Thermochimica Acta, 146 (1989) 215–223
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THERMODYNAMICS OF THE INTERACTION BETWEEN *n*-DODECYLTRIMETHYLAMMONIUM BROMIDE AND CATALASES

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(Received 11 November 1988)

ABSTRACT

The thermodynamic parameters for the interaction of the cationic detergent *n*-dodecyltrimethylammonium bromide (DTAB) with bovine liver catalase at pH 3.2, 6.4 and 10.0 and with *Aspergillus niger* catalase at pH 10.0 have been measured at 25 °C by a combination of equilibrium dialysis to determine the Gibbs energies of detergent binding and microcalorimetry to determine the enthalpies of interaction. The binding data have been analysed in terms of two theoretical models (the Hill equation and the Wyman binding potential concept), the calorimetric data show that DTAB interacts either athermally or endothermically with the catalases. The results have been compared with similar data for the interaction of the anionic detergent sodium *n*-dodecylsulphate (SDS) with catalases. For both detergents the interactions are dominated by the increase in entropy on detergent binding. For SDS the binding entropy is larger in acid solution when the detergent and protein are of opposite charge whereas for DTAB the binding entropy is larger in alkaline solution.

INTRODUCTION

Thermodynamic studies on the interaction between anionic detergents such as sodium *n*-dodecylsulphate (SDS) and catalases have revealed a number of species differences, particularly with regard to the enthalpies of interaction [1–3]. Specifically, near neutrality (pH 6.4), the enzyme from bovine liver interacts exothermically with SDS (10 mM) with an enthalpy of -18 J g^{-1} (-4416 kJ mol^{-1}) [4] whereas the fungal enzyme from *Aspergillus niger* has an enthalpy of interaction of -4 J g^{-1} (-1416 kJ mol^{-1}) [3] and the bacterial enzyme from *Micrococcus luteus* interacts athermally [1]. It

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0040-6031/89/$03.50

is interesting that these enzymes also differ with regard to their enzymic activities in the presence of SDS in that the bacterial enzyme retains activity, the bovine enzyme is denatured [1] and the fungal enzyme can be activated [2]. It is well established that the interaction between anionic detergents and proteins involves initial binding of the detergent molecules to cationic amino acid residues on the surface of the protein followed by more extensive hydrophobic binding as the critical micelle concentration (cmc) of the detergent is approached [5]. At a given pH the initial binding stage is influenced by the state of surface charge of the protein. Chemical blocking of cationic amino acid residues [6] or reducing the extent of the inhibitory effect of anionic amino acid residues [7] by chemical modification can markedly change both the affinity of an anionic detergent for binding sites and the associated enthalpy of interaction. It is also noteworthy that the enthalpy of interaction of anionic detergents is dependent on the alkyl chain length [6,8] so that the overall interaction involves both the detergent head group binding to a cationic site and the alkyl chain binding to an adjacent hydrophobic area on the protein surface.

Studies of catalase–detergent interaction have been confined to anionic detergents. The data reported here are concerned with the thermodynamics of the interaction between catalases from two sources (bovine liver and *Aspergillus niger*) and the cationic detergent *n*-dodecyltrimethylammonium bromide (DTAB) in which the enthalpies of interaction have been measured by microcalorimetry and the Gibbs energies of binding by equilibrium dialysis.

EXPERIMENTAL

Materials

Bovine liver catalase was obtained as a suspension in water containing 0.1% thymol from Sigma Chemical Co. Ltd. (product no. C100). Before use it was exhaustively dialysed against the required buffer. *Aspergillus niger* catalase was from Sobenco Ltd. (Haverfordwest, Dyfed, S. Wales), a kind gift from Dr. J.H.F. Biffen. The characterisation of this enzyme has been previously described [3]. *n*-Dodecyltrimethylammonium bromide was obtained from Sigma (product no. D8638).

