Biochemical Pharmacology, Vol. 35, No. 14, pp. 2407–2410, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

INFLUENCE OF TEMPERATURE ON THE BINDING OF SODIUM AUROTHIOSULPHATE TO HUMAN SERUM ALBUMIN

SUSANNE MØLLER PEDERSEN

Department of Clinical Chemistry, Odense University Hospital, Odense and Rheumatism Unit, Aarhus University, Aarhus, Denmark

(*Received* 25 *October* 1985; *Accepted* 20 *January* 1986)

Abstract—The influence of temperature on the binding of aurothiosulphate by human serum albumin was studied in unbuffered solutions at pH 7.4 and ionic strength 0.15 M by means of equilibrium dialysis. It was found that the high affinity association constant was temperature dependent. The thermodynamic characteristics of binding $\Delta G_1^\circ < 0$, $\Delta H_1^\circ > 0$ and $\Delta S_1^\circ > 0$ indicated that the binding process was endothermic and entropically driven. It was concluded that electrostatic interaction was predominantly involved in the binding of aurothiosulphate to the high affinity binding site on albumin. This is consistent with the molecular mechanism that the ligand binds as Au^+ to a sulfhydryl group of albumin by replacing a hydrogen ion.

Although gold compounds, such as aurothiosulphate and aurothiomalate, have been used successfully in the treatment of rheumatoid arthritis for more than 50 years, their mode of action is still unknown. *In vivo* and *in vitro* studies have shown that "gold" in serum is bound mainly to albumin [1–6], but only little quantitative information concerning the mechanism of this interaction is available [5,7,8]. It is, however, of fundamental pharmacological interest to get further information of the molecular nature of the binding process. Such information can be obtained by studying the influence of temperature on the gold–albumin interaction. This effect has been studied in only one prior work [5], where binding of aurothiomalate to bovine serum albumin was investigated in the temperature range 25°–37° at pH 7.45 in phosphate buffered medium. It was found that the association constants did not depend significantly on the temperature ($P < 0.025$).

In the present investigation the physicochemical factors responsible for the interaction between aurothiosulphate and albumin were studied by a thermodynamic analysis of the temperature dependence of aurothiosulphate binding in unbuffered human serum albumin solutions at ionic strength 0.15–0.16 M and pH 7.4. The interpretations of the resulting thermodynamic data are discussed in conjunction with the molecular mechanisms presented previously [7,8], and a binding theory for the interaction of aurothiosulphate with albumin is proposed.

MATERIALS AND METHODS

Materials. The albumin preparation was purified, lyophilized human albumin (Behringwerke AG, Marburg, F.R.G.). Polyacrylamide gradient gel (PAA 4/30, Pharmacia, Uppsala, Sweden) electrophoresis of a 0.5% albumin solution showed only one distinct band of monomer albumin and one very faint band due to dimer albumin. Crossed-immunoelectrophoresis performed against rabbit antihuman serum (DAKO, Copenhagen, Denmark) showed that no peaks attributable to proteins other than albumin were detectable [9]. The sodium aurothiosulphate, Sanocrysin®, was purchased from Ferrosan (Søborg, Denmark). The visking seamless cellophane tubing (8/32 in., Union Carbide, Chicago) was used for dialysis. All initial solutions of albumin, sodium aurothiosulphate (and blanks) were unbuffered solutions prepared in distilled, sterile water containing 0.15 M NaCl and with pH adjusted in order to obtain pH = 7.4 at equilibrium. During dialysis a decrease in pH (≈0.16) was observed.

Equilibrium dialysis. The binding of sodium aurothiosulphate to human albumin in unbuffered solutions at three different temperatures in the range 6°–37°, was studied in an equilibrium dialysis system previously described [6]. The ionic strength was 0.15–0.16 M, and pH 7.4 when measured at the equilibrium temperature. A second set of experiments were performed identically at 6° and 24° except for pH being 7.4 when measured at 37°. The range of concentration of total sodium aurothiosulphate in the initial solutions was 82–2.041 μM at 6° and 22°(24°) and 40–4082 μM at 37°.

