



A Thermodynamic Interaction of Nanosulfonamide with Human Serum Albumin

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ABSTRACT

Binding parameters of the N-phenyl benzene sulfonyl hydrazide, sulfonamide, and nanosulfonamide interaction with Human serum albumin were determined by calorimetry method. The obtained binding parameters indicated that sulfonamide in the second binding sites has higher affinity for binding than the first binding sites. The binding process of sulfonamide to HSA is both enthalpy and entropy driven. The association equilibrium constants confirm that sulfonamide binds to HSA with high affinity (2.2×10^6 and $3.86 \times 10^5 \text{ M}^{-1}$ for first and second set of binding sites, respectively). The obtained results indicate that sulfonamide increases the HSA anti-oxidant property. Nanosulfonamide has much more affinity for HSA ($3.6 \times 10^6 \text{ M}^{-1}$) than sulfonamide.

Key words: Isothermal Titration Calorimetry, Sulfonamide, Nanosulfonamide, Human serum albumin, Binding sites.

INTRODUCTION

Physicochemical properties of nanoparticles such as their small size, large surface area, surface charge and ability to make them potential delivery systems for effective treatments. The pharmacokinetic parameters of therapeutic drugs against the diseases show limitations in their efficacy. The poor bioavailability, side effects due to the high doses administered, long treatment and the emergence of drug resistant strains are the disadvantages of ordinary drugs. The advances that nanotechnology based drug delivery systems have made in improving the pharmacokinetics and efficacy of therapeutic drugs¹⁻⁴.

Sulfonamides were the first chemical substances systematically used to treat and prevent bacterial infections in humans. Sulfonamides are bacteriostatic drugs; they work by inhibiting the growth and multiplication of bacteria without killing them. Currently, their most common use in humans is treating urinary tract infections⁵⁻⁸. They are estimated to be 16-21% of annual antibiotic usage, making them the most important group of antibiotics consumed by humans⁹. Sulfonamides are compounds that contain sulfur in a SO_2NH_2 moiety directly attached to a benzene ring. The term "sulfa allergy" is often incorrectly applied to all adverse reactions that occur with sulfonamide-containing medications and not just to those due to

hypersensitivity mechanisms. Patients who experience side effects such as nausea and vomiting may interpret this as an allergy and subsequently report that they are allergic to sulfas⁷. The binding of the sulfonamides to serum albumins, an important factor of the pharmacokinetic of these drugs, has been extensively studied by several workers. Especially regarding the extent of binding. The stoichiometry, and the influence of the chemical structure on the binding. But only little information is available on the mechanism of the binding and on the nature of the sulfonamide-albumin complex. Some workers have shown a correlation between the partition coefficients of the sulfonamides and the extent of the binding and concluded that the binding is mainly hydrophobic¹¹. In this work, we compared the most comprehensive study on the interactions of sulfonamide and nanosulfonamide (N-phenyl benzene sulfonyl hydrazide) with HSA for further understanding of their effects of on the stability and the structural changes of the HSA molecules.

MATERIALS AND METHODS

Human Serum Albumin (HSA; MW=66411 gr/mol) and Tris buffer used were analytical grade with the highest purity available without any purification. Sulfonamide derivative (N-phenyl benzene sulfonyl hydrazide) was synthesized. The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system. Sulfonamide and nanosulfonamide solutions (1612.9 μM) were injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL HSA (60.22 μM). Injection of sulfonamide solution into the perfusion vessel was repeated 29 times, with 10 μL per injection. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of each injection was calculated by the "Thermometric Digitam 3" software program. The heat of dilution of the sulfonamide and nanosulfonamide solutions were measured as described above except HSA was excluded. The microcalorimeter was frequently calibrated electrically during the course of the study.

