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A Thermodynamic Study on the Binding of Human Serum Albumin with New synthesized Anticancer Pd(II) Complex

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ABSTRACT

The thermodynamics interaction between new synthesized anti-cancer drugcomplex, Pd_2Py_2, and HSA wasinvestigated at pH 7 by isothermal titration calorimetry. The extended solvation model was used to reproduce the enthalpies of HSA interaction withPd_2Py_2. The solvation parameters obtained from the new model was attributed to the structural change and anti-oxidant property of HSA. The binding parameters for the interaction of Pd_2Py_2 and HSA indicated that the considerable conformational changes in HSA were not observed after binding toPd_2Py_2.

Key words: Human serum albumin,Anti-cancer,Isothermal titration calorimetry, Binding parameters.

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in the systemic circulation, with HSA comprising 60% in plasma. This protein can play a dominant role on the drug disposition and the efficiency. HSA has a high affinity to an extraordinarily diverse range of materials, such as drugs, metabolites, fatty acids and metal ions[1-7]. HSA can bind and carry through the bloodstream many drugs, which are poorly soluble in water and it is responsible for the maintenance of blood pH, the drug disposition and efficacy, and the contribution of colloid osmotic blood pressure[8-9]. The unique feature of albumin is its ability to bind a wide variety of compounds, mainly because of the availability of hydrophobic pockets inside the protein network and the flexibility of the albumins to adapt its shape. The crystallographic analysis of HSA revealed that this protein is a single-chain 66 kDa protein, which is largely α-helical, and consists of three structurally homologous domains that assemble to form a heart-shaped molecule. Each domain is a product of two subdomains, which are predominantly helical and extensively cross-linked by several disulfide bridges[10-13].

The development of palladium anticancer drugs has not been promising and their design has mainly been based on the structure-activity relationship used for platinum anticancer drugs as well as good models for the analogous Pt(II) complexes in solution. Pd (II) complexes are expected to have lower kidney toxicity than cisplatin due to the inability of proteins in the kidney tubules to replace the tightly bound chelate ligands of Pd (II) with sulfydril groups[14-17]. Studies have shown that Pd (II) complexes are very effective in inhibiting proliferation in several tumor cell lines[18-21].

This work represents the most comprehensive study on the interactions between a new design anticancer drug of Pd_2Py_2 with carrier blood protein of HSA and provides new evidence for validity of the extended solvation model and more insights into the interactions of Pd_2Py_2 with HSA for further understanding of the effects of metal based drugs on the stability and the structural changes of carrier proteins.

MATERIALS AND METHOD

HSA was obtained from Sigma-Aldrich (Taiwan, China) and Pd_2Py_2 was synthesized in our laboratory[17]. Protein concentrations were determined from absorbance measurements at 277 nm in 1 cm quartz cuvettes. All other materials and reagents were of analytical grade, and solutions were made in 50 mM buffer phosphate using double-distilled water.

The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra sensitive titration calorimeter (MicroCal, LLC, Northampton, MA). The microcalorimeter consists of a reference cell and a sample cell of 1.8mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with HSA solution (60 μ M) and the reference cell contained buffer solution. The solution inthe cell was stirred at 307 rpm by the syringe (equipped with micro propeller)filled withPd_2Py_2solution (1.2mM) to ensure rapid mixing. Injections werestarted after baseline stability had been achieved. The titration of HSA withPd_2Py_2 solution involved 30 consecutive injections of Pd_2Py_2 solution, thefirst injection was 5 µL and the remaining ones were 10 µL. In all cases, each injection wasdone in 6 s at 3-min intervals. To correct the thermal effects due to Pd_2Py_2 dilution, control experiments were done in which identical aliquotswere injected into the buffer solution with the exception of HSA. In the ITC experiments, the enthalpy changes associated with processes occurring at a constant temperature are measured. The measurements were performed at a constant temperature of 27.0±0.02 ˚C and the temperature was controlled using a Poly-Science water bath.

