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The sodium ion affinities of asparagine, glutamine, histidine and arginine

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Abstract

The sodium ion affinities of the amino acids Asn, Gln, His and Arg have been determined by experimental and computational approaches (for Asn, His and Arg). Na^+-bound heterodimers with amino acid and peptide ligands (Pep_1, Pep_2) were produced by electrospray ionization. From the dissociation kinetics of these Pep_1–Na^+–Pep_2 ions to Pep_1–Na^+ and Pep_2–Na^+, determined by collisionally activated dissociation, a ladder of relative affinities was constructed and subsequently converted to absolute affinities by anchoring the relative values to known Na^+ affinities. The Na^+ affinities of Asn, His and Arg, were calculated at the MP2(full)/6-311+G(2d,2p)//MP2/6-31G(d) level of ab initio theory. The resulting experimental and computed Na^+ affinities are in excellent agreement with one another. These results, combined with those of our previous studies, yield the sodium ion affinities of 18 out of the 20 α-amino acids naturally occurring in peptides and proteins of living systems.

Keywords: Amino acids; Sodium ion affinities; Sodium ion complexes; Kinetic method; Ab initio calculations

1. Introduction

Sodium ion is the most abundant metal ion in vivo, where it interacts with peptides and proteins in order to perform or facilitate essential biological processes, such as neurotransmission, osmotic balance and cellular metabolism [1–4]. The importance of Na^+ in biology has prompted many studies about its intrinsic binding modes and affinities to simple model systems, mainly amino acids and small peptides. The thermochemical and structural data resulting from these studies help to understand how Na^+ is transferred in biological fluids and how it activates biological reactions. In addition, these data also are helpful for the interpretation of the mass and tandem mass spectra of compounds ionized by Na^+ addition.

One important question raised in recent studies is whether the most stable structure of the sodium ion complexes of amino acids involves charge solvation (no net charge on the amino acid) or a salt bridge (the amino acid is in its zwitterionic form). A number of mass spectrometric techniques have been used to probe the structure of such complexes, including collisionally activated dissociation [5], ion mobility [6], relative binding energetics [7,8] and resonant infrared multiphoton dissociation [9–11]. There is growing consensus that, in most cases, charge solvation is more favorable, with the notable exception of Pro–Na^+, which is the only case so far for which a salt bridge structure has been established [9]. A borderline case appears to be that of Arg–Na^+ [5,11,12], which will be considered computationally in the present work.

Another important issue is the thermochemistry of the interaction between Na^+ and amino acids [5,7,13–29]. For the aliphatic amino acids Gly, Ala, Val, Leu, Ile and Pro, the aromatic amino acids Phe, Tyr and Trp and the side-chain functionalized amino acids Ser and Cys, there has been very good agreement between recent experimental Na^+ binding affinities, obtained by different methods, and/or between measured and computationally predicted affinities, so that it can be concluded that their Na^+ thermochemistry is well established. For instance, the sodium ion binding enthalpies of Gly, Pro, Phe, Tyr and Trp have been

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measured by both the kinetic and threshold dissociation methods. The kinetic method values are 161 ± 8, 196 ± 8, 198 ± 8, 201 ± 8 and 210 ± 8 kJ/mol [17], respectively, while the threshold dissociation values are 166 ± 6 [19], 188 ± 4 [24], 208 ± 7 [25], 212 ± 10 [25] and 220 ± 8 [25] (all at 298 K). Given the uncertainties, the two series of values are certainly compatible. For the side-chain functionalized amino acids Asp, Glu and Thr, the available experimental data [17] appear to be firmly established, even if these molecules have only been studied by the kinetic method so far. In contrast, the Na^+ affinities of methionine as well as of amino acids with amine, amide or imine side chains have not yet been characterized with full accuracy (Asn, Gln and His), or are not available at all (Arg, Lys and Met). This paper reports kinetic method experiments [30–34] and ab initio calculations on the Na^+ complexes of Asn, Gln, His and Arg that unveil the Na^+ binding properties of these basic amino acids and provide new or updated information about their structures and Na^+ affinities.

In a previous study of the sodium ion affinities of amino acids (AA) [17], we applied the kinetic method to heterodimer ions of the type AA_1–Na^+–AA_2. In the higher affinity part of the scale, it was difficult to find enough AA pairs to accurately relate the affinity of a given AA to those of several others. Thus, His could only be connected to Gln, leading to a derived sodium affinity of 219 kJ/mol, while the sodium ion affinity of Arg was too large to be determined in this way [17]. Since then, we have studied a series of peptides spanning a much larger range of Na^+ affinities [35]. This made it possible to generate several heterodimers of the type His–Na^+–Pep or Arg–Na^+–Pep, as will be discussed later, and enabled a more reliable determination of the Na^+ affinity of His, leading to a revised value of 228 kJ/mol (see below). An experimental sodium ion affinity of 185 kJ/mol has recently been obtained for His by Gapeev and Dunbar via sodium ligand exchange experiments [18]. Calculations on His and His–Na^+ by the same authors gave a markedly larger value of 226 kJ/mol for the more strongly binding tautomer of His (vide infra). The latter value is in agreement with the present experimental results.

Arginine is intriguing due to its unique structures and properties in solution and in the gas phase. There has been considerable debate about which type of Arg isomer, canonical or zwitterionic, is the most stable in the gas phase [5,36–38]. In addition, the canonical form of Arg may exist in two tautomeric forms of the guanidine group at the end of its side chain. These variations carry over to the sodium complex Arg–Na^+, for which the most stable isomer may be either of the charge solvation (with two different tautomers) or salt bridge type [5,11]. Taking these subtleties into account, the sodium ion affinity of Arg is determined for the first time in this study, both experimentally and theoretically. Finally, the Na^+ affinities of the amino acids asparagine and glutamine, which in analogy to dipeptides carry one amide bond, are also reevaluated because the present study revealed discrepancies with the previously reported values [17] (vide infra). For Asn and His, binding modes and sodium ion affinities are also calculated using ab initio theory. The computed values agree well with the corresponding experimental Na^+ affinities.

2. Methods

2.1. Mass spectrometry experiments

The sodium ion bound heterodimers (Pep_1–Na^+–Pep_2, where Pep is used as a symbol for amino acids as well as peptides for simplicity) were formed in the gas phase by electrospray ionization (ESI) and their competitive dissociations to the metalated monomers were examined by collisionally activated dissociation (CAD) in a quadrupole ion trap mass spectrometer (Bruker Esquire-LC, Billerica, MA). The ESI solvent used was a 2:1 (v/v) mixture of water and methanol. One milligram of each amino acid, peptide and sodium trifluoroacetate (NaTFA) were dissolved in 1 mL of solvent. Salt, Pep_1 and Pep_2 solutions were combined in the ratio 0.75:1:1 to make the solution sprayed. The latter solution was introduced into the ion source by a syringe pump at a rate of 240 μL/h. The spraying needle was grounded and the entrance of the sampling capillary was set at −4 kV. Nitrogen was used as the nebulizing gas (10 psi) and drying gas (8 L/min, 160 °C) and He as the buffer gas in the ion trap. For CAD, the precursor dimer ions were isolated and excited to fragment in the ion trap with a RF frequency that was resonant with their frequency of motion. The excitation time was 40 ms and the RF amplitude ($V_{p\text{-}p}$) was adjusted in the 0.32–0.50 V range to maximize the abundances of the sodiated monomers without causing appreciable competitive and/or consecutive dissociations. Thirty scans per spectrum were collected and the experiments were reproduced three times. The solvents (water, methanol; HPLC grade) were supplied by Sigma–Aldrich (St. Louis, MO) and NaTFA by Aldrich (Milwaukee, WI). Trp, GlyLeu, SerGly, Asn, Gln, His, AlaTrp, TrpAla, GlyGlyGly, AlaGlyGly, GlyHis, Arg and LeuGlyPhe were purchased from Sigma (St. Louis, MO) and GlyPhe, PheGly, AlaAlaGly and AlaAlaAla from BACHEM (King of Prussia, PA). All chemicals were used without further purification. The amino acids and peptides used in this study were chosen based on their side chains and their ability to form Na^+-bound heterodimers.

