ZYGMUNT WASYLEWSKI

PROTEIN-CATIONIC DETERGENT INTERACTION

EQUILIBRIUM DIALYSIS STUDY OF THE INTERACTION OF BOVINE SERUM ALBUMIN
AND OTHER PROTEINS WITH ALKYLpyRIDINiUM BRoMIDE

Institute of Molecular Biology, Department of Animal Biochemistry, Jagiellonian University,
Grodzka 33; 31-001 Kraków, Poland

The binding isotherms of native bovine serum albumin with cationic detergents, such as octyl, decyl, dodecyl and tetradecylpyridinium bromides were determined at pH 6.8 and 3.4 at 25°C. The isotherms for dodecyl and tetradecylpyridinium bromides were also determined at 3°C. The average number of detergent cations bound increased with increasing hydrocarbon chain length. At low detergent concentration the binding of all alkylpyridinium bromides was smaller at pH 3.4 than at pH 6.8. Dodecylpyridinium bromide was bound to native β-lactoglobulin, aldolase, ovalbumin, haemoglobin, myoglobin, lysozyme, trypsin and ribonuclease at pH 6.8. No binding occurred to α-chymotrypsin and chymotrypsinogen. The free enthalpy change, $\Delta G^\circ$, calculated from intrinsic association constants $K$ was determined.

The interaction of anionic detergents, especially sodium dodecylsulphate, with bovine serum albumin and other proteins has been studied extensively as a model for the interaction between amphipathic substances and proteins. The binding of cationic detergents to albumin and other proteins has received far less attention. Few et al. (1955) found that bovine albumin binds up to 109 dodecyltrimethylammonium cations and binding affinities are much lower than for anionic detergents. Nozaki et al. (1974) have shown that tetradecyltrimethylammonium chloride binds with albumin and other proteins.

This paper presents a more detailed investigation of the binding of cationic detergents with different hydrocarbon chain length such as alkylpyridinium bromides, to bovine serum albumin and several other proteins tested by means of equilibrium dialysis method. Three main aspects of the protein-detergent interaction were examined: I, binding of octyl, decyl, dodecyl and tetradecylpyridinium bromides to native bovine serum albumin at pH values above and below the isoelectric point
of the protein; II, influence of temperature on the binding of dodecyl and tetradecylylpyridinium bromides to albumin; III, binding of dodecylpyridinium bromide to several native proteins.

MATERIALS AND METHODS

The following reagents, obtained from the indicated sources, were used: bovine serum albumin, cryst., ribonuclease ex bovine pancreas, 4 x cryst., human haemoglobin, 2 x cryst., lysozyme, 2 x cryst., bovine β-lactoglobulin, 3 x cryst., horse myoglobin, cryst., and ovalbumin, 5 x cryst. (Koch-Light, Colnbrook, England); aldolase, cryst., and α-chymotrypsin, 3 x cryst. (Serva, Heidelberg, F.R.G.); chymotrypsinogen ex bovine pancreas, 6 x cryst. (Sigma Chem. Comp., U.S.A.).

The n-alkylypyridinium bromides were synthesized from ultra-pure alkyl bromides, purchased from Eastman-Kodak (Rochester, U.S.A.) and pyridine in alcohol solution according to the method of Czarnecki & Kowal (1968). The alkylypyridinium bromides were recrystallized six times from boiling acetone and stored in a desiccator over solid sodium hydroxide.

Equilibrium dialysis. Detergent binding measurements were carried out by the equilibrium dialysis method using a shake dialyser consisting of 32 units. The equilibrium dialysis was carried out in phosphate buffer of ionic strength 0.1 and pH 6.8 or in citrate-phosphate buffer of ionic strength 0.1 and pH 3.4. The Visking membrane was placed in boiling water for one hour and rinsed thoroughly with distilled water. One side of the dialysis unit was filled with 1 ml or 0.5 ml of 1% (w/v) or 0.5% (w/v) protein solution in an appropriate buffer. One or 0.5 ml of the detergent solution of a desired concentration was introduced into the other side of the dialysis unit. Dialysis was carried out in thermostated bath at 25 or 3°C with an accuracy of ±0.05°C. The number of detergent moles bound per mole of protein, \( \bar{f} \), was calculated by determining the concentrations of detergent in the outside protein solution before and after dialysis.

