

## 593—INTERACTION BETWEEN GLUTATHIONE AND AMPICILLIN: AN *a.c.* POLAROGRAPHIC METHOD

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### SUMMARY

The interaction of glutathione with ampicillin was investigated by a new *a.c.* polarographic procedure. Ampicillin was found to interact strongly with glutathione. The stability constants were ascertained from the polarographic data, and the free energy change,  $\Delta G^\circ$ , the enthalpy change,  $\Delta H^\circ$ , and the entropy change,  $\Delta S^\circ$ , of the interaction were computed from the data at different temperatures.

The introduction of the polarographic method to the study of interactions between molecules of biological significance (other than metal-ion complexes), and the possibility of GSH binding to ampicillin *in vivo* are the principal contributions of this work.

### INTRODUCTION

*d.c.* polarographic methods using measurements involving half-wave potentials are commonly used to examine complex ion systems. The most frequently used methods for reversible electrode reactions are probably those proposed by De Ford and Hume [1] and Lingane [2], although many other methods are available [3]. Gupta and Chatterjee [4] proved the validity of *a.c.* measurements in evaluating complex ion systems. For reversible electrode reactions the peak potential,  $U_p$ , from *a.c.* polarograms is equal to the reversible half-wave potential,  $U_{1/2}$ . Thus a modified version of Lingane's equation can be used.

Although a large number of papers dealing with the polarography of metal-ion complexes are available, only a few papers have been devoted to the study of another type of interaction.

In a previous preliminary note [5] we demonstrated electrochemical evidence for the interaction between glutathione and ampicillin using *a.c.* polarography. In this paper we wish to complete this preliminary information.

## EXPERIMENTAL

*Chemicals*

Ampicillin was obtained as a pure drug (100 % chromatographically pure, 89.8 % activity) from Chile Laboratories, Santiago, Chile. Glutathione (reduced form, GSH) was obtained from Aldrich Chemical Company. All other chemicals were of analytical reagent grade. Sorensen phosphate buffer at a constant ionic strength (1.0 M) was used for the polarographic studies.

*Apparatus*

The equipment and operation conditions were similar to those used previously [5].

## RESULTS AND DISCUSSION

We have postulated the following treatment to account for the observed shift [5] in the peak potential,  $U_p$ , of GSH produced by the presence of ampicillin.

The polarographic behaviour of GSH has been studied by several authors [6–8], and the following equation accounts for the GSH electrode process at the d.m.e.

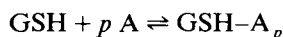


If this process takes place reversibly, the potential of the d.m.e. at all points on the polarographic anodic wave may be given by

$$U = U^\circ + \frac{RT}{\mathcal{F}} \ln \frac{[\text{GSHg}]_{x=0} [\text{H}^+]_{x=0}}{[\text{GSH}]_{x=0}} \quad (2)$$

where  $[ ]_{x=0}$  indicates the concentration at the electrode surface.

We have described the GSH–A interaction by the following equation:



where A is ampicillin and  $p$  is the co-ordination number. The stability constant of the GSH–A $_p$  species is given by

$$K = \frac{[\text{GSH-A}_p]}{[\text{GSH}][\text{A}]^p}$$

At the electrode surface this species will have a concentration given by

$$[\text{GSH-A}_p]_{x=0} = K [\text{GSH}]_{x=0} [\text{A}]_{x=0}^p \quad (3)$$

Substituting equation (2) into equation (1), and considering

$[\text{A}]_{x=0} = [\text{A}]$  and  $[\text{H}^+]_{x=0} = [\text{H}^+]$  we obtain:

$$U = U^\circ + \frac{RT}{\mathcal{F}} \ln \frac{[\text{GSHg}]_{x=0} [\text{H}^+] K [\text{A}]^p}{[\text{GSH-A}_p]_{x=0}} \quad (4)$$

If the  $\text{GSH-A}_p$  species arrives at the d.m.e. by diffusion only, the mean current at any part of the wave may be given by

$$I = kI_{\text{GSH-A}_p} \left( [\text{GSH-A}_p] - [\text{GSH-A}_p]_{x=0} \right) \quad (5)$$

where  $k$  and  $I_{\text{GSH-A}_p}$  are the capillary constant and the diffusion current constant of the  $\text{GSH-A}_p$  species, respectively.

