Biochimica et Biophysica Acta, 598 (1980) 456—462

BBA 78716

THERMODYNAMICS OF OUABAIN BINDING TO HUMAN ERYTHROCYTES

JOACHIM J. HERMANN

University of New Mexico, College of Pharmacy, Albuquerque, NM (U.S.A.)

(Received August 15th, 1979)

Key words: Ouabain; Membrane binding; Langmuirian kinetics; Thermodynamics; (Erythrocyte)

Summary

A previous study reported that the uptake and release kinetics of ouabain by human erythrocytes in suspension could well be explained by a physical model which involves the slow Langmuir binding of the drug to the erythrocyte membrane. The purpose of the present investigation was to assess quantitatively the thermodynamics of this drug-membrane receptor interaction in order to evaluate the consistency of these parameters with the proposed kinetics model.

Cellular drug uptake and release experiments were conducted at 20, 30 and 40°C, and the Langmuir adsorption and desorption rate constants as well as the Langmuir adsorption isotherms determined from the rate data. With the knowledge of these Langmuir parameters, it was possible to estimate the magnitude of all relevant thermodynamic properties by the use of established physicochemical theories.

The activation energies and entropies for the ouabain adsorption and desorption processes were computed as 105 kJ/mol, 231 J/K per mol, 180 kJ/mol and 245 J/K per mol, respectively. The kinetic and isosteric heats of adsorption were found to be −75.0 and −72.4 kJ/mol, respectively. These findings suggest that the ouabain-erythrocyte membrane interaction represents a case of activated chemisorption which follows the Langmuir isotherm, thus, further underscoring the appropriateness of the Langmuir binding kinetics model.

Introduction

Earlier studies have demonstrated that ouabain and other cardiotonic steroids bind nonlinearly and reversibly to the plasma membrane of human erythrocytes [1—3], that this binding inhibits the active movements of Na^+ and K^+ [1—5], and ultimately, since ($Na^+ + K^+$)-ATPase is believed to be an integral

part of the alkali cation transport system of most mammalian tissues, it has been suggested that cardiac glycosides bind specifically to this erythrocyte membrane-associated enzyme [6].

A more recent investigation evaluated the kinetics of ouabain binding to human erythrocytes in suspension [7]. Using the physical model approach, it was demonstrated that only one model, viz. that which involves the slow Langmuir binding of the drug to the erythrocyte plasma membrane, could well explain both the cellular ouabain uptake and release data. To further appraise the appropriateness of the proposed Langmuir binding kinetics model, it was considered fundamental to evaluate the thermodynamics of this system.

Theoretical considerations

The Langmuir adsorption kinetics model will be briefly reviewed here since it provides the theoretical basis for the present study. The rate of cellular ouabain adsorption is given by:

$$R_{ads} = k_f C L(1 - \theta) \tag{1}$$

where k_f is the Langmuir adsorption rate constant, C is the drug concentration in the suspension medium, L represents the maximal number of moles of ouabain that can bind to 1 ml of compacted erythrocytes and $(1 - \theta)$ is the fraction of unoccupied membrane receptor sites.

The rate of cellular ouabain desorption is given by:

$$R_{des} = k_r L \theta \tag{2}$$

where k_r is the Langmuir desorption rate constant and θ represents the fraction of occupied membrane receptor sites.

The overall rate of cellular ouabain binding is equated to the rate of adsorption minus the rate of desorption, hence:

$$\frac{dC_c}{dt} = L[k_f C(1 - \theta) - k_r \theta] \tag{3}$$

where C_c is the cell-associated ouabain concentration. The effect of the aqueous diffusion layer on the rate of binding is assumed to be negligible.

The cell concentration at any time during a flux experiment is given by:

$$C_c = L\theta \tag{4}$$

Mass balance for the drug requires that:

$$T = VC + HC_c \tag{5}$$

where T is the total drug concentration in the suspension, and V and H represent the volume of medium and erythrocytes in 1 ml of suspension, respectively. All concentrations in this study are expressed in units of moles of ouabain per ml of suspension medium, compacted erythrocytes or whole suspension.

