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Short communication

Calorimetric determination of enthalpy changes for proton ionization of *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethane sulfonic acid] (HEPES) and *N*-[2-hydroxyethyl]piperazine-*N*′-[2-hydroxypropane sulfonic acid] (HEPPSO) in water–methanol mixtures

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Abstract

Enthalpies for the two proton ionizations of the biochemical buffers *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethane sulfonic acid] (HEPES) and *N*-[2-hydroxyethyl]piperazine-*N*′-[2-hydroxypropane sulfonic acid] (HEPPSO) were obtained in water–methanol mixtures with methanol mole fraction (X_m) from 0 to 0.360. With increasing methanol, the ionization enthalpy for the first proton (ΔH_1) of HEPES increased steadily from 8.4 to 15.3 kJ mol^{-1} whereas that for HEPPSO rose to a maximum of 21.0 kJ mol^{-1} at $X_m = 0.123$ before dropping to 18.4 kJ mol^{-1} at $X_m = 0.360$. The ionization enthalpy for the second proton (ΔH_2) of HEPES varied from 20.8 kJ mol^{-1} in water to 13.6 kJ mol^{-1} at $X_m = 0.360$ with a maximum of 24.8 kJ mol^{-1} at $X_m = 0.194$. For HEPPSO, ΔH_2 increased steadily from 23.4 to 29.2 kJ mol^{-1}. The solvent composition was selected so as to include the region of maximum structure enhancement of water by methanol. The results were interpreted in terms of solvent–solvent and solvent–solute interactions.

Keywords: HEPES; HEPPSO; Proton ionization; Calorimetry

1. Introduction

A previous communication from this laboratory [1] dealt with the enthalpies of proton ionization in water–methanol solutions of two structurally related buffers: *N*,*N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES) and *N*-tris[hydroxymethyl]methyl-2-aminoethansulfonic acid (TES). This paper reports on two buffers containing the piperazine group: *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethane sulfonic acid] (HEPES) and *N*-[2-hydroxyethyl]piperazine-*N*′-[2-hydroxypropane sulfonic acid] (HEPPSO). All four are zwitterionic N-substituted amino sulfonic acid buffers used in biochemistry. Measurements were made in water–methanol mixtures containing up to 50% (w/w) methanol, corresponding to a methanol mole fraction (X_m) of 0.360. Most thermodynamic measurements on buffers in mixed solvents have determined free energies of ionization. ΔH values have mostly been obtained from potentiometric and spectrophotometric measurements [2–7].

2. Experimental

The procedure involving the operation and calibration of the Parr 1455 solution calorimeter has already been described [1] together with the method of data analysis. HEPES and HEPPSO were Sigma chemicals, dried before use. The initial solution temperature was ca. 21 °C. ΔT varied in the range 0–0.2 °C for the addition of HEPES and HEPPSO to the various solutions and was precise to ±0.002 °C. The ionic strength of all solutions was maintained at 0.300 M by addition of NaCl. The method followed was similar to that described by Ramette [8] for enthalpy determinations of the proton ionizations of glycine in water.

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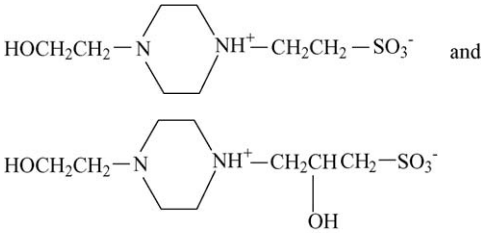
The starting materials in each case were the zwitterionic forms ($HB^{\pm}$) of HEPES and HEPPSO:



Enthalpy changes were measured for

$$HB^{\pm}(c) + H^{+} \rightarrow H_2B^{+}(soln.) \quad \Delta H_A \tag{1}$$

$$HB^{\pm}(c) \rightarrow HB^{\pm}(soln.) \quad \Delta H_B \tag{2}$$

and

$$HB^{\pm}(c) + OH^{-} \rightarrow B^{-}(soln.) + H_2O \quad \Delta H_C \tag{3}$$

The enthalpy change for the ionization of the first proton is given by

$$H_2B^{+}(soln.) \rightarrow H^{+} + HB^{\pm}(soln.) \quad \Delta H_1 = \Delta H_B - \Delta H_A \tag{4}$$

and ΔH_2 for ionization of the second proton is given by

$$\Delta H_2 = \Delta H_C - \Delta H_B - \Delta H_N \tag{5}$$

where ΔH_N is the enthalpy change for the neutralization reaction $H^{+} + OH^{-} \rightarrow H_2O$(soln.). Values for ΔH_N at each solvent composition and ionic strength of 0.300 M were reported in the earlier communication [1].

3. Results and discussion

Table 1 lists values of ΔH_A, ΔH_B, ΔH_C, ΔH_1 and ΔH_2 for HEPES and HEPPSO. These values indicate a general trend of increase in enthalpy with methanol addition. Such behavior is similar to that observed for BES and TES and is consistent with less favorable solvation of H_2B^{+}, $HB^{\pm}$ and B^{-} as the dielectric constant of the medium is lowered by methanol addition ($\varepsilon_{H_2O} = 78.3$ and $\varepsilon_{methanol} = 32.6$, at 25 °C).

