RESEARCH COMMUNICATION

The novel behaviour of interactions between Ni^{2+} ion and human or bovine serum albumin

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We discovered a series of novel behaviours of interactions between Ni^{2+} ion and human or bovine serum albumin. Our results indicated that there exist two closely neighbouring identical prior binding sites in the binding of human or bovine serum albumin with Ni^{2+} ions, not only one. It is very likely that, after the binding of the first Ni^{2+} ion, an induced slow conformational transition happens, which leads to the binding of the second Ni^{2+}

ion and shows itself as a hysteretic effect for a process of non-enzymic protein binding with metal ions. As the concentrations of the 1:1 (molar ratio of Ni^{2+} ion to protein) system increase, an increasing hypochromic effect is observed. Such a hypochromic effect has not been reported previously; however, it is in accord with the mechanism of dipole–dipole interactions between the electric dipole transition moments of chromophores.

INTRODUCTION

After the discovery of Ni-enzymes, the importance in biochemistry of the element nickel has been further confirmed in recent years. Equilibrium dialysis and u.v. spectroscopy have been used to study the interactions between Ni^{2+} ion and human serum albumin (HSA) or bovine serum albumin (BSA), which were usually considered to be physiologically inactive, leading to the discovery of a series of novel behaviours in these two systems. Dixon and Sarkar [1] deduced that there is one prior binding site at N-terminal residues 1–3 in the binding of HSA or BSA with Ni^{2+} ions. We have now discovered that there are actually two closely neighbouring identical binding sites. It is very likely that, after the binding of the first Ni^{2+} ion, an induced slow conformational transition happened, which leads to the binding of the second Ni^{2+} ion. This kind of hysteretic effect [2] is typical for many regulatory enzymes, but has never been reported for a non-enzymic protein.

The molar ratio of Ni^{2+} ion to protein is 1:1. In these systems there actually exist only the metal centres with one or two Ni^{2+} ions. This kind of system shows an increasing hypochromic effect as the concentration increases, and when it reaches approx. $(2.5\text{--}3.0) \times 10^{-4}$ M, the LMCT (ligand to metal charge transition) bands of metal centres disappear almost completely. Such a hypochromic effect has not been reported previously; however, it is in accord with the mechanism of dipole–dipole interactions between the electric dipole transition moments of chromophores [3]. This fact would be reasonable if the two metal centres are close neighbours and possess the accurate relative orientation. Nevertheless, the implication of the novel behaviour described above still needs further clarification.

EXPERIMENTAL

HSA and BSA were purchased from Tianjin Blood Center and Huamei Biological Engineering Co., respectively. Both were of electrophoretic purity. In addition to moisture, contents (in p.p.m.) of impurities, including K^+ , Ca^{2+} , Mg^{2+} , Zn^{2+} , Cd^{2+} and Cu^{2+} ions, were respectively determined to be 2 (60), 240 (17), 18 (29), 8.9 (2.9), 1.7 (0.4) and 14 (15) for HSA (BSA). Traces of

iron, manganese and nickel were present. The total contents of all metal ions in both albumins were less than 0.03% (w/w). The content of fatty acids was determined to be 0.7% (0.4%) (w/w) for HSA (BSA). Both were dialysed in deionized water at 4 °C before use. The buffer Tris and NiCl_2 , NaCl, HCl, EDTA etc. were all of analytical purity. All solutions were prepared with deionized water. There was 0.1 M NaCl in the protein solutions, and 0.1 M Tris buffer was used to maintain their pH at 7.43. The concentration of NiCl_2 was titrated with EDTA. The concentrations of the proteins were calculated from the A_{278} of 0.531 and 0.667 for HSA and BSA respectively (1 mg/ml concentration, 1 cm light path). The molar ratio of Ni^{2+} ion to protein in all the samples is 1:1. The samples for studying the hysteretic effect were all freshly prepared, and the temperature was maintained thermostatically within ± 0.1 °C while recording the spectra. The samples for studying the hypochromic effect were all kept at 4 °C for 12 h after preparation; the spectra were then recorded at room temperature. The absorbance values, accurate to three decimal places, were read from the digital display every 1–2 nm in the absorption range of 230–270 nm. The spectra were recorded with, as references, the corresponding protein solutions of the same concentrations but not containing any Ni^{2+} ion. The spectrometer was a Shimadzu UV-240 spectrophotometer. The experimental arrangements for the equilibrium dialysis resemble that described in [4]. The equilibrium concentrations of Ni^{2+} ions ($[\text{Ni}^{2+}]$) were measured by the dimethylglyoxime spectrophotometric method.