When  $[\text{GSH-A}_p]_{x=0}$  approaches zero, the current reaches its limiting value.

$$I_{\text{lim}} = kI_{\text{GSH-A}_p} [\text{GSH-A}_p] \quad (6)$$

From equations (6) and (5) we obtain

$$\frac{I_{\text{lim}} - I}{kI_{\text{GSH-A}_p}} = [\text{GSH-A}_p]_{x=0} \quad (7)$$

A similar relationship holds in terms of the concentration of the  $\text{GSHg}$  species. Namely,

$$I = kI_{\text{GSHg}} [\text{GSHg}]_{x=0} \quad (8)$$

Thus, substituting for  $[\text{GSH-A}_p]_{x=0}$  and  $[\text{GSHg}]_{x=0}$  in equation (4) the values obtained from equations (7) and (8) we have:

$$U = U^\circ + \frac{RT}{\mathcal{F}} \ln \left( \frac{kI_{\text{GSH-A}_p}}{kI_{\text{GSHg}}} \frac{I}{I_{\text{lim}} - I} [\text{H}^+] K [\text{A}]^p \right) \quad (9)$$

The expression for the half-wave potential for the *complexed GSH* is obtained by substituting the condition  $I = I_{\text{lim}}/2$ . Thus,

$$(U_{1/2})_c = U^\circ + \frac{RT}{\mathcal{F}} \ln \left( \frac{I_{\text{GSH-A}_p}}{I_{\text{GSHg}}} [\text{H}^+] [\text{A}]^p K \right) \quad (10)$$

for the  $\text{GSH-A}_p$  species, while for the *simple GSH* species the half-wave potential is given by

$$(U_{1/2})_s = U^\circ + \frac{RT}{\mathcal{F}} \ln \frac{I_{\text{GSH}} [\text{H}^+]}{I_{\text{GSHg}}} \quad (11)$$

For *a.c.* polarography, equations (10) and (11) are given by

$$(U_p)_c = (U^\circ)' + \frac{RT}{\mathcal{F}} \ln([\text{H}^+][\text{A}]^p K) \quad (12)$$

where  $(U_p)_c$  is the peak potential for  $(\text{GSH-A}_p)$ ,

$$(U^\circ)' = U^\circ + \frac{RT}{\mathcal{F}} \ln \frac{I_{\text{GSH-A}_p}}{I_{\text{GSHg}}},$$

and

$$(U_p)_s = (U^\circ)'' + \frac{RT}{\mathcal{F}} \ln[\text{H}^+] \quad (13)$$

where  $(U_p)_s$  is the peak potential for GSH and

$$(U^\circ)'' = U^\circ + \frac{RT}{\mathcal{F}} \ln \frac{I_{\text{GSH}}}{I_{\text{GSHg}}}$$

If it is assumed that the diffusion current constants  $I_{\text{GSH}}$  and  $I_{\text{GSH-A}_p}$  have approximately the same value for both species the shift in peak potential produced by the presence of ampicillin is given by

$$(U_p)_s - (U_p)_c = - \frac{RT}{\mathcal{F}} \ln(K[A]^p) \quad (14)$$

According to equation (12), an experimental linear relationship between the potential peak of the GSH-A<sub>p</sub> species and the logarithm of the ampicillin concentration was found. This behaviour was obtained using  $1 \times 10^{-4} M$  GSH and different concentrations (between  $5 \times 10^{-5}$  and  $1 \times 10^{-3} M$ ) of ampicillin. A linear relationship was obtained for several temperatures between 15 and 40 °C and for different pH conditions between pH 5 and 9. Figure 1 shows the linear relationship at pH 7.4 for two different temperature values. The slope of the line  $(U_p)_c$  versus  $\log [A]$  allows us to obtain the co-ordination number  $p$  (from equation 12). In Table 1 some of the experimental values of this slope and the corresponding  $p$  values from different temperatures and pH conditions are shown. These results indicate a  $p$  value equal to 1, suggesting a 1 : 1 interaction between GSH and ampicillin.

Moreover, the stability constant,  $K$ , of the complex GSH-A may be calculated according to equation [14], where the value of  $(U_p)_c$  is the potential peak extrapolated to an ampicillin concentration of 1.0 M. We obtained a value of  $3.12 \times 10^4$  for the stability constant under physiological conditions of pH and temperature. Furthermore, we found a linear relationship between  $\ln K$  and  $1/T$ . The parameters of the regression curves for six sets of experimental data at three different pH values

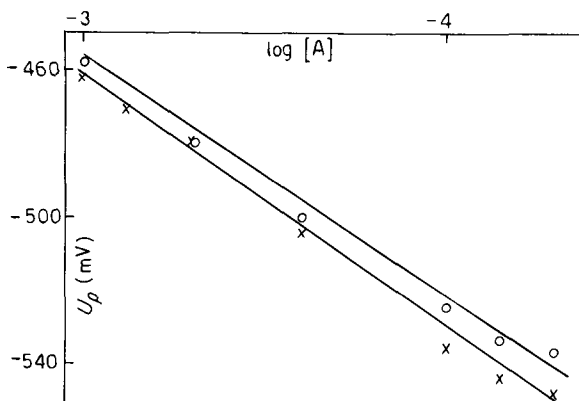


Fig. 1. Relationship between the potential peak of the GSH-A species,  $U_p$ , and the logarithm of ampicillin concentration at pH 7.4. (O) 37 °C, (x) 25 °C.