In order to determine the equilibrium the following experiments were performed: one side of the unit was filled with phosphate buffer and the other side with alkylypyridinium bromide solution of various concentrations. For the range of binding reported here, 48 h were sufficient to obtain an equilibrium state below CMC. Since the total concentration of alkylypyridinium bromides on both sides of the membrane always agreed with the initial concentration in the outside solution, no correction is required for adsorption of alkylypyridinium bromides on membrane.

The bovine serum albumin concentrations were determined from optical density at 279 nm; \( E_{1%}^{1} \) was assumed to be 6.67 (Decker & Foster, 1966). The concentration of other proteins was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

The concentration of alkylypyridinium bromides was measured spectrophotometrically at 257 nm, which is the characteristic band for pyridinium ring, over
a range of concentrations from \(2 \times 10^{-5}\) to \(10^{-4}\) mole per litre using calibration curves prepared for each detergent.

**Critical micelle concentration.** The CMC\(^1\) values of alkylpyridinium bromides were determined by the surface tension dropweight method using a stalagmometer at 20°C. The CMC values in phosphate buffer of ionic strength 0.1 and pH 6.8 of OPB, DePB, DPB and TPB were \(5 \times 10^{-3}\), \(2.8 \times 10^{-3}\), \(1.0 \times 10^{-2}\) and \(9.0 \times 10^{-4}\) mole/litre, respectively.

**RESULTS**

**Binding isotherms.** The binding of DPB and TPB in phosphate buffer, pH 6.8, and ionic strength 0.1 at 3°C with native albumin as a function of the detergent concentration is shown in Fig. 1. The slopes of the binding isotherms for DPB and TPB changed at points \(A\) and \(B\) at which the number of detergent moles bound per mole of protein, \(n\), were 5 and 10 for DPB and TPB, respectively. Deviation from linearity at higher \(n\) values suggests the existence of a second set of binding sites on the protein molecule. Equilibrium dialysis was also carried out at 25°C and at a more acidic pH than the isoelectric point of albumin. The binding isotherms for TPB at 25°C and pH 6.8 and pH 3.4 are shown in Fig. 2a. At each temperature and at low detergent concentrations, the slopes of the binding isotherms for TPB

---

\(^1\) Abbreviations used: OPB, octylpyridinium bromide; DePB, decylpyridinium bromide; DPB, dodecylpyridinium bromide; TPB, tetradecylpyridinium bromide; CMC, critical micelle concentration.
at pH 6.8 were similar and the differences in \( \bar{v} \) between isotherms at different temperatures were not large.

On the other hand, at pH 3.4 the net charge of albumin changed from negative to positive and marked differences in \( \bar{v} \) between isotherms at pH 6.8 and 3.4 became visible (Fig. 2a); the values of \( \bar{v} \) are smaller for pH 3.4 than for pH 6.8 at low detergent concentrations and the slopes of binding isotherms are different.

The binding isotherms for DPB at pH 6.8 and 3.4 at 25°C are shown in Fig. 2b. The slope of the binding isotherm at pH 6.8 changed slightly at point \( B' \) at which the number of detergent bound (moles/mole of protein), \( \bar{v} \), was 20 at a free detergent concentration of \( 1.8 \times 10^{-3} \) moles/litre. The binding of DPB to native albumin at pH 3.4 was smaller than at pH 6.8 at low detergent concentrations.