ΔH_1 for HEPES is seen to increase from 8.4 to 15.3 kJ mol^{-1} as methanol increases. For HEPPSO, the change from 16.7 to 18.4 kJ mol^{-1} has a maximum of 21.0 kJ mol^{-1} at $X_m = 0.123$. These values contrast sharply with those previously reported for BES and TES [1], where ΔH_1 for both of those buffers varied between −2.1 and 1.0 kJ mol^{-1}. These results can be correlated with the acid dissociation constants for the first proton (pK_{a1}). Reported values for pK_{a1} in compounds that bear structural similarity to BES and TES are in the range 1.5–2.0. For example, pK_{a1} at 25 °C is 1.5 for 2-aminoethanesulfonic acid and 1.3 for DL-cysteic acid [9]. By contrast, pK_{a1} at 25 °C for HEPES is 3.0 [10]. No corresponding value has been reported for HEPPSO. The ΔH_1 dependence on methanol content is in accord with observations and compilations indicating that, within a given class of acids, higher pK entails increased dissociation enthalpies [11–13]. Notwithstanding the low value of pK_{a1}, reaction (1) can be considered essentially complete because of the large excess of HCl used (0.300 M HCl versus ∼0.02 M HEPES and HEPPSO).

Table 1 also shows that ΔH_2 for HEPES rises from 20.8 in water to 24.8 kJ mol^{-1} at $X_m = 0.194$, then drops to 13.6 kJ mol^{-1} at $X_m = 0.360$. For HEPPSO, ΔH_2 rises from 23.4 in water to 29.2 kJ mol^{-1} at $X_m = 0.194$ then remains essentially constant. The lower ΔH_2 values for HEPES, when compared to those for HEPPSO, can be attributed to the higher acid strength of HEPES and are in agreement with observations and compilations [11–13] indicating that ΔH for the ionization of the N-bound proton in protonated amines increases with increasing pK. Reported pK_2 values for HEPES at 25 °C in water are 7.24 ($I \sim 0.01$ M) [14], 7.285 ($I = 0.11$ M) [15] and 7.45 ($I = 0.1$ M) [16]. Those for HEPPSO are 7.90 ($I \sim 0.012$ M) [10,17], 7.99 ($I = 0.00520$ M) [18], 8.042 ($I = 0$) [19] and 7.79 ($I = 0.1$ M) [20]. The ΔH_2 value of 20.8 kJ mol^{-1} obtained for HEPES in water is

Table 1
ΔH_A, ΔH_B, ΔH_C, ΔH_1 and ΔH_2 for HEPES and HEPPSO in water–methanol mixtures

X_m	ΔH_A (±0.2 kJ mol^{-1})	ΔH_B (±0.2 kJ mol^{-1})	ΔH_C (±0.2 kJ mol^{-1})	ΔH_1 (±0.2 kJ mol^{-1})	ΔH_2 (±0.2 kJ mol^{-1})
HEPES					
0	−1.3	7.1	−29.8	8.4	20.8
0.059	0.6	12.1	−17.5	11.6	22.3
0.123	1.8	16.0	−9.4	14.2	21.9
0.194	2.7	17.6	−1.5	14.9	24.8
0.273	3.5	18.6	−3.0	15.1	19.7
0.360	4.8	20.1	−5.2	15.3	13.6
HEPPSO					
0	10.5	27.3	−7.0	16.7	23.4
0.059	12.7	31.7	4.2	19.0	24.4
0.123	14.2	35.2	13.6	21.0	25.6
0.194	15.6	35.5	21.3	20.0	29.6
0.273	16.5	37.3	24.7	20.8	28.8
0.360	17.8	36.2	26.4	18.4	29.2

in good agreement with reported values. These are, at 25 °C (in kJ mol^{-1}), 20.96 ($I \sim 0.1$ M) [21], 16.4 ($I \sim 0.01$ M) [14], 20.38 [22], 21.68 ($I = 0.11$ M) [15] and 21.01 ($I = 0.1$ M) [16]. Similarly, the ΔH_2 value of 23.4 kJ mol^{-1} obtained in this work for HEPPSO in water is very close to the reported value of 23.70 kJ mol^{-1} at 25 °C ($I = 0$) [19].

For HEPPSO, pK_2 at 25 °C ($I = 0.1$ M) is 7.79 in water and 7.72 at $X_m = 0.360$ [20], corresponding to a very small change (from 5.35 to 5.30 kJ mol^{-1}) in the standard free energy (ΔG°) for the ionization of the second proton. There are no similar reports on the pK_2 of HEPES. The pK behavior of HEPPSO is similar to that for proton ionizations of acids of the charge type $A^{\pm}B^{-}$ in mixed solvents where no change in the number of ions accompanies ionization [2]. Since both HEPPSO and HEPES are zwitterionic and belong to this charge type, the pK behavior of HEPES in water–methanol is expected to be similar to that of HEPPSO.

The relatively small free energy changes with methanol addition indicate that the enthalpy changes are largely entropic in origin. The maximum at $X_m = 0.194$ for ΔH_2 of HEPES (Table 1) suggests a high degree of solvation of the ionized proton at this composition. This proton could perhaps be considered to be at least partially solvated within the "ice-like" solvent structure [23]. As further methanol addition weakens this structure [24], less stringent entropy requirements could ensue. ΔH_2 for HEPPSO also attains its highest value at $X_m = 0.194$ but, in contrast to that for HEPES, remains essentially unchanged up to $X_m = 0.360$ (Table 1). This behavior suggests that the entropy requirements for the ionization of the second proton of this buffer extend over a wider solvent range. However, as was mentioned in the cases of BES and TES [1], ΔH behavior is much more difficult to interpret than that of the free energy, ΔG. Enthalpy changes arise from electrostatic contributions [25] as well as structural and steric factors that are often difficult to assess [24]. On the other hand, free energy changes can be understood in electrostatic and nonelectrostatic terms. The accumulation of proton ionization enthalpies in mixed solvents on structurally similar molecules could shed light on the role of each of those factors.

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