A range of buffer solutions was used with the following compositions: (i) glycine (50 mM) plus hydrochloric acid, pH 3.2, ionic strength $I = 0.0119$; (ii) sodium phosphate (2.5 mM), pH 6.4, $I = 0.0069$ and (iii) glycine (50 mM), plus sodium hydroxide, pH 10.0, $I = 0.0318$. The buffers also contained 0.02% w/v sodium azide to inhibit bacterial growth, which contributed 0.0031 to the ionic strengths, except when activity measurements were being made. The enzymes were transferred to the required buffer systems by

dialysis using Spectrapor dialysis tubing (Spectrum Medical Industries, Inc.) molecular weight cut-off 6000–8000. All the reagents were of analytical grade and solutions were made up with double-distilled water.

Methods

Enthalpy measurements were made at 25 °C with an LKB 10700 batch microcalorimeter [9]. The reaction vessel was charged with 2.0 ± 0.1 g of catalase solution (concentration 0.25% w/v) and 2.0 ± 0.1 g of surfactant solution of the required concentration (0–20 mM). The reference vessel was charged with 2.0 ± 0.1 g of buffer and 2.0 ± 0.1 g of surfactant solution identical to that in the reaction vessel. The catalase samples were always dialysed against the required buffer and the final buffer dialysate was used as solvent to make up the surfactant solutions. On mixing, the enthalpies of dilution of the surfactant cancel and the enthalpies of catalase dilution were negligible. The catalase concentrations were determined from the solution absorptions at 405 nm using an absorption coefficient ($A1\%_{405}$) of 13.2 for bovine catalase [10] and an $A1\%_{405}$ of 5.0 for *Aspergillus niger* catalase in phosphate buffer pH 6.4 [3].

Equilibrium dialysis was carried out at 25 °C using Spectrapor dialysis tubing (as above). The catalase solutions (1 or 2 cm^3) were placed in dialysis bags which were then equilibrated with equal volumes of DTAB solutions covering a range of concentrations up to approximately 10 mM in closed glass tubes. The tubes were placed in a gently shaking incubator and the systems allowed to come to equilibrium ($\lesssim 90$ hours). At equilibrium the concentration of unbound DTAB outside the dialysis bags was assayed by the orange II method of Few and Ottewill [11] with reference to a previously determined calibration curve. In all the calculations a molecular weight of 245 000 was taken for bovine catalase [12,13] and 354 000 for *Aspergillus niger* catalase [3].

The enzymic activities of the catalases were determined from the rate of decomposition of hydrogen peroxide as previously described [4]. One unit of catalase corresponds to the decomposition of 1 μmol H_2O_2 min^{-1}. In the absence of detergent the optimum activity of bovine catalase was 172 000 units mg^{-1} (pH 7) and the activity of *Aspergillus niger* catalase at pH 10.0 was 20 700 units mg^{-1}.

RESULTS

Figures 1 and 2 show the isotherms ($\bar{\nu}$ (number of ligands bound per protein molecule) vs. $\log(DTAB)_{free}$) for the binding of DTAB to bovine liver catalase at pH 3.2, 6.4 and 10.0 and to *Aspergillus niger* catalase at pH 10.0. The curves relate to free (unbound) DTAB concentrations below the