RESULTS AND DISCUSSION

We have shown previously that the heats of the macromolecules + ligands interactions can be reproduced by Eq. 1 in the aqueous solvent systems[19-32].

$$q = q_{max} x'_B - \delta_A^{\theta}(x'_A L_A + x'_B L_B) - (\delta_B^{\theta} - \delta_A^{\theta})(x'_A L_A + x'_B L_B) x'_B \quad ...(1)$$

qis the heat of HSA + Pd_2Py_2interaction at certain ligand concentrations and q_{max} represents the heat value upon saturation of all HSA. The parameters and δ_B^{θ} exhibit the HSA + Pd_2Py_2 stability in the low and high Pd_2Py_2 concentrations respectively. The positive values of and show that HSA is stabilized by Pd_2Py_2.can be expressed as follows:

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

x'_B is a fraction of bound Pd_2Py_2 with HSA and $x'_A = 1 - x'_B$ is the fraction of unbound Pd_2Py_2. x_B δ_A^{θ}can be defined as follows:

$$x_B = \frac{[Pd_2Py_2]}{[Pd_2Py_2]_{max}} \quad ...(3)$$

$[Pd_2Py_2]$is the concentration of ligand after every injection and $[Pd_2Py_2]_{max}$ is the maximum concentration of Pd_2Py_2 upon saturation of all HSA.The heats of HSA+Pd_2Py_2 interactions, q, were fitted to Eq.1 over the whole Pd_2Py_2compositions. In the fitting procedure, *p* was changed until the best agreement between the experimental and calculated data was approached. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity (p>1). Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, exhibits negative cooperativity (p<1). If the ligand binds at each site independently, the binding is non-cooperative (p=1).L_A and L_B are the unbound and bound ligand contributions to the heats of dilution in the absence of HSA. L_A and $L_B$can be calculated from heats of dilution of Pd_2Py_2 in water,

q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B \left(\frac{\partial q_{dilut}}{\partial x_B} \right) \quad ,$$

$$L_B = q_{dilut} + x_A \left(\frac{\partial q_{dilut}}{\partial x_B} \right) \quad ...(4)$$

The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2values (0.99999) support the method (fig. 1). The binding parameters for HSA+Pd_2Py_2interactions recovered from Eq. 1 were listed in Table 2.

For a set of identical and independent binding sites, it is possibleto use Eq. (5) for calculation of K_d and ‘‘g” in a very simple way as follows:

$$\frac{\Delta q}{q_{max}} M_0 = (\frac{\Delta q}{q}) L_0 \frac{1}{g} - \frac{K_d}{g} \quad ...(5)$$

Where. q represents the heat value at a certain ligand and biomolecule concentration.M_0 and L_0 are total concentrations of HSA and ligand, respectively.q_{max} represents the heat value upon saturation of all HSAmolecule.K_d is the dissociation equilibrium constant for the equilibrium:

Table 1 heats of HSA+Pd_2Py_2interaction, *q*, at 300 K in 50mM phosphate buffer solution of pH=7, q_{dilut} is the heat of dilution of Pd_2Py_2 with water. The precision is ± 0.1μJor better

$[Pd_2Py_2]$ / μM	$[HSA]$ / μM	q / kJmol^{-1}	q_{dilut} / kJmol^{-1}
5.525	116.754	-26.28	2.56
10.989	116.113	-62.36	5.97
16.393	115.478	-96.68	9.62
21.739	114.851	-130.15	13.20
27.030	114.229	-163.10	18.78
32.258	113.616	-197.53	26.14
37.433	113.008	-229.05	32.32
42.553	112.407	-259.57	39.06
47.619	111.812	-288.92	45.14
52.6316	111.224	-314.35	50.44
57.592	110.642	-339.43	55.49
62.500	110.065	-361.91	59.96
67.357	109.495	-382.71	64.72
72.165	108.931	-404.70	69.81
76.923	108.372	-424.42	74.02
81.633	107.819	-444.16	78.65
86.294	107.272	-461.54	82.75
90.909	106.730	-479.87	87.51
95.4774	106.194	-497.17	91.92
100.000	105.663	-513.59	96.18
104.478	105.137	-528.85	100.30
108.911	104.616	-623.91	128.65

