Supporting information

Calorimetric studies of interactions between low molecular weight salts and bovine serum albumin in water at *p*H values below and above the isoionic point

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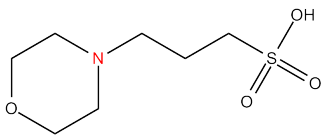
The buffer specific effect on the mixing enthalpy

The role of buffers is to fix solution's *p*H. But buffer ions also interact with the protein surface. Therefore different buffers at the same *p*H value can exhibit different interaction with the (charged) surfaces on the protein. This phenomena is known as buffer-specific effect (for review see Salias and Monduzzi[1]).

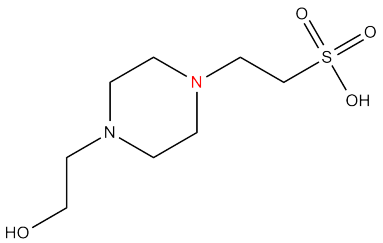
We compare here the enthalpy changes upon mixing aqueous buffer solution of BSA with aqueous buffer solution of NaCl or NaBr at 20 °C for two different buffers with *p*H = 7.35. For this purpose MOPS and HEPES buffers were used (see Figure 1). The first protonation constant ($pK_{a,1}$) for the sulfonic group ($-SO_3H$) is $pK_{a,1} < 3$, while the second constant ($pK_{a,2}$; protonation site marked with red) is 7.2 for MOPS and 7.5 for HEPES. The *p*H of both buffers used in our experiments was set to 7.35 by neutralization with NaOH solution.

MOPS:



3-morpholinopropane-1-sulfonic acid

HEPES:



2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid

Figure 1: Structural formulas and IUPAC names of MOPS and HEPES. The *p*H of both buffer solutions was set to 7.35 by adding NaOH. The second protonation site is marked in red; $pK_{a,2}$ = 7.2 and 7.5 for MOPS and HEPES, respectively.

[1] A. Salias, M. Monduzzi, Not only pH. Specific buffer effects in biological systems, Curr. Opin. Colloid Interface Sci. 23 (2016) 1–9.

Figure 2 shows the cumulative enthalpy changes, $\Delta_{mix}H$, upon addition of NaCl (filled symbols) or NaBr (empty symbols) to BSA solution for the case of MOPS (squares) and HEPES (triangles) buffer at the same nominal *p*H = 7.35 as a function of *r* (salt to protein molar ratio). Same concentrations of the protein and of the added salt were used as in the main paper, i.e. [BSA] = 0.3 mM and [salt] = 150 mM. Total buffer species concentration was 20 mM. ITC measurements were done at 20 °C.

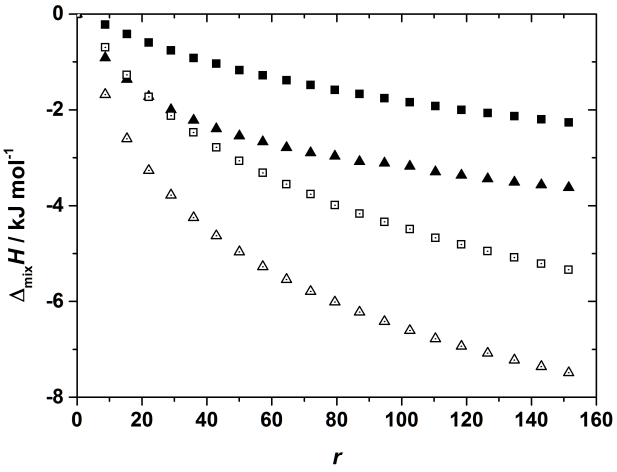


Figure 2: *BSA-salt mixing enthalpies are slightly buffer specific.* Shown are the cumulative enthalpy changes, $\Delta_{mix}H$, at 20 °C as a function of *r* for adding NaCl (filled symbols) and NaBr (empty symbols) solutions to BSA solution with *p*H = 7.35. Results are given for MOPS (squares) and HEPES buffers (triangles).

Heat effects upon addition of salt solution to BSA solution are somewhat more exothermic in HEPES buffer compared to MOPS buffer. The net charge of the BSA at *p*H = 7.35 is negative (*p*H > *p*I ≈ 4.7). At this value of *p*H more buffer molecules are in the zwitterion form in case of HEPES ($pK_{a,2}$ < *p*H) than in case of MOPS ($pK_{a,2}$ > *p*H). It is possible that the interaction of the buffer species with the protein and/or the hydration changes produce an additional exothermic effect.

The difference between heat effects for HEPES and MOPS is more expressed in the case of NaBr compared to NaCl. However, the ion specific effect is larger than the buffer specific effect and is the same in both buffers, i.e. the effects with respect to the added salt remains the same, namely NaCl < NaBr.