# Cation−$\pi$ Interactions and the Gas-Phase Thermochemistry of the $Na^+$/Phenylalanine Complex

**Alexei Gapeev and Robert C. Dunbar***

*Contribution from the Chemistry Department, Case Western Reserve University, Cleveland, Ohio 44106*

*Received February 8, 2001*

**Abstract:** The complex of $Na^+$ with phenylalanine (*Phe*) is a prototype for the participation of cation−$\pi$ interactions in metal-ion binding to biological molecules. A recent comparison of this complex with the $Na^+$/alanine ($Na^+$/*Ala*) counterpart suggested only a small contribution of the phenyl ring interaction to binding, casting doubt on the extent of the cation−$\pi$ effect. The present work reexamines this thermochemistry using ligand-exchange equilibrium measurements in the Fourier transform ion cyclotron resonance (FT-ICR) ion trapping mass spectrometer. An increment of 7 ± 2 kcal $mol^{-1}$ was found in the *Ala*/*Phe* comparison of binding enthalpies, confirming the importance of cation−$\pi$ binding enhancement in the *Phe* case. Absolute $Na^+$ binding enthalpies of 38 ± 2 and 45 ± 2 kcal $mol^{-1}$ were assigned for *Ala* and *Phe*, respectively, using pyridine as the thermochemical reference ligand. All of these results were supported by quantum calculations using both density functional and Hartree−Fock/MP2 methods, improved in several respects over previous calculations. Alanine methyl ester (*AlaMe*) was also observed, and found to have an $Na^+$ ion affinity larger by 2.3 kcal $mol^{-1}$ than *Ala*. New, lower energy conformations of neutral *Phe* were discovered in the computations.

## Introduction

A lively topic of discussion in protein structure and energetics is the importance of cation−$\pi$ interactions.[1−11] In situations where a metal ion performs a structural role, or (more commonly) binds to an accessible surface site on the protein, it is often interesting to consider the possibility that the metal-ion binding involves cation interaction with an aromatic side chain. Gas-phase ion chemistry can back up such possibilities with accurate measurements of the gas-phase binding affinities of aromatic sites. Protein binding will typically involve extensive intramolecular metal-ion chelation, so that particularly relevant model sites are those, like the amino acids themselves, involving extensive chelation.

A recent report of the alkali cation affinities of the aromatic acids *Phe*, *Tyr*, and *Trp* addressed these questions both experimentally and computationally.[12] However, this study left a surprisingly large discrepancy between experimental and computed cation binding energies for these systems. Specifically, for $Na^+$/*Phe*, the computed binding affinity was 6.5 kcal $mol^{-1}$ larger than the experimental result. Another reflection of the same problem showed up in the experimental comparison of binding to alanine and to the aromatic amino acids. The kinetic method experiments of ref 12 showed a relatively small increment of binding going from *Ala* to *Phe* (∼2 kcal $mol^{-1}$), while the quantum calculations suggested a much larger increment (∼7.5 kcal $mol^{-1}$). Similar discrepancies were reported for *Tyr* and *Trp*, and also for the corresponding $K^+$ complexes. A further computational analysis in ref 13, comparing $\pi$-complexed versus rotated conformations, indicated a cation−$\pi$ interaction energy of the order of 5 kcal $mol^{-1}$, and gave further support to the computational predictions of large cation−$\pi$ contributions. Quantitative binding of alkali ions to the benzene $\pi$ face has long been established experimentally,[14] but the importance of this mode of interaction in highly chelated and strained systems such as $Na^+$/*Phe* is a question still worthy of clarification.

Exclusive of cation−$\pi$ interactions, one expects the binding to *Phe* to be slightly stronger (by perhaps 1−2 kcal $mol^{-1}$) than that to *Ala*, because of the greater polarizability of *Phe* (with a similar expectation for the other aromatic amino acids). Thus the experiments showing only 1 or 2 kcal $mol^{-1}$ enhancement between *Ala* and the aromatic amino acids suggested relatively little cation−$\pi$ stabilization of the aromatic complexes, while the calculated DFT results suggested a substantial cation−$\pi$ effect, of the order of 5−7 kcal $mol^{-1}$. These conflicting results left an unsatisfactory uncertainty about the extent of cation−$\pi$ contribution to the thermochemistry, and also about the absolute values of these binding energies.

The most incisive experimental approach to ion thermochemistry is the characterization of true thermodynamic equilibria among the various species. Pursuing this approach, we have achieved good equilibria linking the $Na^+$ complexes of *Ala* and *Phe*, leading to new and more satisfactory experimental thermochemistry. The computational attack on this question has also

* To whom correspondence should be addressed.