2.2. Kinetic method

The kinetic method experiments involved formation of gaseous Pep_1–Na^+–Pep_2 dimers and subsequent CAD of these dimers to the sodiated monomers according to Eq. (1). As mentioned above, the abbreviation Pep is used here for both peptides and amino acids. The abundance ratio of the Pep_1–Na^+ and Na^+–Pep_2 fragments resulting from CAD represents an approximate measure of the rate constant ratio (k_1/k_2) of the dissociations leading to these fragment ions. This assumption presupposes that other competing pathways and mass discrimination effects are negligible. Based on the thermodynamic formulation of transition state theory [39], the natural logarithm of k_1/k_2 is a function of the relative free energy of activation of the two competing dissociations of the heterodimers, as shown in Eq. (2), where R is the ideal gas constant and T_{eff} is the effective temperature of the dissociating dimer ions [34]. The enthalpy and entropy components of the free energies are included in Eq. (2). The unimolecular reactions of Eq. (1) involve cleavages of

electrostatic bonds, which generally proceed without appreciable reverse activation energy [13,14,22,32,40–42]. In such cases, the relative enthalpy of activation becomes equivalent (with opposite sign) to the difference in binding enthalpies of Na^+ to Pep_1 and Pep_2, cf. Eq. (3), where the Na^+ binding enthalpy or Na^+ affinity (also called Na^+ binding energy) is defined as the enthalpy change, ΔH_{Na}, of the reaction $Pep–Na^+ \rightarrow Pep + Na^+$. Analogously, the relative activation entropy can be replaced by an apparent relative entropy [43] of Na^+ attachment to Pep_1 vs. Pep_2.

$$Pep_1–Na^+ + Pep_2 \xleftarrow{k_1} Pep_1–Na^+–Pep_2 \xrightarrow{k_2} Pep_1 + Na^+–Pep_2 \quad (1)$$

$$\ln\left(\frac{k_1}{k_2}\right) = -\frac{[\Delta G_1^{\ddagger} - \Delta G_2^{\ddagger}]}{RT_{\text{eff}}} = \frac{[\Delta S_1^{\ddagger} - \Delta S_2^{\ddagger}]}{R} - \frac{[\Delta H_1^{\ddagger} - \Delta H_2^{\ddagger}]}{RT_{\text{eff}}} \quad (2)$$

$$\ln\left(\frac{k_1}{k_2}\right) = -\frac{[\Delta S_{Na}^{app}(Pep_1) - \Delta S_{Na}^{app}(Pep_2)]}{R} + \frac{[\Delta H_{Na}(Pep_1) - \Delta H_{Na}(Pep_2)]}{RT_{\text{eff}}} = -\frac{\Delta(\Delta S_{Na}^{app})}{R} + \frac{[\Delta H_{Na}(Pep_1) - \Delta H_{Na}(Pep_2)]}{RT_{\text{eff}}} \quad (3)$$

$$\ln\left(\frac{k_1}{k_2}\right) \approx \frac{\Delta H_{Na}(Pep_1)}{RT_{\text{eff}}} - \frac{\Delta H_{Na}(Pep_2)}{RT_{\text{eff}}} = \frac{\Delta(\Delta H_{Na})}{RT_{\text{eff}}} \quad (4)$$

Because the Na^+-bound dimers are not in thermal equilibrium with their surroundings and their internal energy distributions are not Boltzmann-shaped [23,33,34,43–45], an effective temperature and an apparent entropy difference are used instead of a thermodynamic temperature and entropy difference. On the basis of recent studies [17,23,43–46] $\Delta(\Delta S_{Na}^{app})$ depends on the identity of the decomposing dimer ions and on T_{eff} and its value can range from ~0 to the corresponding actual (thermodynamic) entropy difference of Na^+ complexation by Pep_1 vs. Pep_2. The amino acids and peptides used will be shown to have very similar apparent Na^+ binding entropies (vide infra); if $\Delta(\Delta S_{Na}^{app}) \approx 0$, Eq. (3) is simplified to Eq. (4), which relates the experimental k_1/k_2 data to relative Na^+ binding affinities. Relative sodium ion affinities obtained through the examination of $Pep_1–Na^+–Pep_2$ dimers can be converted to absolute ΔH_{Na} data if the relative values are anchored to a known Na^+ binding energy.

2.3. Calculations

Ab initio calculations were carried out at levels which have been shown in previous work to provide reasonably accurate geometries and sodium ion affinities [21,47]. Geometries were optimized at the HF/6-31G(d) and MP2/6-31G(d) levels; vibrational analyses were carried out at the same levels to determine zero-point vibrational energies, thermal corrections to total energies and entropies. Final energetics were determined at the MP2(full)/6-311+G(2d,2p) level using either the MP2/6-31G(d) or HF/6-31G(d) geometries. With Asn and His, structures are simple enough, for both the bare and sodiated molecules, that a careful inspection of the stabilizing intramolecular interactions is sufficient to determine which structures are the most stable. The leading interactions are hydrogen bonds and sodium chelation patterns. The conformational space is much more complex for Arg and Arg–Na^+. Thus, we resorted to Monte Carlo sampling using the Amber94 force field, a procedure which has been described previously [35,48]. Monte Carlo calculations were carried out with HyperChem 6.0 [49], while ab initio calculations used the Gaussian03 suite of programs [50].

3. Results and discussion

3.1. Experimental sodium ion affinity scale of amino acids and peptides

Overall, the CAD spectra of forty-six pairs of $Pep_1–Na^+–Pep_2$ heterodimers were evaluated, which showed detectable abundances for both sodiated monomers, $Pep_1–Na^+$ and $Na^+–Pep_2$ with acceptable signal-to-noise ratio. With heterodimers containing Arg, consecutive fragmentation of Arg–Na^+ via losses of H_2O and, to a much lesser extent, NH_3 takes place (Fig. 1). This reactivity is observed from all Arg–Na^+–Pep heterodimers investigated; each one produces upon CAD a readily detectable $[Arg + Na - H_2O]^+$ peak at *m/z* 179, occasionally accompanied by a much weaker $[Arg + Na - NH_3]^+$ peak at *m/z* 180. The abundance of sodiated arginine used for the calculation of $\ln(k_1/k_2)$ was the sum of the peak heights of *m/z* 197 ($[Arg + Na]^+$) and 179 ($[Arg + Na - H_2O]^+$); the peak from NH_3 loss was not considered because of its consistently minuscule abundance. The Na^+ complex of Arg may have a charge solvation or salt bridge structure, depending on whether the metal ion binds the Arg