![Graph showing binding isotherms](image)

Fig. 2. Logarithmic plots of the binding isotherms for cationic detergents and bovine serum albumin at 25°C and (○), at pH 6.8, ionic strength 0.1; and (●), pH 3.4, ionic strength 0.1. Detergent: a, tetradecylpyridinium bromide; b, dodecylpyridinium bromide; c, decylpyridinium bromide; d, octylpyridinium bromide.

The binding of DePB and OPB at 25°C and pH 6.8 and 3.4 as a function of the equilibrium detergent concentration are shown in Figs. 2c and 2d. The difference in \( \bar{v} \) between isotherms at pH 6.8 for DePB and OPB was not large and the slope of isotherms slightly changed at points \( C \) and \( D \) at which \( \bar{v} = 13 \) and \( \bar{v} = 10 \) for DePB and OPB, respectively. For both detergents the binding at pH 3.4 was lower at low detergent concentrations than at pH 6.8.
The binding of DPB in phosphate buffer, pH 6.8 and ionic strength 0.1, at 25°C, to several other native proteins is shown in Figs. 3a and 3b. No binding of detergent to native α-chymotrypsin and chymotrypsinogen was observed at the detergent concentrations examined. The slopes of binding isotherms changed at points E, F, G, H and I for ovalbumin, myoglobin, aldolase, trypsin and lysozyme, respectively, indicating the existence of a second set of binding sites on protein molecules. On comparing the binding isotherms of DPB at 25°C for albumin (Fig. 2b) and for other proteins it can be seen that for the latter the binding is weaker than for albumin. Since it was shown that on gel filtration haemoglobin, aldolase and β-lactoglobulin dissociated into subunits in DPB solution (Wasilewski et al., 1979) the molecular weights of the subunits were used for calculation.

**Thermodynamic parameters.** If the binding sites on protein are equivalent and non-interacting, the binding of detergents to protein is known to follow the equation (Reynolds et al., 1967):

\[
\frac{1}{\tilde{v}} = \frac{1}{nK} \cdot \frac{1}{C_d} + \frac{1}{n}
\]

where \(\tilde{v}\) is the number of detergent moles bound per mole of protein, \(K\) is the intrinsic association constant of each site, \(n\) is number of binding sites in a set and \(C_d\) is the equilibrium concentration of the detergent. Plotting \(1/\tilde{v}\) against \(1/C_d\) enables to calculate the values of \(n\) and \(K\). Deviation from linearity at higher \(\tilde{v}\) values can be explained either in terms of a second set of sites or in terms of conformation changes in protein molecules.
The plot of $1/\nu$ against $1/C_d$ was linear for all alkylpyridinium bromides examined at pH 6.8 and albumin at low detergent concentrations; the plots for DPB and albumin at 3 and 25°C are presented in Fig. 4 as an example. The plots for alkylpyridinium bromides and albumin at pH 3.4 do not intercept the ordinate. The values of $n$ and $K$ for TPB, DPB, DePB and OPB are given in Table 1 together with the standard free enthalpy change calculated from the relation: $\Delta G^o = -RT \ln K$.

![Graph](image1.png)

Fig. 4. Reciprocal plots of the binding isotherms for dodecylpyridinium bromide and bovine serum albumin at pH 6.8, ionic strength 0.1, (○) at 3°C; (●) at 25°C.

![Graph](image2.png)

Fig. 5. Reciprocal plots of the binding isotherms for proteins showing the freely accessible binding sites for dodecylpyridinium bromide at pH 6.8, ionic strength 0.1 and 25°C. (▲) Trypsin; (△) lysozyme; (■) myoglobin; (□) aldolase; (○) ovalbumin. Haemoglobin (●) is presented for comparison.
Table 1

Association constants \( K \), free enthalpy changes \( -\Delta G^0 \), and number of binding sites \( n \), of bovine serum albumin for cationic detergents at pH 6.8, ionic strength 0.1