(1) Cabarcos, O. M.; Weinheimer, C. J.; Lisy, J. M. *J. Chem. Phys.* **1999**, *110*, 8429.
(2) Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 4177.
(3) Choi, H. S.; Suh, S. B.; Cho, S. J.; Kim, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12094.
(4) DeWall, S. L.; Meadows, E. S.; Barbour, L. J.; Gokel, G. W. *J. Am. Chem. Soc.* **1999**, *121*, 5613.
(5) DeWall, S. L.; Barbour, L. J.; Gokel, G. W. *J. Am. Chem. Soc.* **1999**, *121*, 8405.
(6) Donini, O.; Weaver, D. F. *J. Comput. Chem.* **1998**, *19*, 1515.
(7) Dougherty, D. A. *Science* **1996**, *271*, 163.
(8) Gallivan, J. P.; Dougherty, D. A. *PNAS* **1999**, *96*, 9459.
(9) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303.
(10) Minoux, H.; Chipot, C. *J. Am. Chem. Soc.* **1999**, *121*, 10366.
(11) Murayama, K.; Aoki, K. *Inorg. Chim. Acta* **1998**, *281*, 36.
(12) Ryzhov, V.; Dunbar, R. C.; Cerda, B.; Wesdemiotis, C. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1037.
(13) Dunbar, R. C. *J. Phys. Chem. A* **2000**, *104*, 8067.
(14) Sunner, J.; Nishizawa, K.; Kebarle, P. *J. Phys. Chem.* **1981**, *85*, 1814.

10.1021/ja010351t CCC: $20.00 
Published on Web 07/28/2001

been refined and improved, although the net change in the computed values is relatively small. The experimental and computational pictures have been satisfactorily reconciled, giving us confidence in our understanding of the cation−$\pi$ interaction in this class of complexes.

Considering its importance and interest as a prototypical alkali ion/amino acid interaction, the thermochemistry of $Na^+$ binding to *Ala* is surprisingly poorly characterized. The binding energy of 39.4 kcal $mol^{-1}$ from ref 15 that has been cited as an experimental value is actually an estimate, derived by analogy to a kinetic method measurement of the $Li^+$ ion affinity. Given that this is not a measured value, it is hard to assess how much confidence it deserves. The present result appears to be the first experimental measurement of the $Na^+$/*Ala* binding energy using a technique of good quantitative accuracy. Computationally, Marino et al. reported a recent density functional theory (DFT) study with adequate basis sets[16] giving values of 40.4 and 41.9 kcal $mol^{-1}$, depending on basis (values at 298 K). On average, these latter values are slightly higher than the present calculations because of their neglect of BSSE and their use of the B3LYP functional; we would prefer to lower these values by 1−2 kcal $mol^{-1}$ for these reasons. It is clear that DFT computation of this and similar systems at the current level of feasibility is subject to variations of the order of 2 kcal $mol^{-1}$ depending on detailed choices within the method; the comparison with MP2 results below shows that the absolute uncertainties to be placed on computed values of such binding energies are even larger than this.

A recent DFT computation[17] of the $Na^+$ affinity of *Phe* using the B3LYP functional was reported to give an absolute binding energy of 48.1 kcal $mol^{-1}$. The present lower result presumably reflects our correction for BSSE and our use of the B3P86 functional.

## Methods

**Experiments.** The experiments were done on a Nicolet FT-2000 mass spectrometer equipped with an IonSpec Omega data system, as was used in previous experiments.[18]

Sodium cations were generated by laser ablation−desorption from a NaCl target using the fundamental (1064 nm) of a Nd:YAG laser.

Pyridine *(Pyr)* was introduced through a leak valve situated on the high-pressure side of the two-region vacuum system. *Pyr* pressure was monitored by an ion gauge. The ion gauge reading was calibrated through a measurement of the proton-transfer reaction rate from propyl cation to pyridine. This calibration reaction was presumed to proceed with the collisional rate.

Maintaining controllable and steady amino acid pressure was challenging since amino acids have low vapor pressure at room temperature. However, it was found possible to establish a sufficient steady-state pressure at the operating temperature by sample vaporization. To do this, 20−50 mg of an amino acid were placed in a cavity on the side of the tip of the solids insertion probe, which was located 3−5 cm outside the cell trapping plate. The amino acid pressure could be varied by changing the temperature of the vacuum system. It was impossible to use the ion gauge reading as a meaningful indicator of the amino acid pressure. As was done for the pressure calibration of *Pyr*, the proton transfer reaction rate from propyl cation was used to measure the amino acid pressure. Relative pressure measurements were probably accurate to 20%, except for the possibility of larger errors introduced by the assumption of equal efficiencies of the proton-transfer reactions from propyl cation to the different neutral species.