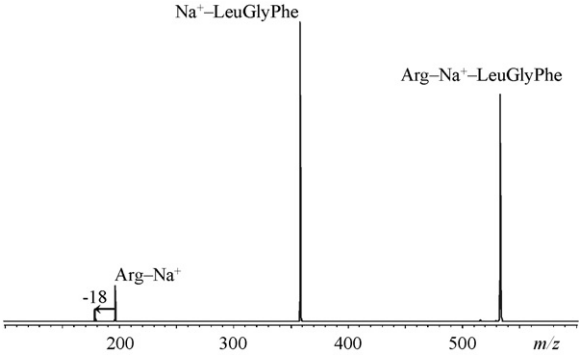

Fig. 1. CAD mass spectrum of heterodimer Arg–Na^+–LeuGlyPhe (*m/z* 532) measured at an excitation amplitude of 0.40 V; the loss of H_2O (18 u) from sodiated Arg is marked on the spectrum.

ligand in its neutral (canonical) or zwitterionic form, respectively [5,7,11,36,51–56]. Jockusch et al. showed that charge solvation complexes of Arg eliminate H_2O, whereas salt bridge complexes eliminate NH_3 [5]. The much higher abundance of *m/z* 179 ($[Arg + Na - H_2O]^+$) vs. 180 ($[Arg + Na - NH_3]^+$) in our spectra (see, e.g., Fig. 1) could thus suggest that the Arg–Na^+ complex arising from heterodimer dissociation predominantly contains the charge solvation isomer [7,8]. The IR spectrum of sodiated arginine reported recently by Williams and co-workers [11] provided evidence for a mixture of the salt bridge and charge solvation isomers. Furthermore, the authors argued that ESI produces mainly (~90%) the salt bridge form of Arg–Na^+, which preferentially loses water because of the lower critical energy needed for tautomerization and subsequent water loss than for ammonia loss directly from the zwitterionic structure [11].

From the $\ln(k_1/k_2)$ data of the 46 Pep_1–Na^+–Pep_2 pairs examined, a Na^+ affinity ladder for 16 amino acids/peptides could be constructed, which is presented in Fig. 2. The Pep_1 and Pep_2 components of the evaluated heterodimers are connected by arrows. The corresponding $\ln(k_1/k_2)$ values are listed at the right side of the arrows and were calculated by assigning k_1 to the dissociation that produced the more abundant sodiated monomer. From the experimental $\ln(k_1/k_2)$ ratios, average cumulative $\ln(k_{Pep}/k_{Trp})$ ratios were calculated through a least-square procedure; the resulting values and corresponding standard deviations are summarized in Table 1 and provide a quantitative measure of the Na^+ affinities of a series of peptides and amino acids relative to Trp.

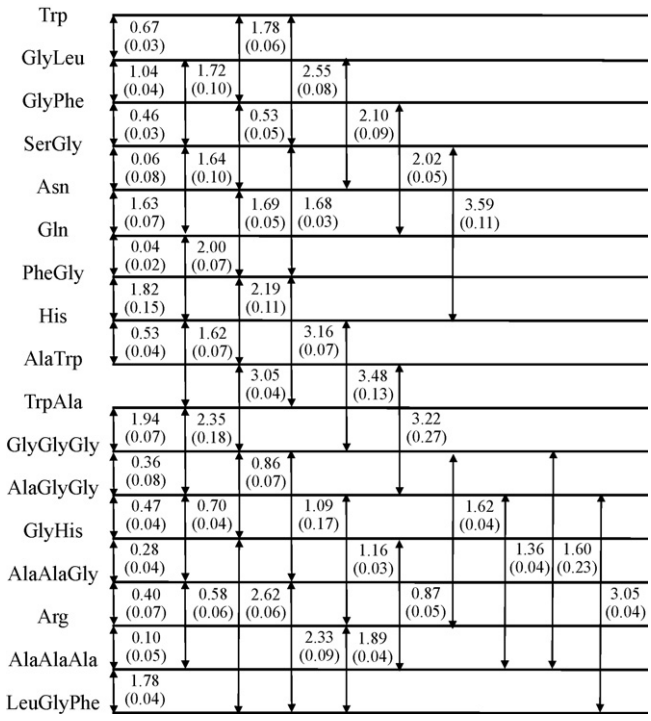

Fig. 2. $\ln(k_1/k_2)$ values calculated from abundances of the sodiated monomers in the CAD spectra of Na^+-bound amino acid/peptide heterodimers. The two components of a sodiated heterodimer are connected by arrows. The corresponding $\ln(k_1/k_2)$ values are shown next to the arrows; k_1 is assigned to the dissociation that produced the more abundant sodiated monomer in order to have positive $\ln(k_1/k_2)$ values. The standard deviations of $\ln(k_1/k_2)$ range between 0.02 and 0.27 and have an average value of 0.16.

Table 1
Experimental and calculated sodium ion affinities (kJ/mol) and calculated Na^+ binding entropies (J/mol K)[a]

Amino acid or peptide	$\ln(k_{Pep}/k_{Trp})$	$\Delta(\Delta H_{Na})$[b]	ΔH_{Na}[c]	ΔH_{Na} theory[d]	ΔH_{Na} theory[e]	ΔS_{Na} theory[d]	ΔS_{Na} theory[e]
Trp	0.00	0.0	210 (8)				
GlyLeu	0.67 (0.03)	2.0 (0.2)	212 (8)				
GlyPhe	1.75 (0.06)	5.2 (0.5)	215 (8)				
SerGly	2.38 (0.08)	7.0 (0.6)	217 (8)				
Asn	2.50 (0.10)	7.4 (0.7)	217 (8)	219	223	108	119
Gln	3.97 (0.11)	11.7(1.0)	222 (8)				
PheGly	4.09 (0.10)	12.0 (1.1)	222 (8)				
His	5.95 (0.15)	17.5 (1.5)	228 (8)	232	235	117	123
AlaTrp	6.38 (0.15)	18.8 (1.7)	229 (8)				
TrpAla	7.41 (0.15)	21.8 (1.9)	232 (8)				
GlyGlyGly	9.40 (0.19)	27.7 (2.4)	238 (8)		242		128
AlaGlyGly	9.71 (0.25)	28.6 (2.5)	239 (8)				
GlyHis	10.22 (0.23)	30.1 (2.6)	240 (8)		245		122
AlaAlaGly	10.47 (0.25)	30.8 (2.7)	241 (8)				
Arg	10.92 (0.24)	32.1 (2.8)	242 (8)	256,[f] 245[g]	256,[f] 251[g]	92,[f] 93[g]	97,[f] 99[g]
AlaAlaAla	11.05 (0.26)	32.5 (2.9)	243 (9)		252		126
LeuGlyPhe	12.81 (0.25)	37.7 (3.3)	248 (9)				

[a] Numbers in parenthesis are standard deviations.
[b] Obtained using $T_{eff} = 354 \pm 30$ K, see text. The standard deviations were calculated from the experimental uncertainties in $\ln(k_{Pep}/k_{Trp})$ and T_{eff}.
[c] Obtained by adding the relative affinities to ΔH_{Na}(Trp). The standard deviations were calculated from the standard deviations of the corresponding relative values and the uncertainty in ΔH_{Na}(Trp) (±8 kJ/mol). The absolute affinities of the peptides have been reported in ref. [35]; a few values have increased, relative to those given in [35], by the insignificant amount of 1 kJ/mol due (a) to the inclusion of the new, amino acid containing heterodimers in the least-square procedure used to generate the cumulative $\ln(k_{Pep}/k_{Trp})$ ratios and (b) to rounding.
[d] MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G(d) values.
[e] MP2(full)/6-311+G(2d,2p)//HF/6-31G(d) values.
[f] Assuming a salt bridge structure for Arg–Na^+, see text.
[g] Assuming a charge solvation structure for Arg–Na^+, see text.