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Temp. (°C)</th>
<th>( K ) (l/mole)</th>
<th>( -\Delta G^0 ) (kcal/mole)</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octylpyridinium bromide</td>
<td>25</td>
<td>4.2 \times 10^2</td>
<td>3.6</td>
<td>10</td>
</tr>
<tr>
<td>Decylpyridinium bromide</td>
<td>25</td>
<td>3.3 \times 10^2</td>
<td>3.4</td>
<td>13</td>
</tr>
<tr>
<td>Dodecylpyridinium bromide</td>
<td>25</td>
<td>1.1 \times 10^3</td>
<td>4.1</td>
<td>20</td>
</tr>
<tr>
<td>Dodecyldimethylammonium bromide</td>
<td>3</td>
<td>5.2 \times 10^3</td>
<td>4.7</td>
<td>5</td>
</tr>
<tr>
<td>Tetradecylpyridinium bromide</td>
<td>25</td>
<td>2.5 \times 10^3</td>
<td>4.7</td>
<td>33</td>
</tr>
<tr>
<td>Tetradecyldimethylammonium bromide</td>
<td>3</td>
<td>1.0 \times 10^4</td>
<td>5.0</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2

Association constants, \( K \), free enthalpy changes, \( -\Delta G^0 \), and the number of binding sites, \( n \), showing the freely accessible binding sites for dodecylpyridinium bromide

Conditions: pH 6.8, ionic strength 0.1, 25°C; molecular weight of the proteins tested is given in parentheses.

<table>
<thead>
<tr>
<th>Protein</th>
<th>( K ) (l/mole)</th>
<th>( -\Delta G^0 ) (kcal/mole)</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>(40 000)*</td>
<td>2.4 \times 10^3</td>
<td>4.6</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>(17 500)</td>
<td>1.3 \times 10^3</td>
<td>4.2</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>(45 000)</td>
<td>8.0 \times 10^2</td>
<td>3.9</td>
</tr>
<tr>
<td>Trypsin</td>
<td>(23 300)</td>
<td>3.0 \times 10^2</td>
<td>3.1</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>(14 400)</td>
<td>2.7 \times 10^3</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Mol. wt. of aldolase subunits was used for calculation because in DPB solution this enzyme dissociates into subunits (Wasylewski et al., 1979).

Plots of \( 1/\theta \) against \( 1/C_2 \) for binding of other proteins with DPB at 25°C and pH 6.8 are presented in Fig. 5. The plots for ovalbumin, aldolase, myoglobin, lysozyme and trypsin intercept the ordinate and give values \( n \) and \( K \). These values and free enthalpy change for that freely accessible set are presented in Table 2.

DISCUSSION

The binding of anionic detergents to native serum albumin is known to be anion specific (Helenius & Simons, 1975). The detergents which contain anionic groups can interact both with hydrophobic areas and positively charged residues of proteins (Swaney & Klotz, 1970). Most of the binding energy is contributed by non-polar interactions (Reynolds et al., 1967).

Nozaki et al. (1974) found that native bovine serum albumin has four highest affinity binding sites for tetradecyltrimethylammonium chloride at pH 5.6, with an association constant \( 1.5 \times 10^4 \) moles per litre.

The data presented here indicate that bovine albumin has a larger number of binding sites for alkylpyridinium bromides at 25°C and pH 6.8 than for alkysul-
phates and tetradeacyltrimethylammonium chloride of a corresponding hydrocarbon chain length and lower binding constants. The values of $a$ obtained for alkylpyridinium bromides at a given temperature increase with increasing hydrocarbon chain length. The correlation between binding and hydrocarbon chain length of alkylpyridinium bromides suggests that the binding energy is derived from hydrophobic interaction. On the other hand, alkylpyridinium binding to albumin at pH values lower than its isoelectric point indicate that, at low detergent concentration, the binding is lower than at pH 6.8 which is probably due to repulsion between the cationic head-groups of detergent molecules and positively charged protein molecules. Increase of detergent concentration up to CMC values at pH 3.4 probably causes an increase of hydrophobic interaction between detergent and protein, and binding is comparable to that at pH 6.8.