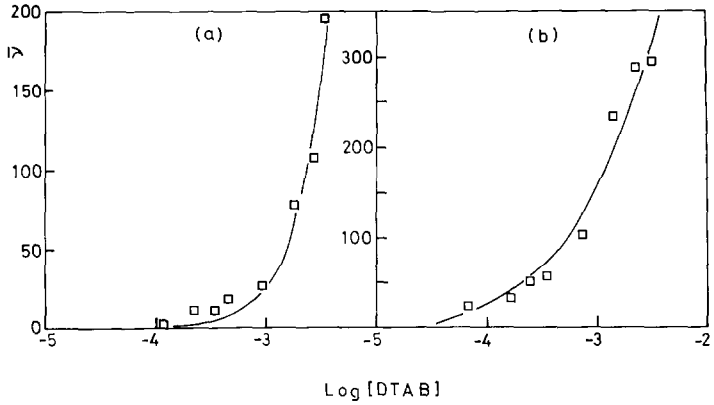

Fig. 1. Binding isotherms (25 °C) for *n*-dodecyltrimethylammonium bromide (DTAB) on bovine liver catalase at pH 3.2 (a) and pH 6.4 (b). The catalase concentrations were 0.125% w/v. The solid lines were calculated from the Hill equation using the parameters given in Table 1.

cmc. The cmc of DTAB at the ionic strengths of the buffers used are in the range 8–12 mM corresponding to values of $\log(\mathrm{DTAB})_{\mathrm{free}} \simeq -2$ [14]. The isotherms can be fitted to a Hill equation of the form [15]

$$\bar{v} = \frac{g(K[S_f])^{n_H}}{1 + (K[S_f])^{n_H}} \tag{1}$$

where $[S_f]$ is the free detergent concentration, g is the maximum value of $\bar{v}$ at saturation, n_H is the Hill coefficient and K the binding constant, provided a value of g is estimated. If it is assumed that at saturation the catalases bind comparable amounts of DTAB to the anionic detergent SDS

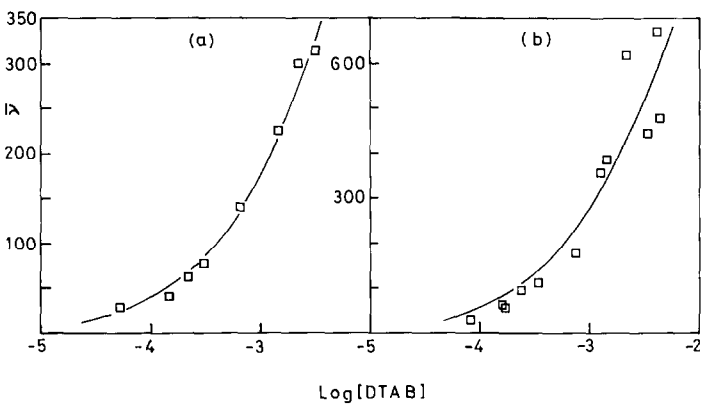

Fig. 2. Binding isotherms (25 °C) for *n*-dodecyltrimethylammonium bromide (DTAB) at pH 10.0 on bovine liver catalase (a) and *Aspergillus niger* catalase (b). The catalase concentrations were 0.125% w/v. The solid lines were calculated from the Hill equation using the parameters given in Table 1.

TABLE 1

Parameters derived from fitting bindings isotherms for DTAB binding to catalase at 25 °C to the Hill equation

System	g	K	n_H	$\Delta G_{\bar{v}}$ (kJ mol^{-1})
Bovine catalase pH 3.2	1200	97.7	1.59	−11.4
Bovine catalase pH 6.4	1200	88.5	0.79	−11.1
Bovine catalase pH 10.0	1200	78.5	0.69	−10.8
Aspergillus niger catalase pH 10.0	1500	136.6	0.74	−12.2

the values of g would be approximately 1200 for bovine catalase [16] and 1500 for *Aspergillus niger* catalase [17]. The data in Figs. 1 and 2 were fitted to the Hill equation by non-linear regression analysis according to a method of Walmsley and Lowe [18] which gave the parameters listed in Table 1. For bovine catalase the Hill coefficients indicate that the binding is positively cooperative ($n_H > 1$) at pH 3.2 but becomes slightly negatively cooperative ($n_H < 1$) at pH 6.4 and 10.0.