Combining Eqns. 3—5 yields the rate equation for the binding process:

$$\frac{dC_c}{dt} = L\left[\frac{k_f T}{V} - \left(\frac{k_f H}{V} + \frac{k_f T}{VL} + \frac{k_r}{L}\right) C_c + \frac{k_f H}{VL} C_c^2\right] \tag{6}$$

The equation for the Langmuir adsorption isotherm for this system is obtained by equating Eqns. 1 and 2 and by the use of Eqn. 4:

$$C_c = \frac{k_f L C}{k_r + k_f C} \tag{7}$$

The equilibrium constant for the ouabain-erythrocyte membrane interaction can be expressed in terms of equilibrium concentrations, i.e.

$$K_{eq} = \frac{C_c}{C} \quad \text{when} \quad \frac{dC_c}{dt} = 0 \tag{8}$$

The true thermodynamic equilibrium constant is obtained by substituting Eqn. 7 into Eqn. 8 and imposing the condition that $C \to 0$; thus:

$$K_{therm} = \frac{k_f L}{k_r} \tag{9}$$

which represents the initial slope of the Langmuir adsorption isotherm.

Materials and Methods

The experimental phase of this study involved the quantitative determination of the uptake and release kinetics of ouabain by human erythrocytes from isotonic media containing a wide range of drug concentrations at 20, 30 and 40°C.

Uptake experiments

Differing aliquots of tritiated ouabain in ethanol/benzene mixture (New England Nuclear Corp., NET-211, Lot 747—186, 12 Ci/mmol) were transferred into vials and the solutions evaporated to dryness. The tracer was subsequently redissolved in 16—20 ml of suspension medium (phosphate-buffered saline, prepared from 0.15 M NaCl and 0.01 M sodium phosphate at pH 7.4). At 'zero' time, 1.0—1.5 ml of a concentrated suspension of previously-washed human erythrocytes were added to the suspension medium. The resulting suspension was maintained at constant temperature and gently agitated. Subsequently, 2-ml samples of the cell suspension were transferred at various times into a centrifuge tube and centrifuged at 5000 × *g* for 10 min. The narrow stem of the centrifuge tube was calibrated to contain 0.1 ml. Following the centrifugation, a 0.1 ml sample of supernatant was collected in a liquid scintillation vial for future assay. The supernatant and compacted erythrocytes in excess of the 0.1 ml calibration mark were aspirated off. The remaining 0.1 ml compacted erythrocyte plug was flushed into a separate scintillation vial with 1 ml of distilled water.

Also collected with each flux experiment were two 0.1 ml samples of whole suspension for use in the computation of the total amount of ouabain per unit volume of suspension. In addition, the volumes of erythrocytes and medium in each suspension were determined by pipetting a 1 ml aliquot of the cell suspension into a hematocrit tube, followed by centrifugation. The volume of erythrocytes per ml of suspension was directly read from the graduated stem of the hematocrit tube.

Release experiments

To study the release kinetics, erythrocytes were incubated at 30°C to saturation in media containing high tracer concentrations. Following saturation, the supernatants were discarded, the cells washed once or twice in medium and then, at zero time, resuspended in fresh suspension medium. Thereafter, the sampling procedures outlined above for uptake experiments were employed to quantitate the release kinetics.

Data evaluation

All samples were counted by means of a liquid scintillation spectrometer (Beckman Instruments, Model LS-100C). The counts, corrected for quenching, background radiation and intercellular drug entrapment error, were then converted to the molar unit via the specific activity. Subsequently, the red cell concentrations for both drug uptake and release were plotted as a function of time and the data analyzed as follows. The integrated form of Eqn. 6 was programmed for a digital calculator (Wang, Model 600) and a set of experimental parameters then substituted into the program. These experimental constants consisted of the previously mentioned parameters T, V, H, L and $C_{c(0)}$. The Langmuir adsorption rate constant was then estimated by adjusting its magnitude until the best visual fit was obtained between the initial experimental uptake data and the theoretically generated uptake profile, all the while setting the Langmuir desorption rate constant equal to zero. Alternately, the Langmuir desorption rate constant was estimated by optimizing the association between the data generated with the model and the initial release data. The adsorption rate constant was set equal to zero while evaluating the desorption rate constant from the initial release profile.

