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THERMODYNAMICS OF THE INTERACTION OF BENZODIAZEPINES WITH HUMAN SERUM ALBUMIN *

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SUMMARY

The binding of 9 structurally related benzodiazepine drugs to human serum albumin has been measured at pH 6 (N conformation of albumin) and pH 9 (B conformation) by equilibrium dialysis and microcalorimetry. From these experiments values for ΔG°, ΔH° and ΔS° are found. It appeared that ΔG° values are hardly influenced by the structure of either the protein or the ligand, in contrast to ΔH° values which showed a much greater variation.

INTRODUCTION

The thermodynamics of the binding of the benzodiazepine drug diazepam to human serum albumin (HSA) have been described and analysed previously (ref. 1). It was found that the conformation of the albumin has a strong influence on the enthalpy of binding ΔH°, whereas the corresponding free energy ΔG° was rather insensitive to changes in the protein structure.

In order to investigate this phenomenon in more detail a series of 9 benzodiazepines (including diazepam) has been studied. Because of the limited amount of material no complete pH profiles could be measured, but experiments were performed at pH 6, where albumin is in the N conformation, and at pH 9, corresponding with the B conformation of albumin.

From binding experiments ΔG° values are obtained, whereas ΔH° values are found from microcalorimetric experiments.

MATERIALS AND METHODS

The structures of the benzodiazepines used are listed in Table 1. These compounds were a gift from Hoffmann-La Roche, Mijdrecht, The Netherlands, except for oxazepam which was obtained from Wyeth Laboratories, Hoofddorp, The Netherlands. The compounds were used without further purification; [$^{14}C_2$]diazepam (54 mCi/mmol) was obtained from Amersham, Utrecht, The Netherlands. All other chemicals were of analytical grade.

Human serum albumin was isolated from human plasma as described before (ref. 1).

Microcalorimetric experiments were performed at 25°C, ionic strength $I = 0.16$ KCl, essentially as described before using the LKB 10700-1 flow system (ref. 1). Experiments were performed at pH 6 and pH 9. Corrections for release of protons were necessary at high pH only. The number of protons released for the compounds 1 to 9 in Table 1 are 0.27; 0.31; 0.23; 0.16; 0.0; 0.33; 0.28; 0.11 and 0.14, respectively.

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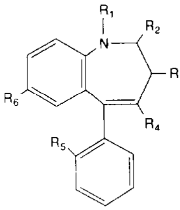
These numbers refer to the binding of one mole of ligand to one mole of HSA. Further details have been described (ref. 1).

Binding experiments using ^{14}C-diazepam were performed at pH 6 (phosphate buffer, I = 0.05, plus KCl to I = 0.16) and pH 9 (borate buffer, I = 0.05, plus KCl to I = 0.16) as described earlier (refs. 2-4). Binding constants of the other benzodiazepines were obtained from displacement studies, assuming that all compounds bind to the same site.

TABLE 1

Chemical formulae of 1,4 benzodiazepines



Nr.	Name	R_1	R_2	R_3	R_4	R_5	R_6
1	Diazepam	$-CH_3$	=O	-H	-	-H	-Cl
2	Desmethyldiazepam	-H	=O	-H	-	-H	-Cl
3	Oxazepam	-H	=O	-OH	-	-H	-Cl
4	Temazepam	$-CH_3$	=O	-OH	-	-H	-Cl
5	Medazepam	$-CH_3$	-	-H	-	-H	-Cl
6	Nitrazepam	-H	=O	-H	-	-H	$-NO_2$
7	Clonazepam	-H	=O	-H	-	-Cl	$-NO_2$
8	Chloordiazepoxide	-	$-NHCH_3$	-H	$\rightarrow O$	-H	-Cl
9	Demoxepam	-H	=O	-H	$\rightarrow O$	-H	-Cl

Errors in the free concentration determinations were estimated to be 5%, which results in an error in $\Delta G°$ of about 0.1 kJmol^{-1}. The estimated error in $\Delta H°$ amounts to 1 to 2 kJmol^{-1}. This results in an error in $\Delta S°$ of about 5 Jmol^{-1}K^{-1}. The relative large error in the number of protons released results in larger errors in $\Delta H°_B$.

RESULTS AND DISCUSSION

Analysis of $\Delta G°$ values

The results of the binding experiments expressed as $\Delta G°$ values, are reported in Table 2. The values found for diazepam correspond very well with those reported earlier, determined under somewhat different conditions (ref. 3). The global view is, that $\Delta G°_N$ and $\Delta G°_B$ values cover a similar range, viz. -26.8 to -29.2 kJmol^{-1} and -27.0 to -31.0 kJmol^{-1}, respectively. From this table it is evident that $\Delta G°$ values for the same compound are hardly influenced by the structure of the HSA, the largest effect being observed for compound 9. In this respect it should be noted that the structures of the benzodiazepines can be assumed to be pH-independent as they will have no pK_a values in this pH region (ref. 5).