RESULTS AND DISCUSSION

The slow conformational transition induced by Ni^{2+} ions

Samples at a protein concentration of 1.0×10^{-4} M were used, with the scanning range being 220–310 nm. The scans were made every 10–30 min. Figures 1(a) and 1(b) are the u.v. spectra of Ni(II)–HSA and Ni(II)–BSA respectively. The absorbance of the sample increases to approx. 6–7 times the initial value within about 3 h. The only reasonable explanation for this is as follows: upon binding Ni^{2+} ions, the serum albumins slowly changed

Abbreviations used: HSA, human serum albumin; BSA, bovine serum albumin; LMCT, ligand to metal charge transition.

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Figure 1 Time-scanning u.v. spectra: (a) Ni(II)-HSA (27 ± 0.1 °C); (b) Ni(II)-BSA (35 ± 0.1 °C)

Table 1 Kinetic parameters of slow conformational transition

Abbreviation: WGHK, wheat-germ hexokinase. —, Not determined.

Kinetic parameters	Ni(II)-HSA	Ni(II)-BSA	WGHK [9]
k (s^{-1})			
(298K)	—	2.77×10^{-4}	—
(300K)	4.01×10^{-4}	—	—
(303K)	5.24×10^{-4}	4.94×10^{-4}	—
(308K)	6.69×10^{-4}	6.67×10^{-4}	0.001
(313K)	8.22×10^{-4}	8.67×10^{-4}	—
E_a ($kJ \cdot mol^{-1}$)	42.2	57.9	41.0
$\Delta H^\ddagger$ ($kJ \cdot mol^{-1}$; 308K)	39.6	55.3	38.5
$\Delta S^\ddagger$ ($J \cdot degree^{-1} \cdot mol^{-1}$; 308K)	—188	—127	—175
$\Delta G^\ddagger$ ($kJ \cdot mol^{-1}$; 308K)	97.5	94.3	92.5

from the original conformational state of weaker affinity (T state) to the final one of stronger affinity (R state) [5], leading to a continual increase in the concentrations of the metal centres until an equilibrium value was reached. Since we scan the LMCT band [6], its absorbance should be in proportion to the concentrations of the metal centres at a given condition. It is known that both HSA and BSA form square-planar NiN_4 metal centres with Ni^{2+} ions at N-terminal residues 1–3 [1]. The maximum

Figure 2 Dependence of Hill coefficient, h , on $[Ni^{2+}]$: (a) HSA system; (b) BSA system

absorbance, A_e , of both systems is about 0.5–0.6 (see Figure 1), so the corresponding molar absorption coefficient for the 1.0×10^{-4} M system is about $10^4 M^{-1} \cdot cm^{-1}$. This is a typical value for the LMCT band of square-planar complexes of Ni^{2+} ions [7]. Through simple deduction, we obtain the following equation:

$$\ln(1 - A/A_e) = -kt \quad (1)$$

where A is the absorbance at time t , A_e is the maximum equilibrium absorbance of the system and k is the rate constant (assuming the conformational transition obeys the first-order reaction rule). We plotted $\ln(1 - A/A_e)$ versus t and obtained straight lines for both Ni(II)-HSA and Ni(II)-BSA at a fixed temperature, and obtained rate constants k , shown in Table 1.

After the rate constants k at various temperatures were obtained, the related activation parameters are easy to calculate [8]; see Table 1. For comparison, we also listed the conformational transition data of wheat-germ hexokinase LI [9]. The relevant data indicate sufficiently that we have observed a typical hysteretic effect for the process of a non-enzymic protein binding with metal ions.