The binding data were also analysed in terms of the Wyman binding potential approach [19] as previously described [16]. Briefly the binding potential Π is given by the expressions

$$\Pi = 2.303RT\int_{\lg[S_f]_{\bar{v}=0}}^{\lg[S_f]_{\bar{v}}} \bar{v}\ \mathrm{d}\log[S_f] \tag{2}$$

$$\Pi = RT\ \ln\left(1 + K[S_f]^{\bar{v}}\right) \tag{3}$$

On integration of the binding isotherm to obtain Π, equation (3) can be solved for the binding constant K at a given value of $\bar{v}$ from which the Gibbs energy per ligand bound $\Delta G_{\bar{v}}$ is determined ($\Delta G_{\bar{v}} = -RT$ in K). To do this the isotherms were fitted by non-linear regression analysis to polynomials for $\bar{v}$ in terms of $[S_f]$ which were integrated to obtain Π and hence K and $\Delta G_{\bar{v}}$. Figure 3 shows $\Delta G_{\bar{v}}$ as a function of $\bar{v}$ for the catalase systems. The shapes of the curves reflect the fact that the initial binding of DTAB is of higher energy and as binding proceeds it becomes progressively weaker. The values of $\Delta G_{\bar{v}}$ in Table 1 are all lower than those of Fig. 3 because fitting isotherms to the Hill equation gives a mean binding constant that is weighted by the large number of weaker binding sites which are occupied as saturation is approached.

The enthalpies of interaction of the catalases with DTAB as a function of the final DTAB concentration after mixing are shown in Fig. 4. Bovine catalase interacts athermally at pH 3.2 and endothermically at pH 6.4 and 10.0. *Aspergillus niger* catalase interacts athermally at pH 3.2 and pH 6.4 (data not shown) and endothermically at pH 10.0. The upper axes in Fig. 4 show the number of DTAB molecules bound per catalase molecule as determined from the binding isotherms. It should be stressed, however, that

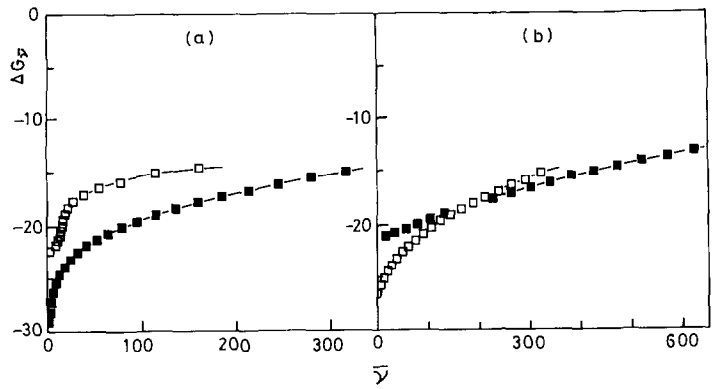

Fig. 3. Gibbs energies per ligand bound ($\Delta G_{\bar{\nu}}$, kJ mol^{-1}) as a function of the number of *n*-dodecyltrimethylammonium bromide (DTAB) ligands bound per catalase molecule ($\bar{\nu}$). □———□, Bovine liver catalase at 25 °C at pH 3.2; ■———■, at pH 6.4. (b) □———□, Bovine liver catalase; ■———■, *Aspergillus niger* catalase, at pH 10.0.

these data refer to equilibrium binding. The calorimetric measurements relate to the enthalpy changes on mixing and covered a time period of 30–40 min, although there is no evidence to suggest that the binding of DTAB does not occur instantaneously. The time required to achieve equilibrium in the dialysis experiments is determined by the slow rate of diffusion of the detergent through the dialysis tubing and not by the rate of DTAB binding to the protein.

