低未占有轨道能量 ( $E_{\text{LUMO}}$ ). 结合自由能与这些描述参数之间的关系用偏最小二乘法进行回归. 结论: 一般说来, 季铵离子  $E_{\text{LUMO}}$  越高, 溶剂化就越弱, 相应的亲和力就越强. 对于比四乙铵大 (TEA) 的季铵离子, 其大的分子尺寸不利于它对通道的亲和力. 结论: 所有季铵离子的通道亲和力与  $\Delta G_{\text{hydration}}$  和  $E_{\text{LUMO}}$  有很好的相关性.