Two strong-coupling Ni(II) centres

Dixon and Sarkar [1] deduced that there is one prior binding site at N-terminal residues 1–3 in the binding of HSA and BSA with Ni^{2+} ions. Our equilibrium-dialysis results indicated that there exist two identical prior binding sites, not only one. Figures 2(a) and 2(b) are the plots of the Hill coefficient, h , versus $\ln[Ni^{2+}]$ for Ni(II)-HSA and Ni(II)-BSA, respectively. The definition of h is:

$$h = \frac{d \log[Y/(1-Y)]}{d \log[Ni^{2+}]}$$

where $Y = \bar{n}/n$ ($n = 8$) is the saturation fraction, where $\bar{n}$ is the average binding number and n is the sum of the binding sites. From Adair fitting [4] of the experimental data, $n = 8$ was obtained. From Figure 2, both HSA and BSA systems reach a maximum value $h_{max} \approx 2$ at a certain $[Ni^{2+}]$ value. The h_{max} can efficiently measure the degree of binding co-operativity. It approximately equals the number of prior-binding identical sites [10]. Table 2 shows the binding constants of the first two steps obtained through fitting. It is notable that all k_2 values are slightly larger than k_1 . This indicates that the binding of Ni^{2+} ions has very strong positive co-operativity, or that the two prior-formed Ni(II) centres are strongly coupled. The calculation also shows that, in the 1:1 systems, there exist only two kinds of (metal-ions-containing) species, which actually contain one and two Ni^{2+} ions.

In explaining the hysteretic-effect data of wheat-germ hexokinase (see Table 1), Buc et al. [9] pointed out that there are

Table 2 First-two-step binding constants for Ni(II)–HSA and Ni(II)–BSA systems

Successive stability constants	$10^4 \times \text{Ni(II)–HSA protein concn. (M)}$				$10^4 \times \text{Ni(II)–BSA protein concn. (M)}$			
	1.0	2.0	2.5	4.0	1.0	2.0	3.0	4.0
k_1	4.04×10^4	3.54×10^4	3.34×10^4	3.31×10^4	1.74×10^5	1.04×10^5	1.74×10^5	1.04×10^5
k_2	4.49×10^4	3.72×10^4	3.39×10^4	3.35×10^4	3.30×10^5	2.40×10^5	9.27×10^4	8.27×10^4

Figure 3 U.v. spectra: (a) HSA system; (b) BSA system

Peaks 1, 2, 3, 4 correspond to the system at concentrations of 1.0×10^{-4} , 2.0×10^{-4} , 3.0×10^{-4} and 4.0×10^{-4} M, respectively. The inserts show enlargements of peak 4.

twofold meanings of the large negative activation entropy: (i) the conformational transition makes more domains in the proteins change from being hydrophobic to hydrophilic, and the solvation increases with it; (ii) the degree of freedom of the movement of the peptide chain in the transition state decreases. As for our systems, the conformational transition leads to the binding with more Ni^{2+} ions. It is probable that the binding of the first Ni^{2+} ion at N-terminal residues 1–3 induces a conformational transition, which makes the IA subdomain [11] of the protein undergo a type of ‘hinged movement’ and change from the relatively hydrophobic ‘valley’ to a more open and more hydrophilic one, leading to the formation of the second Ni(II) centre in this subdomain. The strong coupling of these two Ni(II) centres shows that they must be closely neighbouring in space.

A novel hypochromic effect

The equilibrium absorbance A_e of a given system is steady and repeatable. However, as the concentrations of the system increase,

the equilibrium absorbance first increases slightly, but then decreases rapidly. When the concentrations reach approx. $(2.5\text{--}3.0) \times 10^{-4}$ M and above, the LMCT bands disappear nearly completely (see Figures 3a and 3b). This change indicates that the molar absorption coefficient of the system very likely decreases continuously as the concentrations increase; namely there exists a kind of hypochromic effect. In research on DNA, the decrease in absorbance near 260 nm, resulting from the increase in the degree of order of nucleotides, is a typical hypochromic effect of the $\pi \rightarrow \pi^*$ transition. But the hypochromic effect for LMCT bands which we observed should be the first report of this phenomenon.