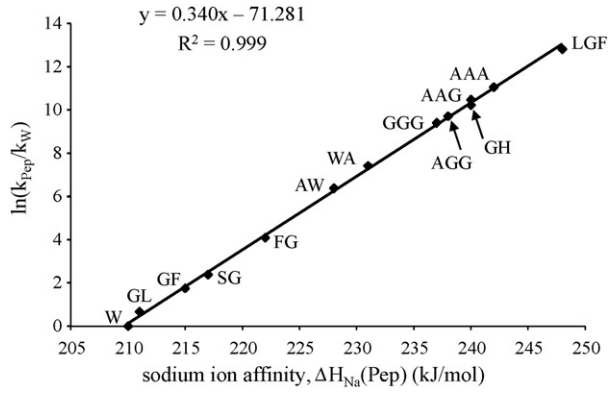

Fig. 3. Plot of cumulative $\ln(k_{Pep}/k_{Trp})$ ratios vs. ΔH_{Na}(Pep). The Na^+ affinity of Trp (W) was taken from ref. [17], and the Na^+ affinities of GlyLeu (GL), GlyPhe (GF), SerGly (SG), PheGly (FG), AlaTrp (AW), TrpAla (WA), GlyGlyGly (GGG), AlaGlyGly (AGG), GlyHis (GH), AlaAlaGly (AAG), AlaAlaAla (AAA) and LeuGlyPhe (LGF) were taken from ref. [35].

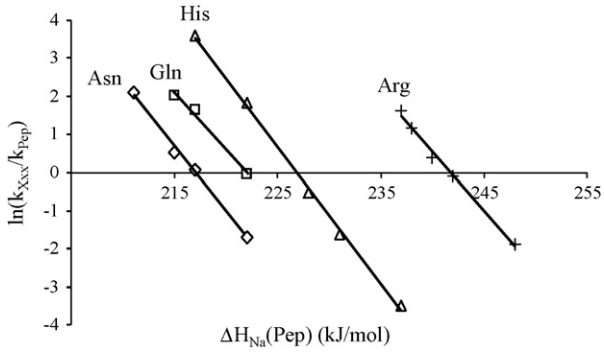

Fig. 4. Plots of $\ln(k_{Xxx}/k_{Pep})$ ratios vs. ΔH_{Na}(Pep) for four sets of Xxx–Na^+–Pep dimers. Xxx = Asn (diamonds), Gln (squares), His (triangles) or Arg (crosses). The peptide reference bases with which each Xxx was paired are given in Table 2.

3.2. Conversion of the $\ln(k_1/k_2)$ scale to Na^+ binding affinities

In order to convert the cumulative $\ln(k_{Pep}/k_{Trp})$ scale into relative Na^+ affinities, the effective temperature of the dissociating heterodimers is needed, cf. Eq. (4). T_{eff} was calibrated from the $\ln(k_{Pep}/k_{Trp})$ values of ligands included in Fig. 2 whose Na^+ affinities were determined in previous studies [17,35], specifically Trp (ΔH_{Na} = 210 kJ/mol), GlyLeu (211), GlyPhe (215), SerGly (217), PheGly (222), AlaTrp (228), TrpAla (231), GlyGlyGly (237), AlaGlyGly (238), GlyHis (240), AlaAlaGly (240), AlaAlaAla (242) and LeuGlyPhe (248). Plotting $\ln(k_{Pep}/k_{Trp})$ of these molecules (Table 1) against the corresponding ΔH_{Na} values gives rise to a regression line with the slope $1/RT_{eff} = 0.340$ (cf. Fig. 3). This slope renders an effective temperature of 354 K, which was used to calculate Na^+ affinities relative to Trp according to Eq. (4), as well as absolute affinities by adding the relative values to ΔH_{Na}(Trp) = 210 kJ/mol (see Table 1).

The standard deviation of the slope of the calibration line in Fig. 3 (0.0033) adds an uncertainty of ca. ±4 K to the derived T_{eff} value. $T_{eff} = 354 \pm 4$ K may be viewed as the mean effective temperature of all 46 Na^+-bound heterodimers examined. The effective temperatures of the individual Pep_1–Na^+–Pep_2 pairs must lie within a broader window, estimated at $\pm(4 \times \sqrt{46}) \approx \pm 30$ K. The latter, higher and more realistic uncertainty was used to calculate the standard deviations of the relative Na^+ affinities listed in Table 1.

3.3. Na^+ binding affinities by pairing each amino acid with a set of peptides

Since each of the amino acids Asn, Gln, His and Arg (Xxx) was paired with at least three peptide reference bases (Pep), cf. Fig. 2, their Na^+ affinities can also be deduced directly, i.e., without first setting up a cumulative $\ln(k_1/k_2)$ ladder. Plotting the $\ln(k_{Xxx}/k_{Pep})$ ratios measured for each Xxx–Na^+–Pep set vs. the corresponding ΔH_{Na}(Pep) leads to the regression lines shown in Fig. 4. The x-intercepts and slopes of these lines yield ΔH_{Na}(Xxx) and the average T_{eff} of Xxx–Na^+–Pep, respectively (Table 2). The effective temperatures of the four sets of Xxx–Na^+–Pep dimers fluctuate between 335 and 398 K but their average value, 368 ± 30 K, is indistinguishable from that obtained using the cumulative $\ln(k_{Pep}/k_{Trp})$ ladder, viz. 354 ± 30 K (vide supra). Despite the T_{eff} variability between the different Xxx–Na^+–Pep sets, the Na^+ affinities obtained by using solely Xxx–Na^+–Pep dimers (Table 2) and those derived from the cumulative Na^+ affinity ladder (Table 1) match within experimental error. The low sensitivity of binding affinities to

Table 2
Na^+ affinities of Asn, Gln, His and Arg based on the dissociations of Na^+-bound heterodimers of these amino acids (Xxx) with peptide reference bases (Pep)[a]

Regression line (Fig. 4)	Xxx–Na^+–Pep			
	Xxx = Asn; Pep = GL, GF, SG, FG	Xxx = Gln; Pep = GF, SG, FG	Xxx = His; Pep = SG, FG, AW, WA, GGG	Xxx = Arg; Pep = GGG, AGG, AAG, AAA, LGF
R^2	0.996	0.989	0.998	0.994
x-intercept = ΔH_{Na}(Xxx)[b]	217 (8)	222 (8)	227 (9)	242 (9)
Slope = $-1/RT_{eff}$[b]	0.3404 (0.0154)	0.3023 (0.0324)	0.3587 (0.0095)	0.3117 (0.0140)
T_{eff}[c]	353 (16)	398 (43)	335 (9)	386 (17)

[a] Numbers in parentheses are standard deviations.
[b] x-Intercept (kJ/mol) and slope of the equation: $\ln(k_{Xxx}/k_{Pep}) = \Delta H_{Na}(Xxx)/RT_{eff} - \Delta H_{Na}(Pep]/RT_{eff}$, which is Eq. (4) with Pep_1 = Xxx and Pep_2 = Pep. The error limits of ΔH_{Na}(Xxx) were calculated from the standard deviations of the x-intercepts (1 kJ/mol), which reflect the fluctuations in relative affinities, and the uncertainties in ΔH_{Na} of the Pep reference bases (8–9 kJ/mol).
[c] Calculated from the respective slopes (K).