If the interactions of alkylpyridinium bromides with albumin were only hydrophobic in nature, then the free enthalpy change, $-\Delta G^\circ$, calculated per one methylene group, should be comparable with the change in cohesive energy occurring when one methylene group is transferred from aqueous phase to the hydrophobic environment. The values of 1.0 - 1.2 $kT$ were calculated for the transfer of one methylene group during micellization of various detergents (Shinoda et al., 1963). As can be seen in Table 1, the values of $-\Delta G^\circ$ at a constant temperature are similar for OPB and DePB and differ from those for alkylpyridinium bromides with longer hydrocarbon chain length. The difference in the free enthalpy change, $-\Delta G^\circ$, between decyl, dodecyl and tetradeacylpyridinium bromide at 25°C is 0.6 - 0.7 kcal mol$^{-1}$, corresponding to about 0.5 $kT$ for 25°C calculated per one methylene group of the detergent tested. This result may be compared with the value of 1.2 $kT$ which has been found for non-ionic alkylglucosides (Wasylewski & Kozik, 1979). The significantly lower value of $-\Delta G^\circ$ calculated per one methylene group for cationic detergents obtained in this study suggests that detergent cations are bound at the areas of the protein molecule exhibiting lower hydrophobic affinity than those interacting with anionic and non-ionic detergents.

The binding isotherms at 3°C and the thermodynamic parameters (Fig. 1 and Table 1) indicate that albumin has only 5 and 10 freely accessible binding sites for DPB and TPB, respectively, and their affinities are higher than at 25°C. The association constant for TPB is very close to the value of $1.5 \times 10^4$ for tetradeacyltrimethylammonium chloride and albumin (Nozaki et al., 1974).

Association to freely accessible binding sites were also observed for ovalbumin, myoglobin, aldolase, trypsin and lysozyme (Figs. 3 and 5 and Table 2). The values of the association constants for aldolase and myoglobin are higher than for albumin. Both proteins have no disulphide bonds which can restrict the binding of detergent. This suggestion is in agreement with the observation of Nozaki et al. (1974) who found that reduction of disulphide bonds is required to raise the availability of hydrophobic residues and obtain maximum binding.

I am indebted to Professor Dr. Maria Sarnecka-Keller for helpful discussion.
REFERENCES


Stechiometry and mechanism of binding of alkylbenzenesulfonates. Biochemistry, 5, 1242 - 1254.


ODDZIAŁYWANIA BIALKA-DETERGENTY KATIONOWE

BADANIE ODDZIAŁYWAN BROMKÓW ALKILÓPIRYDYNOWYCH Z ALBUMINĄ SUROWICY WOŁU ORAZ INNYMI BIAŁKAMI PRZY POMOCY DIALIZY RÓWNOWAGOWEJ

Streszczenie

Wyznaczono izotermę wiązania bromków oktylo, decylo, dodecylo i tetradecylorydynowych z natywą albuminą surowicy wołu przy pH 6,8 i pH 3,4 oraz temp. 25°C. Wyznaczono także izotermę wiązania bromków dodecylo i tetradecylorydynowych w temp. 3°C i pH 6,8. Ilość moli detergenu związanego z albuminą surowicy wzrasta ze wzrostem długości łańcucha węglowodorowego detergenu. Przy niskich stężeniach badanych detergentów wiązanie bromków alkilópydynowych jest niższe przy pH 3,4 aniżeli przy pH 6,8. Wyznaczono izotermę wiązania bromku dodecylopydynowego w roztworze o pH 6,8 i temp. 25°C, z natywą β-laktoglobuliną, aldolazą, owalbuminą, hemoglobliną, miglobliną, lizozymem, trypsyną i rybonukleazą. Nie stwierdzono wiązania bromku dodecylopydynowego do natywnej α-chymotrypsyny i chymotrypsynogenu. Wyznaczono stałe równowagi wiązania oraz zmiany swobodnej entalpii wiązania, ΔG°.

Received 3 August, 1978.