The entire chamber containing the dual ICR cells was jacketed and heated by an external heating mantle. Before each run the temperature inside the chamber was stabilized and measured by a thermocouple mounted on the solids probe. In the configuration used, there was no direct path for neutral amino acid molecules to travel from the sample to the cell without multiple wall collisions, and it was assumed that the neutral molecules were in thermal equilibrium with the cell walls.

The spectra showed no significant peaks other than the expected ions. In particular, there was no observable formation of dimer complexes with any of the neutrals.

**Computations**. All computations were performed using the Gaussian 98 program package.[19] The basis set was 6-31+g(d) on all heavy atoms, 6-31 g(d) on all aromatic ring hydrogens, and 6-31g(d,2p) on the heteroatom hydrogens of the amino acids. The structures were optimized with the full basis of the corresponding energy calculation. Results are reported below for both second-order Moeller−Plesset (MP2) calculations and also density functional (DFT) calculations using the hybrid B3P86 functional. The vibrational frequencies and geometrical parameters (as used in the zero-point energy corrections and in the entropy calculations) were taken from the DFT results. All the binding energy calculations were corrected for zero-point energy (ZPE) effects, which were about 1.5 kcal $mol^{-1}$ (1.0 kcal $mol^{-1}$ for *Pyr*). Basis set superposition error (BSSE) corrections were made using a geometry-consistent counterpoise approach.[20] For the DFT calculations, BSSE corrections were modest, 0.8 kcal $mol^{-1}$ for *Phe*, 0.5 kcal $mol^{-1}$ for *Ala*, and 0.2 kcal $mol^{-1}$ for *Pyr*. With MP2, BSSE was larger, 3.8 kcal $mol^{-1}$ for *Phe*, 1.8 kcal $mol^{-1}$ for *Ala*, and 1.0 kcal $mol^{-1}$ for *Pyr*. An expanded basis set DFT calculation of $Na^+$/*Phe* (putting 6-311+g(d) on Na) gave no significant change in binding energy. Similarly, an MP4//MP2 calculation on $Na^+$/*Ala* showed no significant change from the MP2 result.

DFT energies are somewhat sensitive to the particular functional chosen. The B3P86 functional was chosen here in preference to the popular B3LYP functional based on the recommendation of Armentrout and Rodgers.[21] It has also been suggested that the MPW1PW91 functional may give more accurate binding energies for some types of systems.[22] To survey the scope of this source of uncertainty, several of the key calculations were repeated at the same computational level with the B3LYP functional and with the MPW1PW91 functional (with geometries reoptimized for each functional). $Na^+$ binding energies to *Ala* and to *Phe* were within 1 kcal $mol^{-1}$ of the B3P86 values (tending to be a little higher for both of the other functionals), while the *Ala*/*Phe* differential varied by less than 0.5 kcal $mol^{-1}$. The comparative energies of the low-energy isomers of neutral *Phe* were also reproduced within 1 kcal $mol^{-1}$. It was concluded that the DFT results used here are stable with respect to changes in functional at the relevant level of precision.

## Equilibrium Thermochemistry

The principal achievement of the present study is the observation of equilibrium $Na^+$ transfer thermochemistry for *Ala* and *Phe*. The very low volatility of these molecules,

(15) Bojesen, G.; Brendahl, T.; Anderson, U. *Org. Mass Spectrom.* **1993**, *28*, 1448.

(16) Marino, T.; Russo, N.; Toscano, M. *J. Inorg. Biochem.* **2000**, *79*, 179.

(17) Siu, F. M.; Ma, N. L.; Tsang, C. W. *J. Am. Chem. Soc.* **2001**, *123*, 3397.

(18) Gapeev, A.; Yang, C.-N.; Klippenstein, S. J.; Dunbar, R. C. *J. Phys. Chem. A* **2000**, *104*, 3246.

(19) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *GAUSSIAN 98*, Revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998.

(20) Xantheas, S. S. *J. Chem. Phys.* **1996**, *104*, 8821.

(21) Armentrout, P. B.; Rodgers, M. T. *J. Phys. Chem. A* **1999**, *104*, 2238.

(22) de Oliveira, G.; Martin, J. M. L.; de Proft, F.; Geerlings, P. *Phys. Rev. A* **1999**, *60*, 1034.

particularly *Phe*, makes this a challenging task. Fortunately the FT-ICR ion trapping spectrometer is well suited to observing ion−neutral reactions and equilibria at extremely low reagent pressures and at extremely long reaction time scales, making these experiments feasible.