TABLE 2

Values of thermodynamic parameters related to the binding of benzodiazepines to human serum albumin.

Nr.	$-\Delta G^\circ_N$	$-\Delta G^\circ_B$	$-\Delta H^\circ_N$	$-\Delta H^\circ_B$	ΔS°_N	ΔS°_B
1	29.2	29.2	27	37	7	-26
2	28.6	28.7	35	51	-21	-74
3	27.7	27.9	32	58	-16	-102
4	29.1	28.4	40	88	-38	-200
5	29.1	31.0	10	16	64	49
6	26.8	27.0	16	109	35	-276
7	26.6	27.8	27	98	-1	-235
8	28.8	28.6	16	23	42	18
9	26.8	28.3	11	32	52	-11

Nr. refers to the compounds in Table 1. Units of ΔG° and ΔH° are in $kJmol^{-1}$; units of ΔS° are in $Jmol^{-1}K^{-1}$; the errors in ΔG°, ΔH°_N and ΔS°_N have been discussed above; the error in ΔH°_B for the compounds 1 through 9 amounts to: 3, 4, 4, 5, 2, 6, 6, 2, 3; the corresponding errors in ΔS°_B are: 10, 13, 13, 17, 7, 20, 20, 7, 10.

Correlations between log K (where K is the binding constant) and lipophilic parameters are well known for a large variety of systems (ref. 6) because lipophilicity is assumed to be the major determinant for non-specific binding to proteins. We looked for such a correlation also in this case. As lipophilicity parameters we used log k' values, reported by Hulshoff and Perrin (ref. 7), obtained from HPLC experiments (Porasil, 3% oleylalcohol). The following relationships were obtained:

$$\log K_N = 0.53\ (\pm 0.16) \log k' + 4.01\ (\pm 0.27) \qquad (1)$$

$$n=7 \quad r=0.83 \quad s=0.064$$

$$\log K_B = 0.41\ (\pm 0.05) \log k' + 4.23\ (\pm 0.09) \qquad (2)$$

$$n=7 \quad r=0.96 \quad s=0.037$$

(Compounds 2 and 9 could not be included in these equations because no values for log k' were available). The ranges spanned by log K_N and log k' are 4.66 - 5.12, and 1.26 - 2.08 respectively, which is relatively small. Such a correlation, therefore, can only be observed using values with a sufficiently high accuracy. No significant relationships could be observed, using log P(octanol) values (ref. 8). The slope values given in eqns. (1) and (2) are characteristic for the process of non-specific binding (ref. 6).

A correlation has been reported between the degree of protein binding of benzodiazepines and the lipophilic nature (ref. 9). A comparison with our results is difficult because of the difference between percentage bound and log K.

The benzodiazepines studied here have also affinity for specific receptors. Affinity constants for these receptors have been reported (See e.g. ref. 10). These constants cover a much wider range and are much higher than the affinity towards HSA (log K values from 5.4

to 8). This indicates that other (or additional) forces must be involved in receptor binding, and that therefore the structure of binding sites on HSA and the receptor sites are not the same.

Analysis of ΔH° values

Table 2 also reports values of ΔH° for the interaction between the benzodiazepines studied, and HSA. A comparison with data from literature is difficult, as mostly different reaction conditions have been used (ref. 11).

ΔH° values at one pH value display a much larger variation than the corresponding ΔG° values. The question is which physical-chemical properties of the drug are responsible.

ΔH°_N and ΔH°_B values are clearly different, whereas the structural properties of the small molecules may be assumed to be the same. This demonstrates the influence of the protein structure on ΔH°. In general the data in Table 2 support the statement that an interaction mechanism having an important effect on enthalpy (or entropy) may exert merely a minor perturbation on free energy.

In principle, from the temperature dependence of ΔG°, the corresponding ΔH° values can be found. This implies that from eqn. (1) or eqn. (2) an expression for ΔH° can be obtained. Such an expression will also include the unknown temperature dependence of the constants in these equations and in addition, the temperature dependence of log k' (resulting in a kind of enthalpy of transfer). Anyhow, it will be clear that ΔH° values are determined by many more factors than ΔG°.