Results

Langmuir parameters. Representative kinetic data for the cellular uptake and release of ouabain, including the best-fitting theoretical curves, are shown in Figs. 1 and 2, respectively. The parameters that were used to generate the profiles are also included.

The average values that were obtained for the Langmuir adsorption and desorption rate constants at each temperature, as well as the mean value for the amount of ouabain that can maximally bind to 1 ml of compacted erythrocytes, are listed in Table I. The mean cellular saturation value was found to be independent of temperature and was estimated to be $1.84 \cdot 10^{-11}$ mol/ml erythrocytes which corresponds to 964 receptor sites per cell. This is in close agreement with the findings of Gardner and Conlon [1].

Fig. 3 illustrates the equilibrium data for the cell-associated ouabain concentration as a function of ouabain concentration in the suspension medium as well as the Langmuir adsorption isotherms at 20, 30 and 40°C. The isotherms were computed with Eqn. 7 using the Langmuir parameters listed in Table I.

Energies of activation. The activation energies for adsorption and desorption were computed from the appropriate Arrhenius plots as 105 and 180 kJ/mol, respectively. The activation energies are summarized in Table II.

Entropies of activation. The entropies of activation for adsorption and

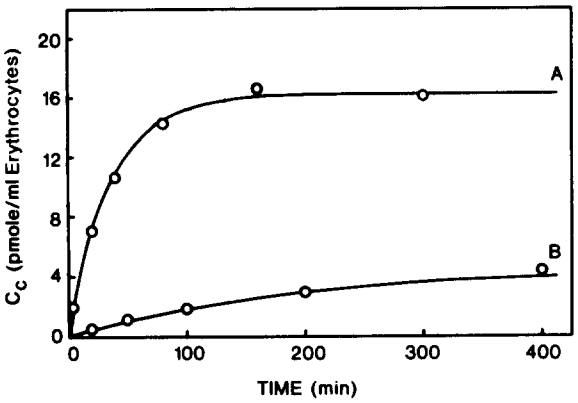

Fig. 1. Theoretical plots for cellular ouabain uptake data collected at 40°C. The media contained different starting concentrations of ouabain. The Langmuir parameters, as determined with the model, as well as the experimental parameters for the uptake experiments are listed as follows: curve A, $k_f = 2.10 \cdot 10^7$ ($cm^3 \cdot mol^{-1} \cdot s^{-1}$), $k_r = 3.64 \cdot 10^{-5}$ (s^{-1}), $L = 1.79 \cdot 10^{-11}$ (mol/ml erythrocytes), $T = 20.0 \cdot 10^{-12}$ (mol/ml suspension), $H = 0.0545$ (ml erythrocytes/ml suspension), $V = 0.9455$ (ml medium/ml suspension), $C_{c(0)} = 0$; curve B, $k_f = 2.20 \cdot 10^7$ ($cm^3 \cdot mol^{-1} \cdot s^{-1}$), $k_r = 3.64 \cdot 10^{-5}$ (s^{-1}), $L = 1.70 \cdot 10^{-11}$ (mol/ml erythrocytes), $T = 9.9 \cdot 10^{-13}$ (mol/ml suspension), $H = 0.067$ (ml erythrocytes/ml suspension), $V = 0.933$ (ml medium/ml suspension), $C_{c(0)} = 0$.

desorption were computed by means of the Eyring equation at 20, 30 and 40°C using the rate constants given in Table I and the activation energies listen in Table II. The mean standard entropies of activation for adsorption and desorption were found to be 231 and 245 kJ/K per mol, respectively. The activation entropies are summarized in Table II.