In fact it was not possible to work directly with the desired ligand exchange

$$Phe + Na^+Ala \rightleftarrows Na^+Phe + Ala \quad (1)$$

since controllable and usable pressures of the two neutrals could not be maintained simultaneously, and also because the free-energy difference is inconveniently large. But it was possible to use alanine methyl ester (*AlaMe*) as a volatile bridge compound, separately observing the two equilibria

$$Ala + Na^+AlaMe \rightleftarrows Na^+Ala + AlaMe \quad (2)$$

and

$$Phe + Na^+AlaMe \rightleftarrows Na^+Phe + AlaMe \quad (3)$$

In combination, these two equilibria are equivalent to the reaction 1 equilibrium, which is the final target. In addition, it was possible to observe the equilibrium between *Ala* and pyridine (*Pyr)*,

$$Ala + Na^+Pyr \rightleftarrows Na^+Ala + Pyr \quad (4)$$

and the equilibrium between *AlaMe* and *Pyr*,

$$AlaMe + Na^+Pyr \rightleftarrows Na^+AlaMe + Pyr \quad (5)$$

The $Na^+$ affinity of *Pyr* is quite well established,[23] providing an anchor for absolute $Na^+$ affinities for this set of compounds.

**Entropy Corrections.** It was not possible to make temperature dependence studies of useful accuracy, so that the equilibrium results yield the free energies of $Na^+$ transfer at a single temperature (usually 370 K). To extract the desired enthalpies, it is necessary to estimate the $Na^+$ transfer entropies for these two reactions. Actually, since the *AlaMe* contributions cancel out, the enthalpy of reaction 1 can be obtained from the present data by estimating the entropy of reaction 1 itself. To obtain absolute $Na^+$ binding enthalpies using *Pyr* as a reference ligand of known affinity, it is also necessary to estimate the entropy of reaction 4.

Contributions to the ligand exchange entropies are of three major types: those arising from molecular motions (translation, vibration, rotation, libration/internal rotation), those corresponding to rotational symmetries, and those arising from multiple conformations.

**(a) Molecular motion contributions:** These entropy contributions are taken from standard statistical-mechanical expressions. Harmonic oscillator frequencies were used for the vibrational entropy contributions: this may be a poor approximation for the low-frequency motions of the metal ion, but it can be hoped that the anharmonicity errors will largely cancel in the calculation of the ligand transfer entropies.

For the transfer of $Na^+$ from *Pyr* to *Ala* there is signficant negative vibrational entropy ($-4.1$ cal mol$^{-1}$ K$^{-1}$) and small rotational ($-0.7$) and translational ($-0.1$) contributions. For the transfer of $Na^+$ from *Ala* to *Phe*, there is a rotational entropy of $-1.6$ cal mol$^{-1}$ K$^{-1}$, and small vibrational ($-0.1$) and translational ($-0.3$) contributions.

(23) Amunugama, R.; Rodgers, M. T. *Int. J. Mass Spectrom.* **2000**, *195/196*, 439.

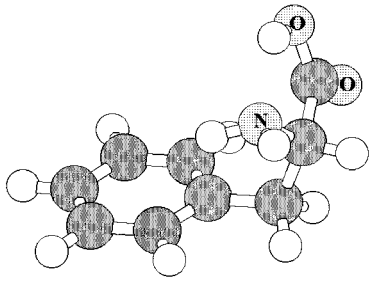


**Figure 1.** Lowest energy conformation located for *Phe*. (A similar conformation was also found with nearly the same DFT energy as the one shown, differing in a rotation of the side chain while retaining the H−$\pi$ stabilization involving the amino hydrogen.)

**(b) Rotational symmetries:** The 2-fold rotational symmetry of pyridine cancels the similar symmetry of the pyridine/$Na^+$ complex, and these symmetries can be ignored. The other species have no rotational symmetries.

**(c) Conformational contributions:** Different conformations of a molecule or complex make comparable contributions to the entropy as long as they are within *kT* of the lowest energy, but drop off rapidly if they are more energetic than this. Unfortunately neither calculations nor existing experimental results are good enough to make good estimates of the numbers of accessible conformations for many of the species participating in these equilibria. An effort was made to estimate the most likely effects. (1) Considering *Ala* in comparison with *Pyr*, there are various similar-energy conformational possibilities of the COOH and $NH_2$ groups of the amino acid that have no counterpart for *Pyr*. It was assumed that *Ala* has an excess of 4 effective conformations of this type. (2) Considering the comparison of neutral *Ala* and *Phe*, the benzyl side chain of *Phe* can orient itself in two nearly equal-energy positions while maintaining the internal H-chelation with the ring, so *Phe* was taken to have twice as many conformations as *Ala*. (The structure displayed in Figure 1 is one of these two conformations.) (3) The $Na^+$ complexes with *Pyr*, *Ala*, and *Phe* each appeared to be constrained to a single low-energy conformation, so no corrections were made for the complexes.