After equilibrium was reached within 72 hr, the concentration of albumin was measured on the inside of the dialysis membrane. The pH and the concentrations of sodium and gold were measured on both sides of the membrane.

At the actual temperatures of these experiments, spectrophotometric and electroimmunoassay determinations of albumin showed that the albumin solution was stable during the time required for attaining equilibrium. No bacterial growth was observed after dialysis in any of the solutions.

Measurements. The pH measurements were performed with a Radiometer pH meter PHM 72 supplied with the electrode system BMS 2 MK2 blood Micro System. The instrument was calibrated to the recommended values of two standard phosphate

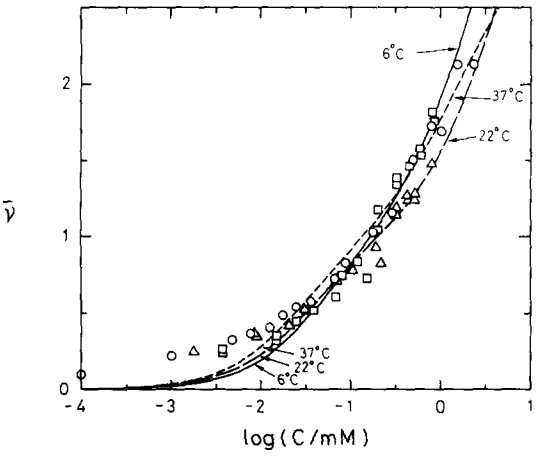

Fig. 1. The effect of temperature on the binding isotherms of aurothiosulphate to human serum albumin at ionic strength 0.15 M and pH 7.4. The curves represent the best fit to equation (1) using $n = 4$. The data points with $C < 10$ μM are not included in the analysis.

buffers at the respective temperatures. A quantitative electroimmunoassay technique according to the principle of Laurell [10] was used for albumin determinations. (Albumin standard: Standard-Human-Serum Behringwerke AG). The gold concentrations were determined with a flameless atomic absorption spectrophotometer (Beckman model 485 fitted with a Masmann Cuvette model 1268) [11]. The sodium concentrations were measured with a IL 343 Digital Flame Photometer.

RESULTS

Binding of aurothiosulphate to albumin at different temperatures

Figure 1 shows the experimental data for the binding of aurothiosulphate to human serum albumin at three different temperatures in the range 6°–37°. The ionic strength was 0.15–0.16 M and pH 7.4, the latter measured at the equilibrium temperature. The binding data are plotted as $\bar{\nu}$ versus log C, where $\bar{\nu}$ is the average number of gold atoms bound per albumin molecule, and C is the concentration of unbound gold at equilibrium. In the calculations a molecular weight of albumin is assumed to be 67,000 throughout.

The lines in Fig. 1 are the best fit to the equation

$$\nu = \sum_{i=1}^{n} \frac{K_i C}{1 + K_i C} \qquad (1)$$

using a non-linear least-square curve-fitting procedure. The summation is over all n sites of the albumin molecule and K_i is the association constant for site i. Equation (1) implies that there is no interaction between the sites. According to previous publications [6–8] $n = 4$ is assumed in the calculations at each temperature.

The apparent association constants calculated from the experimental data of Fig. 1 are displayed in Table 1. The binding data for small concentrations of C ($C < 0.01$ mmol/l) could not be fitted to equation (1) and were therefore not included in the analysis. However, it should be noted that inclusion of these binding data only affect K_1 which is increased by approximately 10–20%. It is conceivable that a better fit for low drug concentrations, i.e. $\bar{\nu} < 0.5$ can be obtained using other binding models which give a different behaviour in that region. This is under further investigation. Table 1 also displays the binding results obtained from the different set of experiments where, pH was kept constant when measured at 37°.

The Donnan distribution ratios for sodium ions (Na/Na') across the semipermeable membrane at equilibrium at 37°, 22° and 6° were 1.01, 1.01 and 1.00, respectively. The primed symbol indicates the side of the membrane free from macro-ions. No correction for the Donnan effect was therefore made as the concentration of added salt was sufficiently high to suppress the Donnan effect.