Heats of adsorption. The heat of adsorption for the ouabain-enzyme interaction was estimated from the data in two ways.

The idea of the activated complex is generally presented on a plot of the energy of the system versus the reaction coordinate. These plots qualitatively

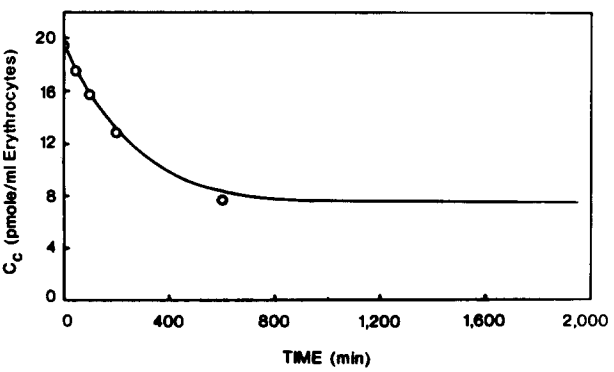

Fig. 2. Theoretical plot for cellular ouabain release data collected at 40°C. The Langmuir parameters, as determined with the model, as well as the experimental parameters for this release experiment are: $k_f = 2.32 \cdot 10^7$ ($cm^3 \cdot mol^{-1} \cdot s^{-1}$), $k_r = 3.60 \cdot 10^{-5}$ (s^{-1}), $L = C_{c(0)} = 1.95 \cdot 10^{-11}$ (mol/ml erythrocytes), $T = 1.486 \cdot 10^{-12}$ (mol/ml suspension), $H = 0.074$ (ml erythrocytes/ml suspension), $V = 0.926$ (ml medium/ml suspension).

TABLE I

MEAN VALUES AND STANDARD DEVIATIONS FOR THE LANGMUIR PARAMETERS AS DETERMINED FROM OUABAIN UPTAKE AND RELEASE DATA

The numbers within square brackets indicate the number of uptake (for k_f) or release (for k_r) experiments from which the mean value of each rate constant was computed. No uptake experiment was carried out at 37°C.

Temperature (°C)	L (mol/ml erythrocytes)	k_f ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)	k_r (s^{-1})
20	$(1.84 \pm 0.16) \times 10^{-11}$	$(1.48 \pm 0.22) \times 10^6$ [10]	$(3.49 \pm 1.23) \times 10^{-7}$ [4]
30	$(1.84 \pm 0.16) \times 10^{-11}$	$(5.59 \pm 0.11) \times 10^6$ [8]	$(3.64 \pm 0.33) \times 10^{-6}$ [5]
37	$(1.84 \pm 0.16) \times 10^{-11}$	—	$(2.16 \pm 0.21) \times 10^{-5}$ [4]
40	$(1.84 \pm 0.16) \times 10^{-11}$	$(2.32 \pm 0.19) \times 10^7$ [22]	$(3.64 \pm 1.41) \times 10^{-5}$ [8]

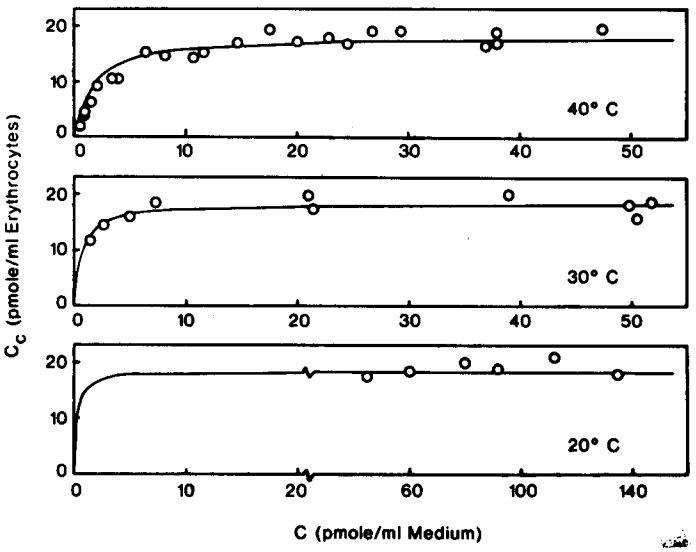

Fig. 3. Equilibrium data for the cell-associated ouabain concentration as a function of the ouabain concentration in the suspension medium at 20, 30 and 40°C. The adsorption isotherms were computed via Eqn. 7 using the respective Langmuir parameters listed in Table I.