**AlaMe.** While the $Na^+$ affinity of *AlaMe* does not need to be assigned in carrying out the main purposes of this study, it may be interesting in its own right. Quantum calculations were carried out at the same level as for the amino acid cases to assess the entropy corrections for reactions 2, 3, and 5. Conformationally *AlaMe* is expected to be similar to *Ala*, and the same conformational entropy assumptions were made as for the *Ala* equilibria.

## Results and Discussion

Confidence in the thermochemistry derived from equilibrium measurements depends totally on the assurance that equilibrium is indeed established among all the participating species. A frequent serious problem in metal-ion transfer systems such as reactions 2−5 is the leakage of metal ions to other species (like the dimers $M_1M_2Na^+$), which can preclude the establishment of the desired $Na^+$ transfer equilibrium. Fortunately $Na^+$ is a poorly clustering ion in this respect, and no such clustering reactions, nor any other extraneous reactions, were observed here.

The definitive test of whether equilibrium is reached in the ICR cell is the use of ion ejection to approach the equilibrium from both sides. This was demonstrated for all of the reactions of interest here. The most difficult of them was reaction 3, for

**Table 1.** Equilibrium Results for the Neutral Molecule *M* Exchanging $Na^+$ with a Reference *Base* (either *Pyr* or *AlaMe*) (The equilibrium reaction is $M + Na^+Base \rightleftarrows Na^+M + Base$)

A. *Ala* and *AlaMe* vs *Pyr*

| *M* | pressure of *M* (Torr) | *Pyr* pressure (Torr) | temp (K) | ratio of $Na^+M$:$Na^+Pyr$ | $K_{eq}$ | $\Delta G$ (kcal mol$^{-1}$) |
|---|---|---|---|---|---|---|
| *Ala* | $2.0 \times 10^{-10}$ | $1.0 \times 10^{-6}$ | 326 | 1.2 | $6 \times 10^3$ | −5.0[a] |
| *AlaMe* | $3.5 \times 10^{-10}$ | $1.3 \times 10^{-6}$ | 370 | 5 | $1.9 \times 10^4$ | −7.2 |

B. *Ala* and *Phe* vs *AlaMe*

| *M* | pressure of *M* (Torr) | *AlaMe* pressure (Torr) | temp (K) | ratio of $Na^+M$: $Na^+AlaMe$ | $K_{eq}$ | $\Delta G$ (kcal mol$^{-1}$) |
|---|---|---|---|---|---|---|
| *Ala*[b] | $6 \times 10^{-9}$ | $4 \times 10^{-10}$ | 370 | 0.63 | $4.2 \times 10^{-2}$ | 2.3 |
| *Phe* | $1.0 \times 10^{-9}$ | $6 \times 10^{-8}$ | 370 | 1.5 | 90 | −3.3 |

[a] This value, at 326 K, is not strictly comparable to the other (370 K) values. For plotting on Figure 2 it has been adjusted to −4.8 kcal mol$^{-1}$ at 370 K using the calculated $T\Delta S$ correction. [b] The *Ala/AlaMe* experiment was done in the presence of *Pyr* as a buffer gas at a pressure of $2.2 \times 10^{-6}$ Torr, which assisted in the initial capture of $Na^+$. Because of the relatively low $Na^+$ affinity of *Pyr*, no measurable $Na^+Pyr$ was present when the system approached equilibrium.

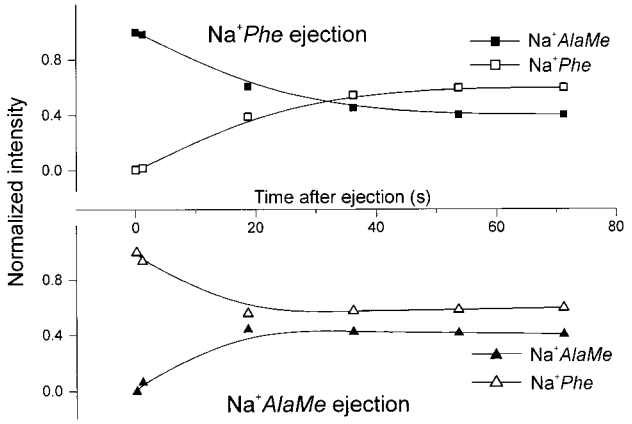


**Figure 2.** Approach to equilibrium from both directions for the transfer of $Na^+$ between *AlaMe* and *Phe*.

**Table 2.** Equilibrium Thermochemistry

| | $\Delta G_{transfer}$ (kcal mol$^{-1}$) | $\Delta S_{transfer}$[a] (cal mol$^{-1}$ K$^{-1}$) | $\Delta H_{transfer}$ (kcal mol$^{-1}$) |
|---|---|---|---|
| *Pyr*/*Ala* | −5.0 | −7.7 | −7.5 |
| *Ala*/*Phe* | −5.6 | −3.4 | −6.8 |