Thermodynamics of binding of aurothiosulphate by human serum albumin

The thermodynamic functions given here are based on the high affinity association constant K_1 as the measurements were not carried out to values of $\bar{\nu}$ large enough to determine the number of lower affinity binding sites and the corresponding constants with sufficient precision.

Table 1. The association constants for binding of sodium aurothiosulphate to human serum albumin at different temperatures*

T (K)	pH† Mean ± S.D.	K_1 ($M^{-1} \times 10^{-3}$)	K_2 ($M^{-1} \times 10^{-3}$)	K_3 ($M^{-1} \times 10^{-3}$	K_4 ($M^{-1} \times 10^{-3}$)	rms‡
279	7.38 ± 0.03	22.4	0.46	0.46	0.46	0.08
279	(7.38 ± 0.02)	21.7	0.57	0.57	0.05	0.07
295	7.40 ± 0.02	28.8	0.24	0.24	0.24	0.06
297	(7.34 ± 0.03)	30.6	0.36	0.36	0.20	0.05
310	7.37 ± 0.03	36.8	0.66	0.66	0.00	0.07

* For each value of T, assuming the number of binding sites, $n = 4$, the association constants were obtained by analysis of a complete binding isotherm consisting of 12–17 experimental points. Albumin concentration 0.54–0.58 mmol/l. Ionic strength 0.15–0.16 M.

† The pH measured at the equilibrium temperature. The numbers in parenthesis indicate that pH was measured at 37°.

‡ The S.D. of $\bar{\nu}$ on C from the best least-square fit to equation (1).

Table 2. The high affinity constants (K_1) and the corresponding thermodynamic data for the reaction of human serum albumin with aurothiosulphate

T (K)	K_1 ($M^{-1} \times 10^{-3}$)	$\Delta G_1°$	$\Delta H_1°$ (experimental)*	$\Delta H_1°$ (electrostatic)*	$\Delta S_1°$ (experimental)†	$\Delta S_1°$ (electrostatic)†
279	22.4	−23.235	11.36	8.13	0.124	0.112
295	28.8	−25.184		8.81	0.124	0.115
310	36.8	−27.097		9.48	0.124	0.118

* Expressed in kJ mol^{-1}.
† Expressed in kJ $mol^{-1} K^{-1}$.

The standard free energy change, $\Delta G°$, and the equilibrium constant K of a reversible reaction are related by the expression

$$\Delta G° = -RT\ln K \quad (2)$$

where R is the gas law constant and T is the absolute temperature. According to equation (2) the corresponding $\Delta G_1°$ values of the investigated aurothiosulphate–albumin interaction are calculated from the K_1 values of Table 1. These $\Delta G_1°$ values are given in Table 2.

The variation of K with temperature is given by the van't Hoff equation

$$\frac{d\ln K}{d(1/T)} = -\frac{\Delta H°}{R} \quad (3)$$

Thus, if $\ln K$ is plotted against $1/T$, the slope of the curve at any point is equal to $\Delta H°/R$ and the corresponding enthalpy value $\Delta H°$ can easily be estimated. To illustrate this treatment, data for variation with temperature of the thiosulphate–albumin equilibrium are plotted in Fig. 2. The curve is practically a straight line, indicating that $\Delta H_1°$ is constant for the reaction over the experimental temperature range. The $\Delta H_1°$ value calculated from the slope is displayed in Table 2. The positive value of $\Delta H_1°$ indicates that the reaction is endothermic.

From the free-energy of binding, $\Delta G°$, and the enthalpy, $\Delta H°$, one can also obtain the entropy, $\Delta S°$, of binding from the equation

$$\Delta G° = \Delta H° - T\Delta S° \quad (4)$$

The corresponding $\Delta S_1°$ values are displayed in Table 2.