The theoretical treatments of the hypochromic effect are already very exhaustive. The equations from many works all resemble the following equation, which was based on the dipole–dipole interactions between the electric dipole transition moments and was first proposed by Tinoco [3]:

$$\frac{F_{oa}}{f_{oa}} = 1 - \frac{k\lambda_{oa}^2}{N} \sum_{i=1}^N \sum_{j \neq i}^N G_{ij} e_i e_j - \frac{4k\lambda_{oa}^2}{N} \sum_{a' \neq a}^N \sum_{i=1}^N \sum_{j \neq i}^N G_{ij} e_i e_j f_{oa'} \frac{\lambda_{oa'}^2}{\lambda_{oa}^2 - \lambda_{oa'}^2} \quad (2)$$

In the equation: oa and oa' are the observed absorption band and any other absorption band respectively; f and F are oscillator strength per monomeric band for the monomers and the polymers, respectively; N is the number of chromophores in the polymers; k is a constant; G_{ij} is a parameter that is in inverse proportion to the third power of r_{ij} ; r_{ij} is the distance between the centres of groups i and j ; e_i, e_j are the unit vectors in the direction of the respective transition moment in the related chromophores. As for our systems, because there exist only two chromophores, and every chromophore has only one allowable transition [6], we may use 1 and 2 to express these two transitions, respectively. The equation can be simplified as:

$$\epsilon_i = A_i - B_i \frac{\lambda_1^2 \lambda_2^2}{\lambda_1^2 - \lambda_2^2} (i = 1, 2) \quad (3)$$

Here we have considered that both f_1 and f_2 are constant values

Table 3 Gaussian resolution of experimental spectrum of Ni(II)–HSA and Ni(II)–BSA systems

C, total concentration.

$10^4 \times C_{\text{HSA}} \text{ (M)}$	λ_1	A_1	λ_2	A_2	$10^4 \times C_{\text{BSA}} \text{ (M)}$	λ_1	A_1	λ_2	A_2
1.0	246.8	0.094	252.1	0.106	1.0	246.6	0.209	254.1	0.256
1.2	248.1	0.047	251.0	0.063	1.2	247.5	0.176	252.9	0.312
1.5	248.3	0.232	254.5	0.404	1.5	249.0	0.286	254.5	0.282
1.8	249.2	0.209	254.1	0.325	1.8	249.8	0.130	254.2	0.107
2.0	249.7	0.251	255.0	0.242	2.0	249.7	0.057	253.4	0.069
2.2	250.3	0.158	254.8	0.122	2.2	250.3	0.041	254.5	0.026
2.5	250.0	0.060	254.2	0.065	2.5	250.1	0.017	255.3	0.013
3.0	251.3	0.012	255.4	0.009

Figure 4 Plot of ϵ_i versus $\lambda_1^2 \lambda_2^1 / (\lambda_1^2 - \lambda_2^2)$: (a) Ni(II)-HSA system; (b) Ni(II)-BSA system

Key: ○, band 1; ●, band 2.

of the order of magnitude of 1, and used the molar absorption coefficient ϵ_i instead of the oscillator strength F_i . A_i includes the factors relating to λ_i^2 , but, after we resolve the experimental band into two peaks of Gaussian type (see Table 3), we discover that the error in A_i resulting from the variation of λ_i is smaller than 5%, completely falling in the range of data error in this paper.

From Gaussian resolution of the experimental bands, the absorbances A_1 and A_2 of two Ni(II) centres can be obtained. From the concentrations of the system and the k_1 and k_2 value (Table 2), the concentrations of the species containing one and two Ni(II) centres can be calculated, and then their ϵ_1 and ϵ_2

values can be calculated. Plotting ϵ_i versus $(\lambda_1^2 \lambda_2^1) / (\lambda_1^2 - \lambda_2^2)$, we should obtain straight lines. Figures 4(a) and 4(b) are the results for Ni(II)-HSA and Ni(II)-BSA respectively. From the plots, this hypochromic effect also obeys the dipole-dipole interactions mechanism. This shows that the two Ni(II) centres not only are closely neighbouring in space, but also possess accurate relative orientation. From this arises an interesting question: would the structure consisting of the two metal centres, which is formed by Ni^{2+} ions (usually of catalytic activity) near the surface of the protein and possesses accurate relative orientation, be inert, or on the contrary would it show a certain kind of still undiscovered activity in biochemistry?

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