T_{eff} fluctuations probably results from the fact that the variable probed experimentally, viz. $\ln(k_1/k_2)$, is a relative and, hence, naturally small quantity [32,33,57]. A T_{eff} window of 60 K (±30 K) causes a change of <0.5 kJ/mol per $\ln(k_1/k_2)$ unit. Only Na^+-bound dimers with $\ln(k_1/k_2) < 3.6$ were evaluated in this study (cf. Fig. 2) to minimize the effect of T_{eff} variations among individual dimers.

3.4. Experimental Na^+ affinity trends

The sodium ion affinity of Arg was determined for the first time and the agreement between our experimental (242 kJ/mol) and ab initio affinities (245 or 256 kJ/mol, discussed below) is good.

Our recent study on the Na^+ affinities of simple di-, tri- and tetrapeptides [35] produced a database of twenty new Na^+ affinities spanning the range 203 kJ/mol (GlyGly) to 265 kJ/mol (AlaAlaAlaAla). The availability of these reference bases allowed us to cross-check and determine more accurately the Na^+ affinities of the amino acids examined in our earlier study (in which only amino acid containing heterodimers were probed) [17]. Here, a markedly larger number of Pep_1–Na^+–Pep_2 pairs containing both amino acid as well as peptide ligands were used to derive new $\ln(k_1/k_2)$ orders and relative affinities for the amino acids Pro, Thr, Phe, Asp, Glu, Trp, Asn, Gln and His, which were selected as test cases. New and old results are summarized in Table 3 and agree very well with each other, except for Asn, Gln and His, whose sodium ion affinities appear to have been underestimated in our earlier study. For this reason, these three amino acids were reexamined in multiple heterodimer combinations (Fig. 2), which led to upward revision of their Na^+ affinities by ~10 kJ/mol (Tables 1 and 2).

Inspection of the data in Table 3 reveals that the Na^+ affinity increments between Asn, Gln and His have not changed, but that the affinity levels of these three amino acids relative to Trp and the other five amino acids tested have increased (by the mentioned ~10 kJ/mol). This increase suggests that higher energy conformers of sodiated Asn, Gln and His were probed in our earlier study. In order to verify that the most stable complexes were sampled in this study, ab initio calculations were performed on Asn and His and their Na^+ complexes, as will be discussed later.

Table 3
Comparison of relative Na^+ affinities determined previously [17] and in the current study[a]

Amino acid	$\Delta(\Delta H_{Na})$ from ref. [17][b]	$\Delta(\Delta H_{Na})$ from this study[c]
Pro	0	0
Thr	1	1
Phe	2	2
Asp	7	5
Glu	8	7
Trp	14	14
Asn	10	21
Gln	16	26
His	22	32

[a] All values in kJ/mol.
[b] Obtained using Pep_1–Na^+–Pep_2 heterodimers, in which both ligands were amino acids (±<4 kJ/mol) [17].
[c] Obtained using Pep_1–Na^+–Pep_2 heterodimers, in which one ligand was an amino acid (that listed in the left column) and the other ligand a peptide of known Na^+ affinity (±<3 kJ/mol) [35], see text.

The availability of peptide reference bases in the present study made it possible to pair Asn, Gln and His with molecules of quite similar Na^+ binding affinity (cf. Figs. 2 and 4 and Tables 1 and 2), which was impossible in our previous study [17]. It has been well documented that kinetic method experiments are most likely to yield accurate thermochemical data if the molecules combined in a heterodimer have small affinity differences [32,33]. The lack of suitable pairing partners is a possible reason for the previously underestimated affinities.

It is worth noting that the position of tryptophan in the Na^+ affinity ladder of our earlier study was determined from the dissociations of Na^+-bound dimers involving asparagine and glutamine [17]. The relative affinities of Asn and Gln changed in our new study, but not that of Trp (Table 3). This result suggests that consistently the higher energy conformers of Asn–Na^+ and Gln–Na^+ were probed in the old experiments, but the corresponding most stable conformers in the new ones, with the result that both studies yielded the same relative Na^+ affinity of Trp.

The revised sodium ion affinities of Asn and Gln deduced from the new experiments are 217 and 222 kJ/mol, respectively (Tables 1 and 2). It is noteworthy that Asn, an isomer of GlyGly (both $C_4H_8N_2O_3$), binds Na^+ more strongly than GlyGly, by 14 kJ/mol. Similarly, Na^+ is bound more strongly by Gln than the isomeric dipeptides AlaGly and GlyAla, by 17 and 15 kJ/mol, respectively. Hence, an amide side chain affords a superior coordination environment for Na^+ as compared to a backbone amide group. It is likely that Na^+ chelation requires less steric constraints from the branched backbones of Asn and Gln than the linear backbones of GlyGly and AlaGly/GlyAla.

Our revised sodium ion affinity of His, 228 kJ/mol (mean of values in Tables 1 and 2), is 43 kJ/mol higher than the affinity obtained by Gapeev and Dunbar using the ligand exchange equilibrium method [18]. The much lower ΔH_{Na}(His) measured by the latter method was attributed to the exclusive sampling of a higher-energy His–Na^+ tautomer in ligand exchange experiments, which involved thermal desorption of neutral His (vide infra).

3.5. Ab initio calculations on the amino acids His, Arg and Asn and their sodium ion complexes

In order to strengthen the anchoring of the absolute sodium ion affinity ladder, to assess the relative entropies of binding of amino acids to Na^+ and to provide a detailed picture of the low energy structures and modes of interaction between amino acids and the sodium ion, ab initio calculations were performed on bare and sodiated His, Asn and Arg.

3.5.1. Histidine

As argued by Gapeev and Dunbar [18], the two tautomers of His shown in Fig. 5 must be considered for both His and

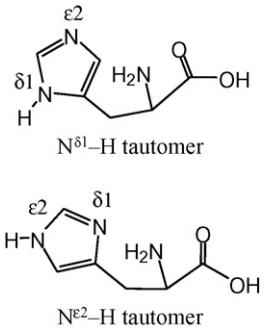

Fig. 5. The $N^{\varepsilon 2}$–H and $N^{\delta 1}$–H tautomers of histidine. The former binds Na^+ at $N^{\delta 1}$ and the latter at $N^{\varepsilon 2}$. Na^+ interaction with $N^{\delta 1}$, which involves the $N^{\varepsilon 2}$–H tautomer, generates the most stable His–Na^+ complex.

His–Na^+. While the $N^{\delta 1}$–H tautomer is much more frequently found in organic and biological molecules, both $N^{\delta 1}$–H and $N^{\varepsilon 2}$–H tautomers may coexist in general, and have been detected in histidine itself [58], the dipeptides GlyHis and HisGly [48], proteins [59] and as metal ligands in a metalloprotein [60]. In agreement with a previous exploration of the structures of isolated His [61], our computations indicate that the $N^{\varepsilon 2}$–H tautomer is intrinsically more stable for both His and His–Na^+ in the gas phase.