Table 2 binding parameters for HSA+Pd_2Py_2 interactionrecovered from Eq. 1at pH=7. The positive valueof δ^{θ}_{A} show that stabilizes the HSA structure in the low concentration of Pd_2Py_2 . The binding process for HSA+Pd_2Py_2 interactionisenthalpy-driven,indicating that electrostatic interaction plays an important role in the interaction of HSAwith Pd_2Py_2 . The positive value of δ^{θ}_{A} show that the anti-oxidant property of HSA was increased as a result of its interaction with

p	1.00±0.01
g	1.00±0.03
K_a / M^{-1}	1.79×105±2500
q / kJmol^{-1}	-14.50±0.03
ΔG / kJmol^{-1}	-30.18±0.07
ΔS / kJmol^{-1}K^{-1}	0.05±0.01
δ^{θ}_{A}	2.32±0.07
δ^{θ}_{B}	-0.011±0.002

$$M + L \Leftrightarrow ML \qquad K_d = \frac{[M][L]}{[ML]} \qquad ...(6)$$

If *q* and q_{max} are calculated per mole of biomacromolecule then the molar enthalpy of binding for each binding site (ΔH) will be:

$$\Delta H = \frac{q_{max}}{g}$$

The standard Gibbs free energy, ΔG°, can be calculated from association constant $(K_a = \frac{1}{K_d})$ as follows:

$$\Delta G^{\circ} = -RTLnK_a \qquad ...(7)$$

Where is the appearance association equilibrium constant as a function of Pd- anti-cancer concentration.Therefore for the first time, we managed to calculate and values with using one set of experimental data in one temperature.All thermodynamic parameters of ligand binding to HSA are summarized in Table2.

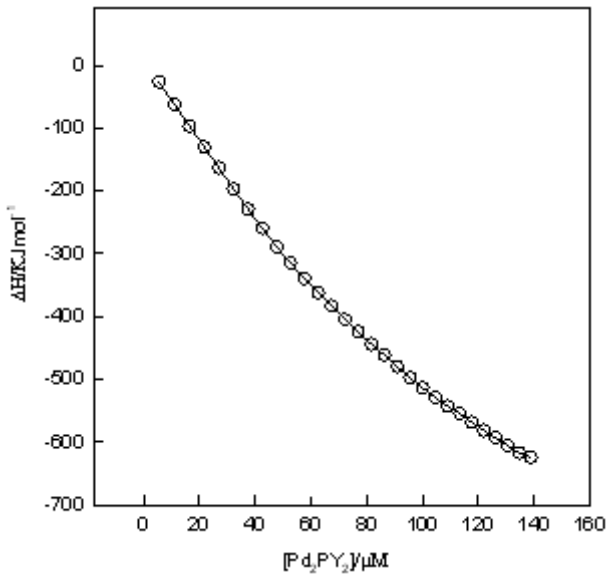

Fig. 1: Comparison between the experimental heats, *q*, (O), for HSA+ Pd_2Py_2 interactions and calculated data (lines) via Eq. 1.$[Pd_2Py_2]$ is concentration of Pd_2Py_2 in μM at pH=7

CONCLUSION

It has been confirmed that the extended coordination model, via equation 1 will satisfactorily reproduce the heats of HSA+Pd_2Py_2 interaction. Prediction of number of binding sites on HSA molecule, structural changes, determining the binding enthalpies and associated binding constants for such a complicated system accurately, make this theory the most powerful one. δ_A^θ and δ_B^θ values are positive and negative respectively (table 2), indicate that considerable conformational changes in HSA were observed due to the interaction with Pd_2Py_2.The negative value of shows that the HSAstructure is destabilizedin the high concentration ofPd_2Py_2.The negativevalueof molar enthalpy (-17889kJ mol^{-1} at 300 K) suggest thatthe binding process is only enthalpy-driven, indicating that electrostatic interaction is more important in the interaction.

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