RESULTS AND DISCUSSION

We have shown previously that the heats of the ligand + HSA interactions in the aqueous solvent mixtures, can be calculated via the following equation⁹⁻¹⁴:

$$q = q_{\max} x'_B - \delta'_A (x'_A I_A + x'_B I_B) - (\delta'_A - \delta'_B) (x'_A I_A + x'_B I_B) x'_B \dots (1)$$

q are the heats of sulfonamide + HSA or nanosulfonamide+HSA interactions and q_{\max} represents the heat value upon saturation of all HSA. The parameters δ'_A and δ'_B are the indexes of HSA stability in the low and high sulfonamide concentrations respectively. Cooperative binding requires that the macromolecule has more than one binding site, since cooperativity results from the interactions between identical binding sites with the same ligand. If the binding of a ligand at one site increases the affinity for that ligand at another site, then the macromolecule exhibits positive cooperativity. Conversely, if the binding of a ligand at one site lowers the affinity for that ligand at another site, then the enzyme exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative. $p > 1$ or $p < 1$ indicate positive or negative cooperativity of a macromolecule for binding with a ligand, respectively; $p = 1$ indicates that the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \dots (2)$$

x'_B is the fraction of bound sulfonamide or nanosulfonamide to HSA, and x_B is the fraction of unbound sulfonamide or nanosulfonamide. We can express x_B fractions, as the sulfonamide concentrations divided by the maximum concentration of the sulfonamide or nanosulfonamide upon saturation of all HSA as follows:

$$x_B = \frac{[\text{sulfonamide}]}{[\text{sulfonamide}]_{\max}}, \quad x_A = 1 - x_B \dots (3)$$

Table 1: Binding parameters for HSA+sulfonamide interaction

The interaction is both enthalpy and entropy-driven but the electrostatic interactions are more important than hydrophobic forces. K_a values show that sulfonamide in the second class of binding sites has higher affinity for binding than the first class of binding sites. The positive values of δ_A^θ and δ_B^θ indicate that the anti-oxidant property of HSA increased as a result of its interaction with sulfonamide

| Parameters | First binding sites | Second binding sites |
|-------------------------------|---------------------------|----------------------------|
| p | 1 | 1 |
| g | 1 | 4 |
| $K_a / L.mol^{-1}$ | $2.2 \times 10^6 \pm 250$ | $3.86 \times 10^5 \pm 750$ |
| $\Delta H / kJmol^{-1}$ | -24.63 ± 0.08 | -12.45 ± 0.06 |
| $\Delta G / kJmol^{-1}$ | -30.57 ± 0.08 | -32.09 ± 0.11 |
| $\Delta S / kJmol^{-1}K^{-1}$ | 0.02 ± 0.003 | 0.06 ± 0.005 |
| δ_A^θ | 4.86 | |
| δ_B^θ | | 4.76 |

Table 2: Binding parameters for HSA+nanosulfonamide interactions. The interaction is both enthalpy-driven indicating that the electrostatic interactions are dominant. K_a values show that nanosulfonamide has high affinity for binding to HSA. The positive value of δ_A^θ indicates that the anti-oxidant property of HSA increased as a result of its interaction with nanosulfonamide. The negative δ_B^θ value proves that nanosulfonamide dampened the anti-oxidant property of HSA in the high concentration of nanosulfonamide

| Parameters | |
|-------------------------------|---------------------------|
| p | 1 |
| g | 1 |
| $K_a / L.mol^{-1}$ | $3.6 \times 10^6 \pm 650$ |
| $\Delta H / kJmol^{-1}$ | -36.43 ± 0.12 |
| $\Delta G / kJmol^{-1}$ | -37.63 ± 0.15 |
| $\Delta S / kJmol^{-1}K^{-1}$ | 0.004 ± 0.001 |
| δ_A^θ | 2.65 ± 0.06 |
| δ_B^θ | -38.14 ± 0.09 |