We find that the most stable structure of His–Na^+ (see Fig. 6) is of the charge solvation type, and involves sodium chelation to the carbonyl oxygen, amino nitrogen and $N^{\delta 1}$ nitrogen of the imidazole ring in its $N^{\varepsilon 2}$–H tautomer; a similar finding was reported by Gapeev and Dunbar [18]. Another charge solvation structure with bidentate chelation of Na^+ to the carbonyl oxygen and $N^{\delta 1}$ nitrogen is 16 kJ/mol less stable. It has the same energy as the most favorable salt bridge isomer, in which there is a Na^+/carboxylate/ammonium triad (Fig. 6). Based on the most stable geometries of the $N^{\varepsilon 2}$–H tautomer for both His and His–Na^+, the computed 298 K sodium binding enthalpy of histidine is 235 or 232 kJ/mol, depending upon whether the HF/6-31G(d) or MP2(full)/6-31G(d) geometry is used, respectively. These values are in good agreement with the experimental value of 228 kJ/mol obtained in this work, and with the 225 kJ/mol value calculated by Gapeev and Dunbar at the B3P86/6-311+G(2df,2pd) level of density functional theory (DFT) [18].

The values mentioned above are 40–50 kJ/mol larger than that derived from ligand exchange equilibria [18]. In order to iden-

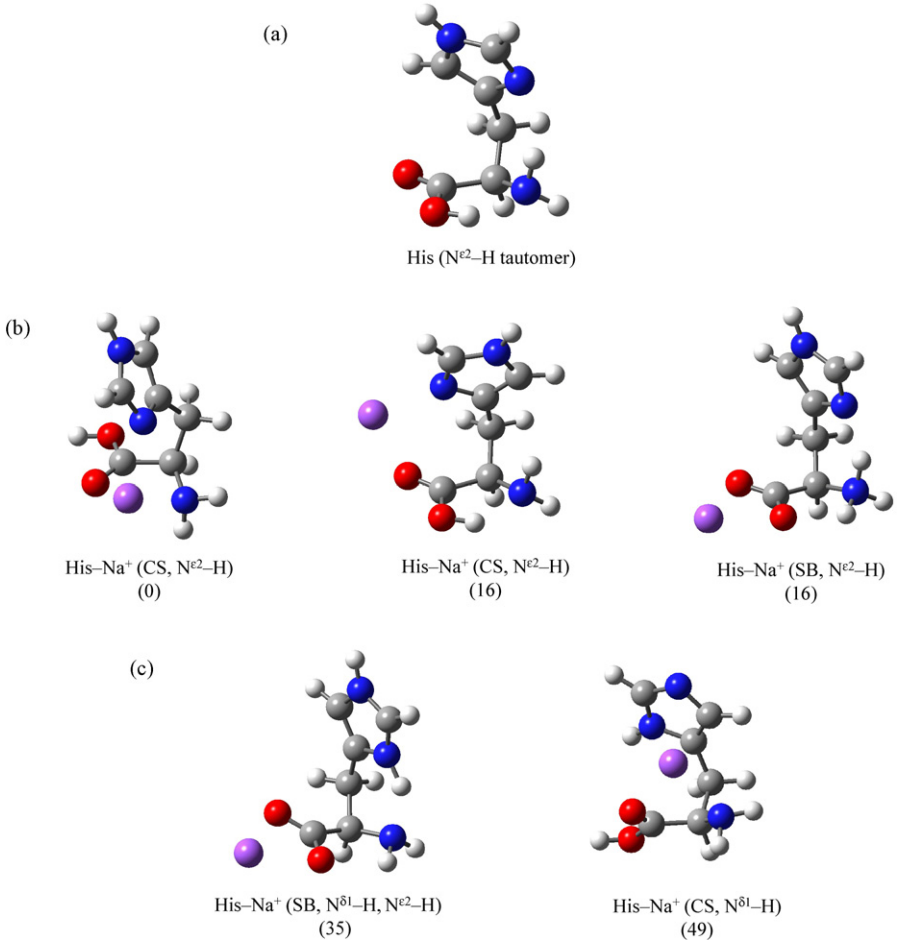

Fig. 6. Geometries of the lowest-energy conformers/isomers of His and the His–Na^+ complex: (a) the most stable conformer of His, tautomer $N^{\varepsilon 2}$–H; (b) the most stable structures of His–Na^+ in its $N^{\varepsilon 2}$–H tautomer; (c) the most stable structures of His–Na^+ in its $N^{\delta 1}$–H tautomer. Relative energies in kJ/mol are given in parentheses.

tify the possible origin of such a large discrepancy, the $N^{\delta1}$–H tautomers of His and His–Na^+ were considered. Simultaneous chelation of Na^+ to imidazole, carbonyl oxygen and amine nitrogen is less favorable in the $N^{\delta1}$–H than the $N^{\varepsilon2}$–H tautomer, because the $N^{\varepsilon2}$ position is much less accessible than $N^{\delta1}$. It leads to a CS structure that lies 49 kJ/mol higher in energy than the most stable $N^{\varepsilon2}$–H-based isomer. The most stable sodium ion complex with $N^{\delta1}$–H was found to bear a salt bridge in which the acid is deprotonated and the imidazole, rather than the amine group, is protonated (Fig. 6). It has a relative energy of 35 kJ/mol, i.e., 19 kJ/mol higher than the $N^{\varepsilon2}$–H salt bridge described above, to which it is related by a simple proton transfer from the amine to the imidazole $N^{\delta1}$ site. Detachment of Na^+ from this salt bridge structure is very likely to trigger proton transfers from imidazole $N^{\delta1}$ to the amine and from the amine to the carboxylate, leading to the most stable conformer of the $N^{\varepsilon2}$–H tautomer of histidine. Assuming that such a rearrangement occurs, the sodium ion affinity of the $N^{\delta1}$–H tautomer of His is computed to be 197 kJ/mol, in reasonable agreement with the experimental value of 185 kJ/mol [18]. These results suggest that different histidine tautomers are involved in the complexes probed in ligand exchange equilibria vs. kinetic method experiments. In the former case, gaseous histidine is supplied by thermal sublimation of a powder, and it seems reasonable that a zwitterion is formed since it is the most stable isomer in the condensed phase. The present experiments produced His–Na^+–Pep dimers from a liquid sample via ESI; these ions were then collisionally fragmented to produce His–Na^+ ions. Since the His–Na^+ interaction is much more favorable with the $N^{\varepsilon2}$–H tautomer, it is a reasonable hypothesis that this tautomer is favored in solution (where tautomerization can occur through intermolecular proton transfers, contrary to the dilute gas phase), and then left unchanged when extracted to the gas phase. If the $N^{\delta1}$–H tautomer of His–Na^+ is considered instead, dissociation into either the $N^{\delta1}$–H or $N^{\varepsilon2}$–H tautomer of free His would lead to poor agreement between the computed and experimental sodium ion affinities.

3.5.2. Asparagine

Ab initio calculations were carried out on Asn and Asn–Na^+. Conformers of Asn have already been explored in great detail before [62,63]. The present work used these results to carry out a limited structural search, yielding five conformers at the HF/6-31G(d) level, the three most stable of which were reoptimized at the MP2/6-31G(d) level. Their energies are within less than 1 kJ/mol at the HF/6-31G(d) level, however refined energetics of the MP2/6-31G(d) geometries lead to larger differences, such that there is little doubt that **1** (Fig. 7) is the most stable (for a complete picture of Asn conformers, see ref. [63]). In this conformer, the acid is trans, allowing the donation of a H bond to the amino terminus, itself donating a H bond to the side chain carbonyl oxygen. This network of H bonds is completed by one from the side chain amide to the acid O=C.