[a] Calculated as described in the text.

which the ejection results are displayed in Figure 2. This experiment goes as follows: First an initial preparation period is allowed (not displayed) during which $Na^+$ ions attach to ligands. Then (at zero time on the plot) all ionic species are ejected from the cell except the one ion of interest, either $Na^+Phe$ (upper section of the figure) or $Na^+AlaMe$ (lower section). The subsequent evolution of the ion intensities is followed by FT-ICR detection, eventually leveling off to a constant ratio of the two equilibrating species ($Na^+Phe$:$Na^+AlaMe$). As is seen in Figure 2, this ratio leveled off within about 50 s to the same value (1.5) for approach to equilibrium from either direction.

Table 1 displays the experimental parameters and results for the equilibrium trials. Table 2 shows the thermochemical values obtained from these equilibrium constants. The free energy thermochemical ladder is displayed in Figure 3. As is seen, the relationship among *Pyr*, *Ala*, and *AlaMe* is fixed by a redundant set of measurements, increasing the confidence level of this portion of the ladder. The enthalpies resulting from the calculated $T\Delta S$ corrections are shown in Table 2, and the resulting $\Delta H$ ladder is displayed in Figure 4. It is seen that the entropy corrections to the free-energy values are substantial. This largely reflects chelation effects, both the loss of freedom

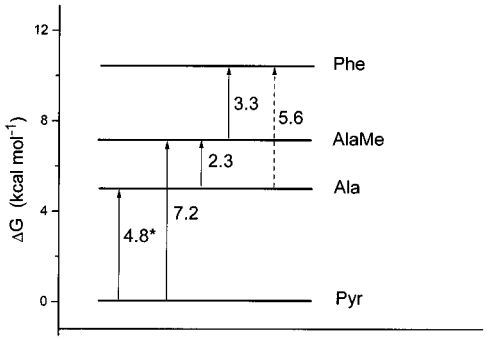


**Figure 3.** Ladder of ligand-transfer free energies at 370 K. (The starred value was measured at 326 K but for plotting has been corrected to the value expected at 370 K.) The dashed arrow for reaction 1 is not a directly measured value, but is derived from the measured ladder values.

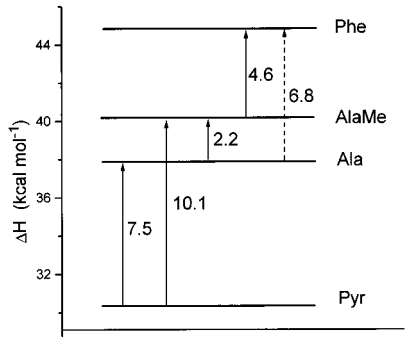


**Figure 4.** Ladder of ligand-transfer enthalpies and absolute $Na^+$ binding enthalpies, obtained from Figure 3 by applying computed entropy corrections to the free energy measurements, anchored to the literature *Pyr* value.

of the chelated *Ala* complex compared with *Pyr*, and also the greater chelation of $Na^+Phe$ compared with $Na^+Ala$.

There are small enthalpy corrections (of the order of 0.2 kcal mol$^{-1}$) for cooling to 0 K, and applying these gives the final 0 -K binding energies listed in Table 3. We can suggest the uncertainty of the *Ala*/*Phe* comparison as ±2 kcal mol$^{-1}$, and similarly estimate the $Na^+$ affinity of *Ala* relative to *Pyr* as uncertain to ±2 kcal mol$^{-1}$. The absolute $Na^+$ affinities might be slightly more uncertain, depending on the uncertainty ascribed to the pyridine reference value. These uncertainties reflect both experimental uncertainties (mainly the relative pressure measurements) and also the uncertainties in making the entropy corrections to the $\Delta G$ values.