In Fig. 1 the values of ΔH°_N and ΔH°_B for this series have been plotted. Except for compounds 6 and 7, a linear relationship between ΔH°_B and ΔH°_N is observed. In our opinion there are two different ways to explain such a relationship. The first one is starting with eqn. (1), which can be written in a general form as follows:

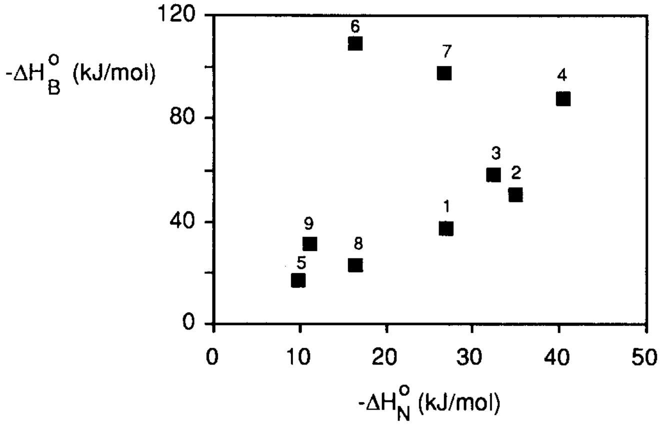


Fig. 1. Enthalpy of binding of benzodiazepines to human serum albumin in the N and in the B conformation.

$$\log K = c_1 \log P + c_2 \qquad (3)$$

where c_1, c_2 etc. are constants. Applying the well-known Van 't Hoff relationship ($d\ln K/d(1/T) = -\Delta H/R$) to eqn. (3), assuming that $dc_1/d(1/T)$ is constant (for which assumption no reasons are known), we arrive at:

$$\Delta H° = c_3 d\log P/d(1/T) + c_4 \log P + c_5 \qquad (4)$$

This equation once again shows that $\Delta H°$ is determined by more factors than $\Delta G°$; the term $d\log P/d(1/T)$ represents the enthalpy of transfer. If eqn. (4) applies to both $\Delta H°_N$ and $\Delta H°_B$, then a linear relationship might be expected between these two quantities.

The second approach is, by considering the binding of a ligand L to the N and B form in a cyclic process (see eqn. (5)):

$$\begin{tikzcd} L + N \arrow[r, "4"] \arrow[d, "1"] & LN \\ L + B \arrow[r, "2"] & LB \arrow[u, "3"] \end{tikzcd}$$

(5)

ΔH(N-B) in step 1 will be independent of the type of ligand; if the same holds for step 3, then ΔH_N and ΔH_B will be related in a linear way.

Enthalpy-entropy compensation

From the fact that $\Delta G°$ is nearly constant in this series it directly follows that enthalpy-entropy compensation will be observed. In Fig. 2 this plot is represented. This effect has been described for many systems (see e.g. ref. 12). General models have been presented that account for the existence of enthalpy and entropy changes in protein ligand interactions,

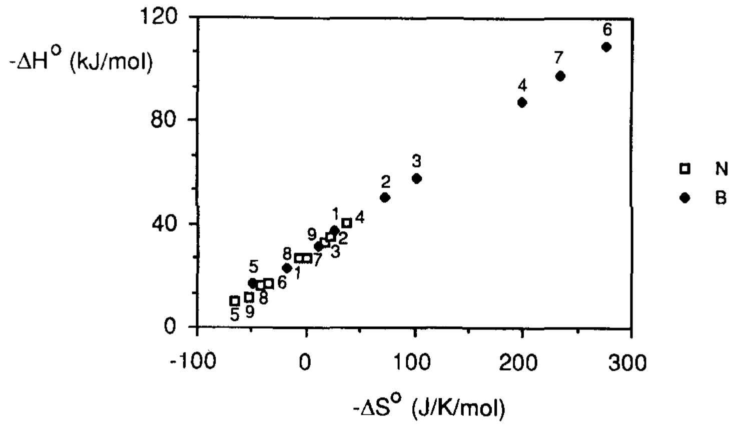


Fig. 2. Enthalpy-entropy compensation plot for the binding of benzodiazepines to human serum albumin in the N and in the B conformation.

based on the coupling between ligand binding and some type of transition in the state of the protein (ref. 13). The question arises what type of transition plays a role here. Experiments have been performed at pH 6 and pH 9, where no detectable conformational change occurs; however, this does not exclude minor local conformational changes. What is clear is that a different interaction occurs at pH 6 and 9, despite similarities in ΔG°. This is also evident from the pH dependence of the binding. At pH 6, no macroscopic pK change leading to a change in protons bound, could be observed. This contrasts the binding at pH 9, where protons are released, the amount depending on the structure of the ligand, which indicates that the interactions are not the same. This might be observed also in Fig. 2: measured points belonging to the N conformation are much more clustered than the corresponding points for the B conformation, the larger deviation being caused by the NO_2-containing derivatives 6 and 7.

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