The potential energy surface for Asn–Na^+ appears not to have been described previously. The most stable complex results from sodium chelation in a tridentate manner by the carbonyl oxygens and the amine group. The most stable salt bridge structure lies 24 kJ/mol higher in energy; it involves a Na^+/carboxylate/ammonium triad (see Fig. 7). Na^+ is coordinated at the negatively charged carboxylate group, while two protons of the positively charged ammonium group form H bonds, one with the amide oxygen and the other with the most proximate carboxylate oxygen. Another conformation of the salt bridge (SB2 in Fig. 7) is found to lie 7 kJ/mol higher in energy. There are also CS structures in the same energy range. Bidentate chelation of sodium, rather than tridentate as in CS1, is found to be less favorable: additional hydrogen bonds, such as that from the trans acid to the amine in CS2, cannot compensate for a weaker Na^+/Asn interaction. Dissociation of the most stable Asn–Na^+ complex to the most stable Asn conformer at 298 K is calculated to require 223 kJ/mol using the structures optimized at the HF level of theory; with the MP2-optimized geometries, this energy decreases to 219 kJ/mol. Both these values are in excellent agreement with the experimentally derived Na^+ affinity of Asn, 217 kJ/mol (see Tables 1 and 2).

3.5.3. Arginine

For Arg and Arg–Na^+, the flexibility of the side chain makes the identification of the most stable structures a computational challenge. Since the pioneering work of Williams and co-workers [52], there have been several attempts at identifying the most stable conformer and isomer of Arg. At the time when this work was started, the most extensive study was that by Simons and co-workers [36,37]. Using their results, extensive explorations of the potential energy surfaces were carried out for both Arg and Arg–Na^+. A total of 24 structures of Arg were fully characterized at the ab initio level, 17 neutral and 7 zwitterionic, while 13 structures of Arg–Na^+ were obtained, 7 of the charge solvation type and 6 salt bridges. In agreement with Simons and co-workers [36,37], we found that the most stable structures of bare Arg are non-zwitterionic. We identified, however, a conformer that was lower in energy than any of those described previously [64]. While this work was near completion, another, much more thorough study of the conformers and isomers of Arg was reported by Liang et al. [38], who presented a large number of new structures that are more stable than any of those previously published. It turned out that our best structure was also found by Liang et al., but was not their most stable. For this reason, we used the two most stable structures found by Liang et al. and reoptimized them at the HF/6-31G(d) and MP2/6-31G(d) levels, since the energy ordering is not necessarily the same at those levels and the ones used by Liang et al. (see structures **1** and **2** in Fig. 8). Their energy difference is very close to that reported [38]. The two conformers are very similar. Both involve the $-N{=}C(NH_2)_2$ tautomer in Arg's guanidine side chain end, a trans acid donating a H bond to the amine and a H bond from the amine to the imino nitrogen of guanidine. The difference between the two conformers is mostly in the relative positions of the two NH_2 groups of guanidine, slightly modifying the local H bonding network. We also report in Fig. 8 the most stable conformer of Arg in its $-NH-C(=NH)(NH_2)$ tautomer (**3**), since it is the likely dissociation product of Arg–Na^+ (vide infra). It is only 5 kJ/mol higher in energy than the overall most stable structure.

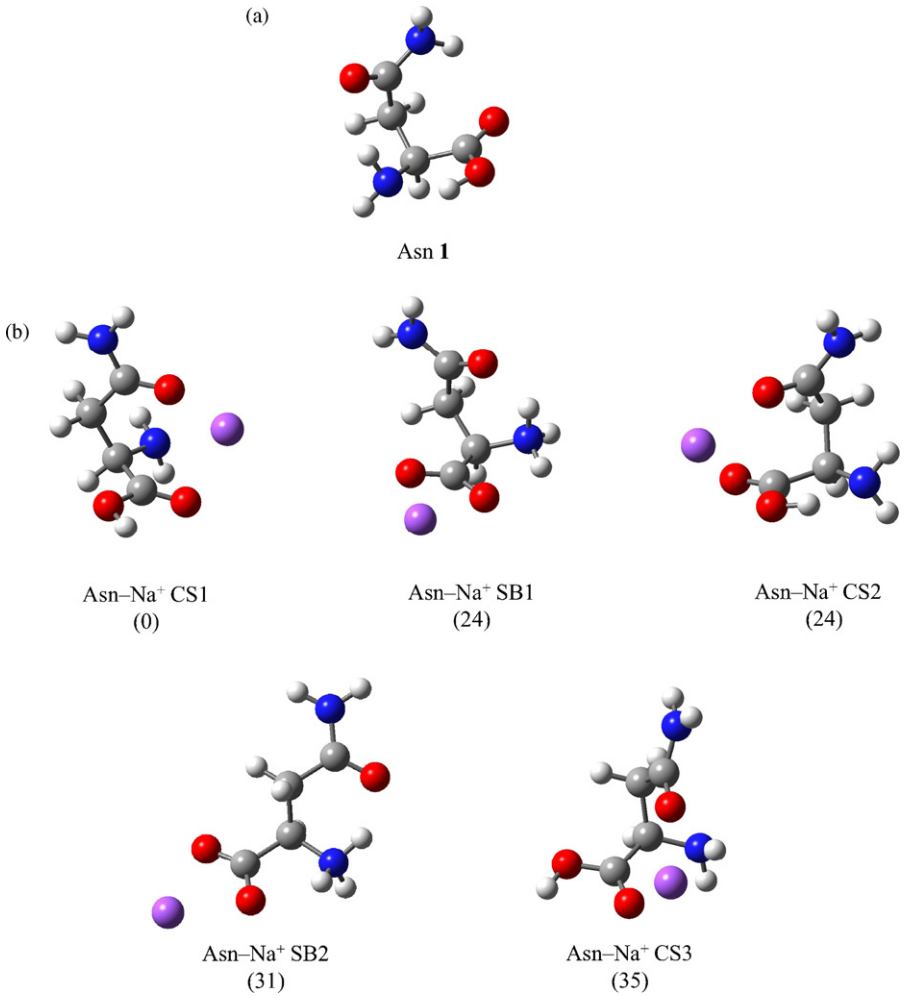

Fig. 7. Geometries of the lowest-energy conformers/isomers of (a) Asn and (b) the Asn–Na^+ complex with a charge solvation (CS) or a salt bridge (SB) structure. Relative energies in kJ/mol are given in parentheses.

For Arg–Na^+, the most stable structures have been reported by Williams and co-workers [11]. Our results follow their trends and therefore only the best SB and CS conformers are depicted in Fig. 8. The more stable between these two (by 10 kJ/mol) is the SB isomer in which the side chain is protonated, establishing two hydrogen bonds to the amino and carboxylate termini. The most stable CS isomer involves the –NH–C(=NH)(NH_2) tautomer of Arg. Sodium chelation is tridentate to the carbonyl oxygen, amino nitrogen and imino nitrogen of the side chain. Since the SB structure is 10 kJ/mol lower in energy, its formation should be favored experimentally. Assuming that sodium detachment from this salt bridge is accompanied by proton transfer from the guanidinium to the carboxylate group along the existing hydrogen bond, Arg–Na^+ bond scission leads to a structure of Arg that is not the most stable (**3** in Fig. 8). This process has a critical enthalpy of 256 kJ/mol at 298 K. If the most stable charge solvation structure is considered instead, its dissociation leads to the same tautomer of Arg and requires an enthalpy of 245 kJ/mol at 298 K. The value associated with the charge solvation isomer is somewhat closer to the experimental result of 242 kJ/mol, but calculations predict the salt bridge to be more stable. Given the small energy difference between these structures, our experimental results are compatible with either structure, or with a mixture of both.