**Table 3.** $Na^+$ Affinities[f]

| $Na^+$ affinity | AlaMe | Pyr | Ala | Phe | $\Delta H$ (*Ala-Phe*) |
|---|---|---|---|---|---|
| GIBMS | | 30.3[a] (*30.5*)[a] | | | |
| equilibrium (present results) | 40.4 ± 1 [b] | | 38.0 ± 2 [c] (*38.6*) | 44.8 ± 2 [c] (*45.3*) | 6.8 ± 2 (*6.7*) |
| kinetic method | | | 39.4 [d] | 41.6 [e] | 2.2 |
| comput. MP2 | | 29.7 | 36.1 | 42.9 | 6.8 |
| comput. DFT | | 30.9 | 39.9 | 45.4 | 5.5 |
| best value | | 30.3[a] | 38 ± 2 | 45 ± 2 | 6.8 ± 2 |

[a] Reference value (GIBMS value from ref 23). [b] Estimated uncertainty relative to *Ala*. Absolute uncertainty is hard to assign. [c] Estimated uncertainty relative to *Pyr* reference value. Absolute uncertainty is hard to assign. [d] Reference 15. It should be noted that this value was estimated, not measured. [e] Reference 12. [f] Binding enthalpy at 0 K, kcal $mol^{-1}$; values in italics are corrected to 298 K.

Computationally, several refinements beyond the results of ref 13 were made. (1) Using DFT, the B3LYP functional was replaced with B3P86, following Armentrout's indication[21] that B3P86-DFT gives $Na^+$ binding energies about 1 kcal $mol^{-1}$ lower than B3LYP, and that these energies tend to agree better with experiment. We observed a similar lowering of the binding energies, and have adopted the B3P86 values as being preferable. (2) The three hydrogen atoms which may participate in hydrogen bonding or aromatic−$\pi$-cloud interactions were augmented with additional basis functions (two sets of three p functions). (3) MP2 binding energy calculations were made with the same expanded basis. (4) Further search of the potential surface of neutral *Phe* uncovered a new structure lying about 2.0 kcal $mol^{-1}$ (0.3 kcal $mol^{-1}$ by MP2) below the ground-state conformation previously assigned in ref 13. This structure, which is stabilized by interaction of one of the amino hydrogens with the aromatic ring, is displayed in Figure 1. (Actually there are a pair of such structures of very similar energy, related by a 120° rotation of the side chain). It is notable that an analogous conformation of *Ala* exists, but is calculated to be about 1 kcal $mol^{-1}$ higher than the *Ala* ground conformation.[24] The stabilization of this conformation in *Phe* can be regarded as reflecting a cation−$\pi$-type interaction of the positively charged H atom with the aromatic ring.

Table 3 summarizes the present thermochemistry along with some previous values. The present individual $Na^+$ affinity values are not in actual conflict with the previous (kinetic method) experimental values within the combined uncertainties. The comparative $\Delta H$(*Ala-Phe*) from ref 12 is, however, much lower than the present result. The present measurement of $\Delta H$(*Ala-Phe*) should be more reliable than the kinetic method result, being based on the essentially direct comparison of the two species, whereas the kinetic method only gave $\Delta H$(*Ala-Phe*) as a difference of two separate values (from different laboratories).

The comparison of alanine with the aromatic amino acids has been taken in previous work as a useful indication of the extent of cation−$\pi$ stabilization in the metal-ion chelates (after taking into account small differences in polarization interactions). The present results make this comparison more problematic, showing that the most favorable conformation of neutral *Phe* differs from that of alanine in a way that reflects substantial interaction of the amino group with the aromatic ring. In effect, neutral *Phe* already benefits from a certain amount of stabilization in its ground configuration due to intramolecular H−$\pi$ chelation with the aromatic ring. This stabilization energy is given up when the metal ion attaches, so the metal cation affinity in comparison with *Ala* is not a fully valid indication of the energy gained by cation−$\pi$ chelation of the metal ion. However, this only amounts to a question of 1 or 2 kcal $mol^{-1}$; the increase in $Na^+$ affinity in going from *Ala* to *Phe* is much larger than this, and the present analysis confirms that this increase is substantially a reflection of the cation−$\pi$ interaction.

The situation regarding the absolute $Na^+$ affinities of this set of compounds seems satisfactory. Table 3 summarizes the absolute $Na^+$ affinities from various sources. Binding energies of 38 ± 2 kcal for *Ala* and 45 ± 2 kcal for *Phe* would be comfortably within the range of uncertainties of most of the experimental and computational results. The kinetic method result for *Phe* is rather low compared with this, although still within the combined absolute uncertainties of the techniques. In the end, it appears that the very low *Ala/Phe* differential obtained in ref 12 can be largely ascribed to a coincidence of a rather high *Ala* value and a rather low *Phe* value, but neither of these individual values now appears to be wrong by more than a modest error of 2−3 kcal $mol^{-1}$. A justification, which may be correct, was given in ref 12 for why the kinetic method $Na^+$/*Phe* result might be systematically too low.