Incubation of the bovine catalases with 10 mM DTAB results in the following percentage losses in enzymic activity: 62% (pH 3.2), 21% (pH 6.4)

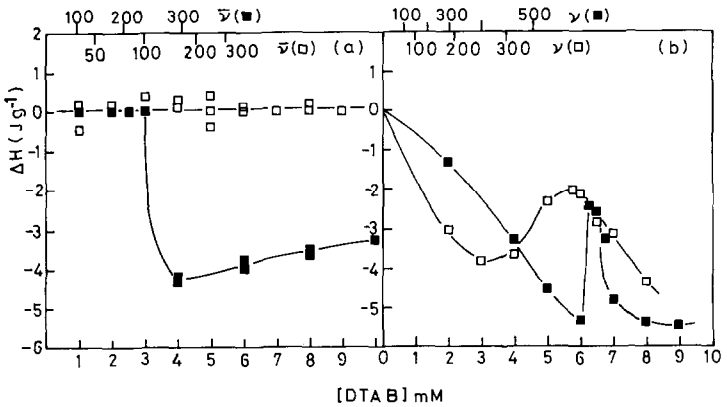

Fig. 4. Enthalpy of interaction between *n*-dodecyltrimethylammonium bromide (DTAB) and catalase at 25 °C. (a) □———□, bovine liver catalase at pH 3.2; ■———■, at pH 6.4. (b) □———□, Bovine liver catalase at pH 10.0; ■———■, *Aspergillus niger* catalase at pH 10.0. The final catalase concentration was 0.125% w/v. The upper axes show the number of DTAB ligands bound per catalase molecule at equilibrium.

and 100% (pH 10.10). *Aspergillus niger* catalase like bovine catalase loses all its activity when incubated with DTAB at pH 10.0.

DISCUSSION

The enthalpies of interaction of DTAB with the catalases are either athermal or endothermic. The curves in Fig. 4 show an increasing endothermic effect as the DTAB concentration is increased, followed by a minimum and, at pH 10, a maximum. In general unfolding of a protein is an endothermic process and detergent binding is exothermic [5]. The curves for the bovine liver catalase at pH 6.4 and 10.0 and the *Aspergillus niger* catalase at pH 10.0 suggest that DTAB binding initiates an endothermic conformational change that predominates over the exothermic binding of detergent. The maxima at pH 10.0 could arise from increasing (exothermic) binding concomitant with endothermic unfolding. The complete loss of enzymic activity at 10.0 is consistent with a major conformational change and possibly subunit dissociation.

The enthalpy data and the binding curves have been used to determine the enthalpy of binding per ligand bound ($\Delta H_{\bar{v}}$) as a function of $\bar{v}$. These data have been used with the $\Delta G_{\bar{v}}$ vs. $\bar{v}$ curves (Fig. 3) to obtain $T\Delta S_{\bar{v}}$; both $\Delta H_{\bar{v}}$ and $T\Delta S_{\bar{v}}$ as a function of $\bar{v}$ are shown in Figs. 5 and 6, from which it follows that the positive entropy term is the major contribution to the interaction.

The thermodynamic parameters for the interaction between DTAB and SDS with catalase are compared in Table 2. At pH 3.2 and 6.4 the Gibbs energies of interaction of SDS are considerably larger than those for DTAB. The isoelectric point of bovine catalase is at pH 5.4 [20] so that at pH 3.2 it

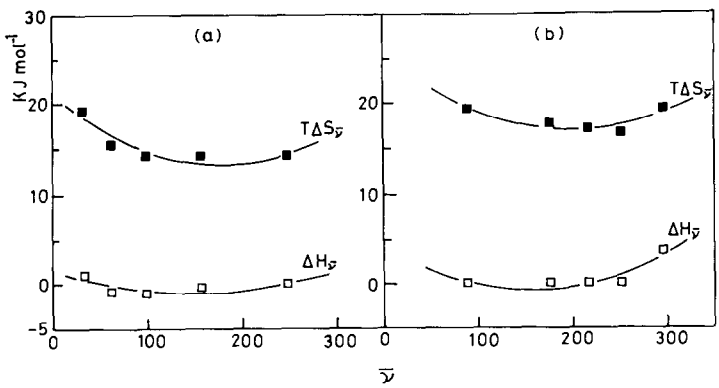

Fig. 5. □——□, Enthalpy ($\Delta H_{\bar{v}}$) and ■——■, entropy ($T\Delta S_{\bar{v}}$) per ligand bound at 25 °C as a function of the number of *n*-dodecyltrimethylammonium bromide (DTAB) ligands bound per catalase molecule ($\bar{v}$). (a) At pH 3.2; (b) at pH 6.4.