3.5.4. Na^+ binding entropies

The sodium binding entropies reported in Table 1 take into account only the rotational, vibrational and translational components of the most stable conformer of each sodium complex and free amino acid. A more precise estimate would include conformational entropy that arises from the equilibrium between several structures for each species. Conformers lying within ca. 5 kJ/mol of the lowest one contribute significantly, and most amino acids have multiple conformers meeting this criterion. This is expected to be less severe for sodium ion complexes, in which ion chelation restrains flexibility. The conformational contribution to the sodium binding entropy of several amino acids at 298 K was evaluated by Gapeev and Dunbar [18]. These authors found small values, in the 10–15 J/mol K range, i.e., less than 10% of the dynamical entropy of the most stable conformation. Thus, this term was neglected in the present study which focuses on relative binding entropies, in which the small conformational entropy terms would largely cancel out.

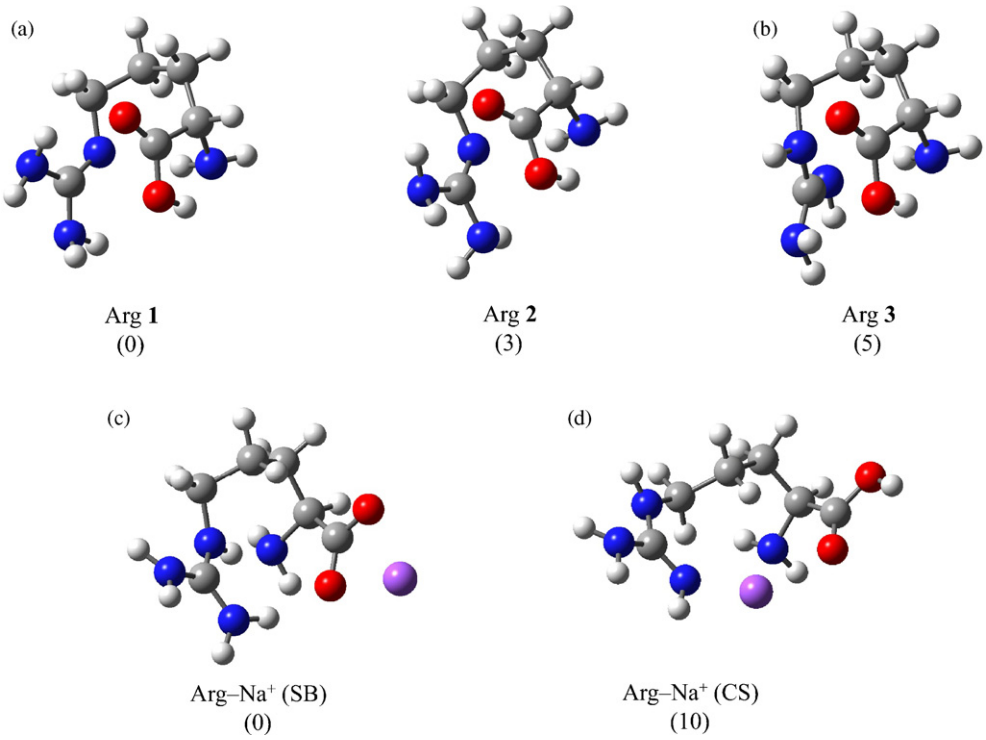

Fig. 8. Geometries of the lowest-energy conformers/isomers of Arg and the Arg–Na^+ complex: (a) the two most stable conformers of the ─N═C$(NH_2)_2$ tautomer of Arg. (b) the most stable conformer of the ─NH─C(═NH)(NH_2) tautomer of Arg; (c) the most stable Arg–Na^+ complex involving a salt bridge (SB) between Na^+ and the Arg zwitterion protonated at the guanidine group; and (d) the most stable among charge solvation (CS) isomers of Arg–Na^+, involving Na^+ and the NH─C(═NH)(NH_2) tautomer of Arg. Relative energies in kJ/mol are given in parentheses.

Our calculations indicate that the entropies of Na^+ complexation of amino acids and simple peptides are quite similar, except for Arg, for which ΔS_{Na} is smaller [17,35]. The more flexible side chain of Arg makes possible a direct interaction between the carboxylic acid and the side chain (impossible in Asn or His); this interaction is disrupted and new ones are established in the sodium ion complex. Thus, Arg is more ordered than the other amino acids and less reorganization occurs upon sodium ion binding, resulting in a smaller entropy change upon formation of the Arg–Na^+ bond. Still, the ΔS_{Na} values calculated in the present study vary by less than ~20% (Table 1). Hence, the relative Na^+ binding entropies of the amino acid/peptide ligands in the Na^+-bound heterodimers studied, $\Delta(\Delta S_{Na})$, are quite small and the corresponding apparent relative entropies, $\Delta(\Delta S_{Na}^{app})$, which range from 0 to $\Delta(\Delta S_{Na})$ (see Section 2), must be negligible, as assumed. That $\Delta(\Delta S_{Na}^{app})$ for formation of Pep_1–Na^+ vs. Pep_2–Na^+ is insignificant is attested by the very good agreement between one-step and cumulative $\ln(k_1/k_2)$ values for the Pep_1/Pep_2 pairs examined (cf. Fig. 2). When significant apparent relative entropies are present, the $\ln(k_1/k_2)$ ratio between a given pair has been shown to depend on the route connecting Pep_1 and Pep_2 [41,65], which is not true for the heterodimers included in Fig. 2.

4. Conclusions

We have used the kinetic method to evaluate the relative Na^+ affinities of the amino acids (AA) Asn, Gln, His and Arg, based on the dissociations of AA–Na^+–Pep heterodimers. The sodium ion affinity of tryptophan (210 kJ/mol) [17] served as an anchor to deduce the corresponding absolute affinities. High level ab initio theory has been used to predict the sodium ion affinities of Asn, His and Arg. Binding enthalpies were computed with the assumption that the dissociation of a sodium ion complex is accompanied by restructuration of the amino acid to its most stable conformer. For Arg–Na^+, we also assume that sodium detachment is accompanied by proton transfer along a hydrogen bond. An effective temperature of 354 ± 10 K for the dissociating complexes yields experimental Na^+ affinities that agree excellently with the theoretical predictions.

The experimental sodium ion affinities of Asn, Gln and His reported in this study (217, 222, and 228 kJ/mol, respectively) correct and thus supersede earlier underestimated values from our laboratory [17]. The calculations in this study predict that the value for His is 232 kJ/mol, which is in very good agreement with the corrected experimental result. This value corresponds to the dissociation of the His–Na^+ complex with the $N^{\varepsilon 2}$–H tautomer to the isolated $N^{\varepsilon 2}$–H tautomer of histidine. The sodium ion affinity of Arg has been presented for the first time; theory and experimental results agree very well (245 and 243 kJ/mol, respectively). While it is now firmly established that isolated Arg has a non-zwitterionic structure, our results indicate that its sodium complex has two degenerate structures, one salt bridge and one charge solvation isomer. It is likely that the composition of the structures formed depends upon the experimental conditions. Finally, our corrected experimental value for Asn

(217 kJ/mol) matches well the ab initio values of 223 kJ/mol (HF geometries) and 218 kJ/mol (MP2 geometries). This agreement gives confidence that our revised value for Gln is accurate as well.

The present results, combined with those of our previous investigations on amino acids [17] and peptides [23,35], lead to the first series of sodium ion affinities of 18 out of the 20 α-amino acids naturally occurring in peptides and proteins. This body of data should serve as a database to help unravel the mechanisms underlying the biochemical activity of sodium ions.

Acknowledgement

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