[sulfonamide] is the concentration of sulfonamide after every injection and [sulfonamide]_{max} is the maximum concentration of the sulfonamide upon saturation of all HSA. L_A and L_B are the relative contributions of unbound and bound sulfonamide in the heats of dilution in the absence of HSA and can be calculated from the heats of dilution of sulfonamide or nanosulfonamide in buffer, q_{dilut} , as follows:

$$\bar{L}_A = q_{dilut} + x_B \left(\frac{\partial q_{dilut}}{\partial x_B} \right), \quad \bar{L}_B = q_{dilut} + x_A \left(\frac{\partial q_{dilut}}{\partial x_B} \right) \quad \dots(4)$$

The heats of sulfonamide + HSA interactions, q , were fitted to Eq. 1 across the entire sulfonamide or nanosulfonamide compositions. In the fitting procedure, p was changed until the best agreement between the experimental and calculated data was approached (Figures 1 and 2). The high r^2 value (0.999) supports the method. The binding parameters for sulfonamide + HSA interactions recovered from Eq. 1 were listed in Tables 1 and 2. The agreement between the calculated and experimental results (Figures 1 and 2) gives considerable support to the use of Eq. 1.

$\delta_A^\#$ and $\delta_B^\#$ values for sulfonamide + HSA interactions are positive, indicating that in the low and high concentrations of the sulfonamide, the HSA structure is stabilized. These results suggest that the antioxidant property of HSA increased. $p=1$ indicates that the binding is non-cooperative.

For a set of identical and independent binding sites, a plot of $\left(\frac{\Delta q}{q_{max}}\right)[HSA]$ versus $\left(\frac{\Delta q}{q}\right)[sulfonamide]$ should be a linear plot by a slope of $1/g$ and the vertical-intercept of $\frac{K_d}{g}$, which g and K_d can be obtained¹⁵⁻¹⁹.

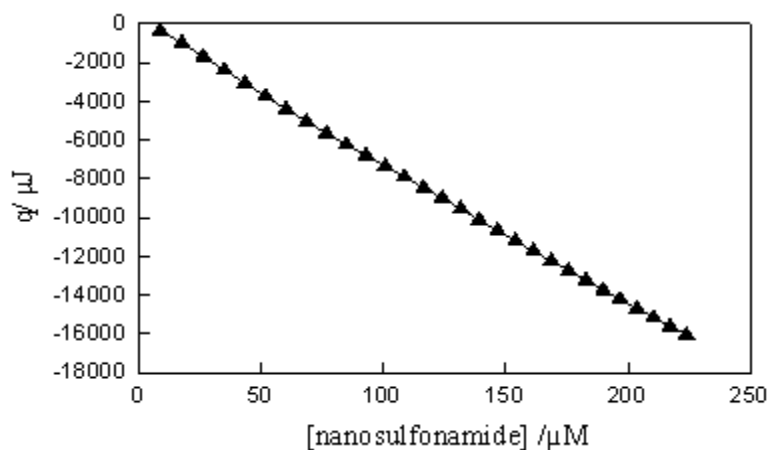


Fig. 1: Comparison between the experimental heats (▲) at 300 K, for (nanosulfonamide + HSA) interactions and the calculated data (lines) via Eq. 1