A recent threshold CID measurement[25] of the $Na^+$ affinity of glycine (39 kcal $mol^{-1}$) is in mild disagreement with the present scale of absolute amino acid values: since *Ala* must have a higher $Na^+$ affinity than *Gly*, there is a modest inconsistency with our best value of 38 kcal $mol^{-1}$ for *Ala*. However, an earlier threshold CID measurement[26] (36.6 kcal $mol^{-1}$) for *Gly* is fully consistent with our results. Rather than trying to resolve such inconsistencies, we would simply take these variations as an indication of the realistic level of uncertainty of absolute metal-ion affinities of chelating ligands using any of the current experimental approaches.

The MP2 computational binding energies are noticeably lower than the DFT values. Some of this difference might be rationalized if the sizable BSSE corrections applied to the MP2 values are actually excessive. However, the only secure conclusion to draw is that quantum calculations on these systems at this computational level, using either DFT or MP2, are uncertain within several kilocalories per mole in absolute value, although comparisons of similar molecules using the same method can be considered more reliable.

The present direct equilibrium comparison shows the $Na^+$ affinity of *AlaMe* to be larger than that of *Ala* by more than 2 kcal $mol^{-1}$. There is no reason to think that the structures of these complexes would be different, since bidentate metal coordination to the nitrogen and the carbonyl oxygen is favorable in both cases. This rather large increment presumably reflects some combination of differential polarizability effects in the ion complexes, and differential hydrogen bonding effects in the neutral compounds. A good supporting analogy to our result is the computational study by Jensen[27] of the comparative $Na^+$ affinities of the glycine and its methyl ester, in which he found the methyl ester to be greater than glycine itself by 2.5−3 kcal $mol^{-1}$. The fact that $Na^+$*AlaMe* (which has no zwitterion form) is more stable than $Na^+$*Ala* argues against a stable zwitterion ground conformation for $Na^+$*Ala*; Wyttenbach et al. have argued convincingly that this would not be a reasonable possibility in any case.[28]

(24) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem.* **1998**, *102*, 4623.

(25) Armentrout, P. B.; Rodgers, M. A. Proceedings of the 48th ASMS Conference, June 11−15, 2000, Long Beach, CA.

(26) Klassen, J. S.; Anderson, S. G.; Blades, A.; Kebarle, P. *J. Phys. Chem. A* **1996**, *100*, 14218.

(27) Jensen, F. *J. Am. Chem. Soc.* **1992**, *114*, 9533.

(28) Wyttenbach, T.; Witt, M.; Bowers, M. T. *J. Am. Chem. Soc.* **2000**, *122*, 3458.

## Conclusions

The leading conclusion from these experiments is that the $Na^+$ affinity of *Phe* is higher than that of *Ala* by at least 5, and probably 7, kcal $mol^{-1}$. This allays doubts raised by the previous lower kinetic method result, and confirms the large expected stabilization of $Na^+$*Phe* by cation$-\pi$ interaction. Since this thermochemistry results from essentially direct equilibirium comparison of the two $Na^+$ affinities, we give it high confidence within the uncertainty. Moreover, the large value of this increment is confirmed by the computational results using both DFT and MP2 methods.

Equilibrium measurements anchor the absolute $Na^+$ affinities to the recent experimental determination of *Pyr*. The absolute values are assigned as 38 $\pm$ 2 kcal for *Ala* and 45 $\pm$ 2 kcal $mol^{-1}$ for *Phe*. The value for *AlaMe* is 2.3 kcal $mol^{-1}$ higher than *Ala*, making it 40 $\pm$ 2 kcal $mol^{-1}$.

The discovery that Figure 1 is a lower energy *Phe* conformation than the previously assigned ground state slightly affects the interpretation of the *Ala/Phe* binding energy increment. This increment is not a fully informative indicator of the cation$-\pi$ interaction energy between the metal ion and the aromatic ring in $Na^+$*Phe*, because the ground conformation of neutral *Phe* is itself somewhat stabilized by intermolecular chelation of an amino H atom with the ring.

It seems that we now have good quantitative understanding of the gas-phase binding of $Na^+$ to the prototypical aromatic amino acid, *Phe*. The tridentate (O,N,Ring) chelating structure described in refs 12 and 13 describes the geometry. A specific contribution of the order of 5$-$8 kcal $mol^{-1}$ to the overall binding energy comes from the cation$-\pi$ interaction with the ring. This cation$-\pi$ interaction, along with small contributions from differential polarizability interactions and internal chelation in neutral *Phe*, accounts for the increase in $Na^+$ affinity between *Ala* and *Phe*.

**Acknowledgment.** This work received support from the donors of the Petroleum Research Fund, administered by the American Chemical Society.

JA010351T