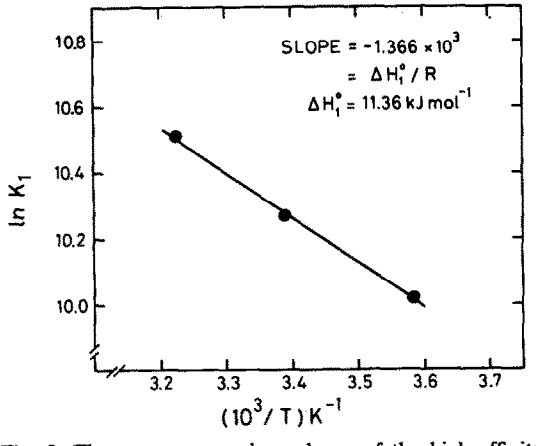

Fig. 2. The temperature dependence of the high affinity constant for aurothiosulphate and albumin. The calculated linear regression line has $r^2 = 0.998$ and yields the displayed value of $\Delta H_1°$ by van't Hoff equation (2).

DISCUSSION

In the present investigation the dependence of temperature on the binding of aurothiosulphate to human albumin was studied by means of equilibrium dialysis using unbuffered solutions. Table 1 shows that the high affinity association constant K_1 decreased with decreasing temperature. The temperature dependence of the lower affinity constants is difficult to interpret properly, since the values of K_2–K_4 could not be precisely determined as the calculated number of lower affinity binding sites varied. Therefore, only the high affinity constant and the corresponding thermodynamics for the reaction of albumin with aurothiosulphate will be discussed.

In a study of the binding of calcium to human serum albumin the apparent association constant was also found to be temperature dependent [12]. But when hydrogen ion competition for imidazole groups was taken into account the binding constant was found to be independent of temperature. A useful procedure for determining whether hydrogen ion competition for imidazole groups is present was applied [12]. That is a second set of binding experiments is performed at different temperatures but with identical pH when measured at 37°. The temperature-induced increase of pH in an albumin solution around physiological pH is mainly caused by increased hydrogen ion affinity of imidazole groups. Thus hydrogen ion competition for imidazole groups remains effectively temperature independent in this type of experiment, while it is temperature dependent in the experiments where pH was identical when measured at the actual temperatures. Consequently, if hydrogen ion competition to imidazole groups is important, the two types of experiments should yield different binding results. The binding results summarised in Table 1 show that this is not the case. This indicates that the binding of aurothiosulphate to the high affinity binding site of albumin involves a group which is not titrated in the investigated pH range. This group is most likely the sulfhydryl group of cysteine (34).

The thermodynamic parameters for the binding of aurothiosulphate by albumin assembled in Table 2

show that $\Delta H_1°$ is positive, i.e. the binding process is endothermic or heat absorbing. This is particularly common among chemical changes and is well known for example for the reaction between a free copper ion and albumin [13]. Furthermore, it is found that $\Delta S_1°$ is positive and that the major contribution to $\Delta G_1°$ arises from the $T\Delta S_1°$ term rather than from $\Delta H_1°$. Consequently the binding process is entropically driven.

There are essentially four types of non-covalent interactions that could play a role in ligand binding by proteins. These are hydrogen bonds, van der Waals forces, hydrophobic bonds and electrostatic interactions [14, 15]. The implications of the present results will be discussed in conjunction with their thermodynamic characteristics. Hydrogen bonding is accompanied by a negative value of $\Delta S°$ [14–16] and van der Waals interactions are accompanied by a large negative value of $\Delta H°$ [14] and both types of interactions are therefore inconsistent with the observed thermodynamic parameters. The present results are consistent with the thermodynamic characteristics of hydrophobic bonding [15, 16] ($\Delta G° < 0$ $\Delta H° > 0$ and $\Delta S° > 0$). But the non-apolar character of gold, Au^+, or gold complex, $AuS_2O_3^{-3}$, makes it unlikely that hydrophobic bonding is a significant factor in the binding process. It is more likely that electrostatic binding processes are involved. It has been shown both theoretically and experimentally that electrostatic binding processes are also entropically driven. Furthermore, if the formation of a ligand–protein complex is entirely of electrostatic origin then the following relations between ΔG_{elect}, ΔS_{elect} and ΔH_{elect} could be derived [14]

$$\Delta S_{elect} = -a\Delta G_{elect} \quad (5)$$

and

$$\Delta H_{elect} = \Delta G_{elect}(1 - aT) \quad (6)$$

where a is $0.00456\ K^{-1}$ for pure water. If the binding is entirely electrostatic then the entropy ΔS_{elect} and enthalpy ΔH_{elect} can be calculated by equations (5) and (6) and the experimentally determined value of $\Delta G°$. The resulting predicted values are displayed in Table 2. A comparison with the experimentally determined values shows that the major part of $\Delta S_1°$ and $\Delta H_1°$ can be accounted for by electrostatic interactions.