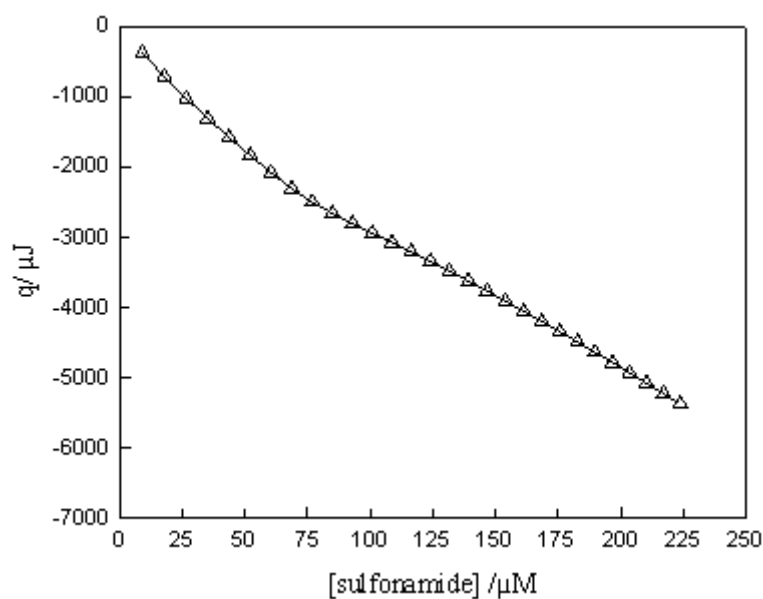


Fig. 2: Comparison between the experimental heats (△) at 300 K, for (sulfonamide + HSA) interactions and the calculated data (lines) via Eq. 1

$$\frac{\Delta q}{q_{\max}} [HSA] = \left(\frac{\Delta q}{q}\right) [sulfon] \frac{1}{g} - \frac{K_d}{g} \quad \dots(5)$$

Where g is the number of binding sites, K_d is the dissociation equilibrium constant, $[HSA]$ and $[sulfon]$ are the concentrations of HSA and sulfonamide or nanosulfonamide, respectively,

$\Delta q = q_{\max} - q$, q represents the heat value at a certain ligand concentration and q_{\max} represents the heat value upon saturation of all HSA. If q and q_{\max} are calculated per mole of biomacromolecule then the molar enthalpy of binding for each binding site (H) will be $H = q_{\max}/g$. The best linear plots with the correlation coefficient value of 0.999 were obtained using amounts of -2670 and -5400 μJ (equal to -24.63, -49.81 kJmol^{-1}) for q_{\max} in the first and second binding sites, respectively. Dividing the q_{\max} amounts of -24.63 kJmol^{-1} by $g=1$, and -49.81 kJmol^{-1} by $g=4$, therefore, gives $H = -24.63$ for the first binding sites, $H = -12.45$ kJmol^{-1} for the second binding sites.

To compare all thermodynamic parameters in metal binding process for HSA, the change in standard Gibbs free energy (ΔG°) should be calculated according to the equation (6), which its value can use in equation (7) for calculating the change in standard entropy (ΔS°) of binding process.

$$\Delta G^\circ = -RT \ln K_a \quad \dots(6)$$

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \quad \dots(7)$$

Where K_a is the association binding constant (the inverse of the dissociation binding constant, K_d). The K_a values are obtained

22.1 $\times 10^5 \pm 250$, 3.86 $\times 10^5 \pm 250$ M^{-1} for the first and second binding sites, respectively.

The results show that there are two sets of binding sites for sulfonamide. The interaction is both enthalpy and entropy driven but the electrostatic interactions are more important than hydrophobic forces. It was found that there is 1 site in the first class of binding sites and 4 sites in the second class of binding sites. K_a values show sulfonamide in the second binding sites has higher affinity for binding than the first binding sites.

Energy of binding ($H = -36.43 \text{kJmol}^{-1}$) for nanosulfonamide with HSA is more negative than that of sulfonamide. Therefore, the energetic interaction between nanosulfonamide and HSA has become more favorable. The affinity of nanosulfonamide is roughly twice of sulfonamide, therefore reduces the drug dosage frequency, treatment time and side effects. K_a values show that nanosulfonamide has higher affinity for binding with HSA than sulfonamide. The more effectiveness of nanosulfonamide can be attributed to its small size which, result in reducing drug toxicity, controlling time release of the drug and modification of drug pharmacokinetics and biological distribution. The positive δ'_i value (table 2) shows that nanosulfonamide (in around 30 μM of nanosulfonamide) stabilizes HSA structure and increases the anti-oxidant property of HSA. The negative δ'_i value indicates that nanosulfonamide dampened the anti-oxidant property of HSA in the high concentration domain (around 250 μM of nanosulfonamide).

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