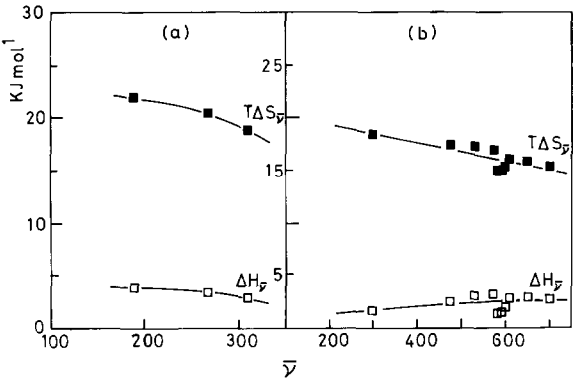

Fig. 6. □———□, Enthalpy ($\Delta H_{\bar{v}}$) and ■———■, entropy ($T\Delta S_{\bar{v}}$) per ligand bound at 25 °C as a function of the number of *n*-dodecyltrimethylammonium bromide (DTAB) ligands bound per catalase molecule ($\bar{v}$) at pH 10.0. (a), Bovine liver catalase; (b), *Aspergillus niger* catalase.

would be expected that the positively charged protein would less readily bind the cationic detergent. Changing the pH from 3.2 to 6.4 would change the net charge on the protein from positive to negative as a result of dissociation of the carboxyl groups of glutamyl and aspartyl residues without affecting the number of cationic sites (apart from a small amount of deprotonation of histidyl residues, $pK_a \simeq 6$). This would be expected to weaken SDS binding as a consequence of coulombic repulsion and strengthen DTAB binding. Comparison of $\Delta G_{\bar{v}}$ for SDS and DTAB at pH 3.2 and 6.4 shows evidence of such effects as do the $\Delta H_{\bar{v}}$ values for SDS. At pH 10 both SDS and DTAB have comparable Gibbs energies of binding but $\Delta H_{\bar{v}}$ differs

TABLE 2

Comparison of the thermodynamic parameters for the binding of DTAB and SDS to catalase

No. of ligands bound ($\bar{v}$)	pH	$\Delta G_{\bar{v}}$ (kJ mol^{-1})		$\Delta H_{\bar{v}}$ (kJ mol^{-1})		$T\Delta S_{\bar{v}}$ (kJ mol^{-1})		$\delta T\Delta S_{\bar{v}}$ (kJ mol^{-1})
		SDS [a]	DTAB	SDS [a]	DTAB	SDS [a]	DTAB	
100(Bovine)	3.2	−34.5	−15.5	−8.5	0	26.0	15.5	10.5
200(Bovine)	3.2	−33.0	−14.4	−8.5	0	24.5	14.4	10.1
300(Bovine)	3.2	−31.5	–	−8.5	0	23.0	–	–
100(Bovine)	6.4	−32.5	−19.4	−1.8	0	30.7	19.4	11.3
200(Bovine)	6.4	−27.3	−17.0	−2.0	0	25.3	17.0	8.3
300(Bovine)	6.4	−24.0	−15.4	−2.6	3.5	21.4	18.9	2.5
300(Bovine)	10.0	−15.0	−14.2	−2.0	3.1	13.0	17.3	−4.3
300(*Asp. niger*)	10.0	−15.7	−16.5	−4.0	1.8	11.7	18.3	−6.6
500(*Asp. niger*)	10.0	−15.2	−14.2	−2.8	2.3	12.4	16.5	−4.1
700(*Asp. niger*)	10.0	−14.5	−12.6	−2.4	2.8	12.1	15.4	−3.3

[a] Data from references 4, 16 and 17.

in sign. There are no marked differences between the two catalase species at this pH.