Moreover, the thermodynamic parameters obtained in the present investigation are essentially identical with those obtained for the interaction between bovine serum albumin and cupric ions [13, 14] which have no apolar character and thus no possibility of hydrophobic bonding. It is then concluded that the positive entropy is mainly a manifestation of electrostatic effects.

Measurements of the thermodynamic changes accompanying protein aggregation frequently show that the reactions are also accompanied by small enthalpy changes and large entropy changes. The possibility that aurothiosulphate induces albumin aggregation i.e. dimerization upon binding to albumin can therefore not be entirely excluded.

In the study of Mason [5] the temperature dependence of the association constants for the binding of *aurothiomalate* to bovine serum albumin could be variously interpreted. In the analysis the sum of the association constants, ΣK_n, and $n = 4$ was used. No distinction between high and lower affinity association constants was made. However, the same conclusion evolves that the binding process was entropically driven and that electrostatic bonding formed the basis of aurothiomalate albumin interaction.

It is concluded that the binding of aurothiosulphate to the high affinity binding site on human serum albumin appears to involve predominantly electrostatic bonding. In a recent study of the influence of ionic strength on aurothiosulphate albumin interaction [8] it is shown that the high affinity constant K_1 decreases by addition of NaCl to the aqueous medium. This support involvement of electrostatic interactions as electrolytes tend to weaken electrostatic forces by producing a stabilising Debye–Hückel atmosphere around the charge groups when they are in the dissociated form, whereas they strengthen hydrophobic bonds by decreasing the solubility in water of non-polar materials. A molecular mechanism consistent with the observed thermodynamics and in accordance with earlier suggestions [7, 8] might be that aurothiosulphate binds as gold ion to a sulfhydryl group of the albumin by replacing a hydrogen ion.

Acknowledgements—I wish to acknowledge Mrs. Inge Bihlet for her skilful technical assistance.

REFERENCES

1. B. R. Mascarenhas, J. L. Granda and R. H. Freyberg, *Arthritis Rheum.* **15**, 391 (1972).
2. R. J. van de Stadt and B. Abbo-Tilstra, *Ann. Rheum. Dis.* **39**, 31 (1980).
3. R. J. Ward, C. J. Danpure and D. A. Fyte, *Clin. chim. Acta* **81**, 87 (1977).
4. C. J. Danpure, *Biochem. Soc. Trans.* **4**, 161 (1976).
5. R. W. Mason, *Pharmacology* **15**, 536 (1977).
6. S. M. Pedersen, *Biochem. Pharmac.* **30**, 3249 (1981).
7. S. M. Pedersen, *Biochem. Pharmac.* **32**, 2485 (1983).
8. S. M. Pedersen, *Biochem. Pharmac.* **34**, 4319 (1985).
9. B. Weeke, *Scand. J. Immun. Suppl.* **1**, 47 (1973).
10. C.-B. Laurell, *Analyt. Biochem.* **15**, 45 (1966).
11. S. M. Pedersen and P. M. Graabæk, *Scand. J. clin. Lab. Invest.* **37**, 91 (1977).
12. K. O. Pedersen, *Scand. J. clin. Lab. Invest.* **30**, 89 (1972).
13. I. M. Koltz and H. A. Fiess, *J. phys. Chem.* **55**, 101 (1951).
14. I. M. Klotz, *Ann. N.Y. Acad. Sci.* **226**, 18 (1973).
15. S. N. Timasheff, in *Protides of the Biological Fluids* (Ed. H. Peeters), p. 511. Pergamon Press, Oxford (1972).
16. W. Kauzmann, *Advan. Protein chem.* **14**, 1 (1959).