TABLE 1

Experimental  $p$  values obtained from the regression lines:  $p$  versus  $\log [A]$  at different values of pH and temperature. (Each curve was calculated from seven pairs of data)

$T(^{\circ}\text{C})$	pH	$\frac{\Delta U_p (mV)}{\Delta \log [A]}$	Correlation coefficient	$p$
37	7.4	59.14	0.996	0.97
37	6.0	55.52	0.975	0.91
37	8.0	71.28	0.986	1.16
25	7.4	70.09	0.997	1.18
25	5.0	71.48	0.999	1.20
25	9.0	73.11	0.997	1.23
40	7.4	71.05	0.994	1.14
40	8.0	68.49	0.998	1.10
40	7.4	69.66	0.996	1.19
20	9.0	78.30	0.994	1.35

are given in Table 2. Assuming that there is no significant temperature dependence on enthalpy change,  $\Delta H^{\circ}$ , within the temperature range in which the interaction was studied (15–40  $^{\circ}\text{C}$ ), it is possible to estimate the standard enthalpy change for the association of 1 mole of GSH with 1 mole of ampicillin from equation (15),

$$\ln K = \frac{-\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} \quad (15)$$

which is the equation of the straight line

$$\ln K = m \frac{1}{T} + n$$

where  $m (= -\Delta H^{\circ}/R)$  is the slope and  $n (= \Delta S^{\circ}/R)$  is the intercept. The values of  $m$  and  $n$  are given in Table 2.

TABLE 2

Parameters of the regression curve  $\ln K = m(1/T) + n$  at different pH values. Temperature range: 15–40  $^{\circ}\text{C}$

pH	Slope $\times 10^{-4}$	Intercept	Correlation coefficient
7.4	1.47	- 37.08	0.999
8.0	1.52	- 37.00	0.997
9.0	1.29	- 30.13	0.999

TABLE 3

Thermodynamic data for the GSH-A interaction at pH 7.4

$T$ (°C)	$K \times 10^{-4}$ (dm <sup>3</sup> /mole)	$\Delta H^\circ$ (kcal/mole)	$\Delta G^\circ$ (kcal/mole)	$\Delta S^\circ$ (kcal/mole)
37	3.12	-29.28	-6.35	-73.96
20	64.69	-29.28	-7.76	-73.44

The standard free energy,  $\Delta G^\circ$ , for complex formation is estimated from

$$\Delta G^\circ = -RT \ln K \quad (16)$$

and the entropy change,  $\Delta S^\circ$ , can again be obtained by substituting  $\Delta H^\circ$  and  $\Delta G^\circ$  into equation (17):

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (17)$$

Thermodynamic parameters for the interaction at physiological pH are reported in Table 3. From the negative enthalpy change of 29.28 kcal/mole, it can be concluded that complex formation is an exothermic process, and the negative sign for  $\Delta G^\circ$  indicates that the binding process is spontaneous. It is not possible, at present, to draw further conclusions concerning the nature of the interaction between glutathione and ampicillin.

We also studied the dependence between the peak current,  $I_p$ , and the temperature for three different pH values. We obtained a linear relationship between  $\log I_p$  and the reciprocal of the temperature,  $1/T$ , according to equation (19) [9]:

$$\log I_p + \frac{1}{2} \log T = \text{const} - \frac{\Delta G_{act}}{4.606R} \frac{1}{T} \quad (19)$$

From the slope of the linear diagram corresponding to equation (19), the diffusion activation free energy,  $\Delta G_{act}$ , was calculated to be 4.42 kcal/mole (pH = 7.4) for the GSH-A species. The resultant  $\Delta G_{act}$  values were observed to be pH-dependent, and increased as the pH increased (Table 4). This observation implies that the conformation of the GSH-A species and, hence, its diffusivity are sensitive to the variation of proton  $[H^+]$  concentration in the solution. As the proton concentration decreased, the conformation of the GSH-A molecule changed so that its molecular diffusion required a higher value of diffusion activation free energy.

TABLE 4

Diffusion activation free energy values for the GSH-A species

pH	$\Delta G$ (kcal/mole)
7.4	4.42
8.0	5.61
9.0	6.25

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