The increase in entropy on detergent binding is a consequence of the hydrophobic bonding contribution to the interactions and arises from the liberation of structured water molecules around the alkyl chains and in the case of DTAB also the trimethylammonium head group. In acid solution the entropy increase for SDS binding is significantly larger than for DTAB binding, as the difference $\delta T\Delta S_{\bar{v}}$ ($= (T\Delta S_{\bar{v}})_{SDS} - (T\Delta S_{\bar{v}})_{DTAB}$) shows (Table 2, column 9). This suggests stronger hydrophobic binding (with release of more structural water) between SDS and the positively charged protein than between DTAB and the protein. In alkaline solution stronger hydrophobic bonding would be expected between the negatively charged protein and DTAB. The negative values of $\delta T\Delta S_{\bar{v}}$ at pH 10.0 would seem to support this argument. The thermodynamic data suggest a model for detergent binding dominated by hydrophobic interactions that are modulated by the overall charge on the protein and the charge on the surfactant. When the protein and surfactant are of opposite charge the hydrophobic interactions are strengthened and when protein and surfactant are of the same charge they are weakened.

REFERENCES

1 M.N. Jones, P. Manley, P.J.W, Midgley and A.E. Wilkinson, Biopolymers, 21 (1982) 1435.
2 M.N. Jones, A. Finn, A.A. Mosavi-Movahedi and B.J. Waller, Biochem. Biophys. Acta, 913 (1987) 395.
3 A.A. Mosavi-Movahedi, A.E. Wilkinson and M.N. Jones, Int. J. Biol. Macromol., 9 (1987) 327.
4 A. Finn, M.N. Jones, P. Manley, M.I. Paz-Andrade and L. Nunez Reguiera, Int. J. Biol. Macromol., 6 (1984) 284.
5 M.N. Jones, in M.N. Jones (Ed.), Biochemical Thermodynamics, Elsevier, Amsterdam, 1988, Chap. 5.
6 M.N. Jones and P. Manley, J. Chem. Soc. Faraday I, 76 (1980) 654.
7 M. Nogueira, A. Finn and M.N. Jones, Int. J. Biol. Macromol., 8 (1986) 270.
8 M.I. Paz-Andrade, M.N. Jones and H.A. Skinner, J. Chem. Soc. Faraday I, 74 (1978) 2923.
9 I. Wadso, Acta Chem. Scand., 22 (1968) 927.
10 A. Takeda, A. Hachimori, M. Murai, K. Sato and T. Samejima, J. Biochem., 78 (1975) 911.
11 A.V. Few and R.H. Ottewill, J. Colloid Sci., 11 (1956) 34.
12 T. Samejima and J.T. Yang, J. Biol. Chem., 238 (1963) 3256.
13 N.A. Kiseler, C.L. Shpitzberg and B.J. Vainstein, J. Mol. Biol., 25 (1967) 433.
14 M.N. Jones and J. Piercy, J. Chem. Soc. Faraday I, 68 (1972) 1839.
15 A.V. Hill, J. Physiol., 40 (1910) 4P.
16 M.N. Jones and P. Manley, Int. J. Biol. Macromol., 4 (1982) 201.
17 A.A. Mosavi-Movahedi, M.N. Jones and G. Pilcher, Int. J. Biol. Macromol., 10 (1988) 75.
18 A.R. Walmsley and A.G. Lowe, Comput. Method. Prog. Biomed., 21 (1985) 133.
19 J. Wyman, J. Mol. Biol., 11 (1965) 631.
20 T. Samejima, M. Kamata and K. Shibata, J. Biochem., 51 (1962) 181.