TABLE II

THERMODYNAMIC PARAMETERS FOR THE ADSORPTION AND DESORPTION OF OUABAIN TO THE HUMAN ERYTHROCYTE MEMBRANE

E_a, activation energy; $\Delta S^{0\dagger}$, standard entropy of activation; ΔH^{kin}_{ads}, kinetic heat of adsorption; ΔH^{isos}_{ads}, isosteric heat of adsorption.

Parameter	Temperature (°C)	Adsorption	Desorption
E_a (kJ/mol)	—	105	180
$\Delta S^{0\dagger}$ (J/K per mol)	20	231	246
	30	230	245
	40	231	244
Mean $\Delta S^{0\dagger}$ (J/K per mol)	—	231	245
ΔH^{kin}_{ads} (kJ/mol)	—	−75.0	—
ΔH^{isos}_{ads} (kJ/mol)	—	−72.4	—

illustrate that the internal energy change associated with the formation of product(s) from reactant(s) is given by the difference between the activation energies for the forward and reverse reactions. Since the ΔPV term in this system must be small, the internal energy change can be equated to the heat of adsorption. Specifically, by means of its origin, this heat of adsorption will be called the kinetic heat of adsorption. Its value was computed as −75.0 kJ/mol.

The true equilibrium constants for the ouabain-erythrocyte interaction at temperatures 20, 30 and 40°C were calculated with Eqn. 9 using the Langmuir parameters given in Table I and found to be 78.0, 28.3 and 11.7, respectively. The isosteric heat of adsorption was calculated from the van't Hoff isochore as −72.4 kJ/mol.

Conclusions

The results of the present study allow for a better understanding of the proposed Langmuir binding kinetics model. The unusually large and positive values for the entropies of activation imply that both adsorption and desorption are strongly entropy-driven processes. Yet, in spite of these favorable activation entropies, the overall rates for adsorption and desorption are quite slow due to the presence of surprisingly large energies of activation. Moreover, these large activation barriers, combined with the relatively strong ouabain-enzyme bond ($\Delta H_{ads} \approx 73.7$ kJ/mol), suggest that the binding phenomenon in question represents a case of activated chemisorption which follows the Langmuir isotherm. In conclusion, the close agreement that was found between the kinetic and isosteric heats of adsorption, as well as the observed unfavorable activation parameters, demonstrate a high degree of self-consistency and hence, strongly support the appropriateness of the Langmuir binding kinetics model for this drug-receptor interaction.

Acknowledgements

The author is grateful to Mrs. Ona Bailey for the typing of this manuscript and to the Research Allocations Committee of the University of New Mexico for financial support of this investigation.

References

1 Gardner, J.D. and Conlon, T.P. (1972) J. Gen. Physiol. 60, 609—629
2 Smith, T.W., Wagner, H., Jr., Markis, J.E. and Young, M. (1972) J. Clin. Invest. 51, 1777—1789
3 Gardner, J.D., Kiino, D.R., Swartz, T.J. and Butler, V.P., Jr. (1973) J. Clin. Invest. 52, 1820—1833
4 Perrone, J.R. and Blostein, R. (1973) Biochim. Biophys. Acta 291, 680—689
5 Hoffman, J.F. (1969) J. Gen. Physiol. 54, 343—353
6 Glynn, I.M. (1964) Pharmacol. Rev. 16, 381—407
7 Hermann, J.J. (1975) Ph.D Dissertation, University of Michigan, Ann Arbor