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METAL–ATP BINDING.

I. THERMODYNAMIC DATA FOR ADENOSINE–SILVER BINDING

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SUMMARY

A potentiometric method using silver concentration cells was used to follow free Ag^+ in silver–adenosine titrations at pH 5.6–5.8.

A computer method was developed for the calculation of K_1 and K_2 in the formation of ML_2 when only free M is known. This method was applied to the silver–adenosine titration data at 10°, 17°, 25°, 33° and 40° to give $\Delta G°$, $\Delta H°$, and $\Delta S°$ for each of the two steps in the formation of Ag^+(adenosine)$_2$ at 25°. The implications of these data with regard to Ag–ATP binding are that metal-ring and metal-bridge forms would predominate in the Ag–ATP complex.

INTRODUCTION

Recent work has made it clear that in ATP–metal ion binding the nitrogen atoms of the adenosine ring of ATP play a secondary but important role in the binding[1].

The degree of binding to the negatively charged phosphate chain and/or to the neutral adenosine ring of ATP depends on the charge and electronic structure of the metal ion bound. To the present alkali, alkali earth, doubly charged transition metal ions, and Fe^{3+} have been studied[1]. It is proposed that the Ag^+ ion would differ from any of the previously studied ions in its ATP-binding behaviour since it has a single positive charge and strong nitrogen-chelating ability—a combination that is not found in any of the above mentioned ions. Therefore, as a first step towards elucidating the nature of Ag^+–ATP binding (and therefore, something of the nature of the ATP ring binding site), thermodynamic data for Ag^+–adenosine binding have been determined, using a potentiometric technique designed for computer data analysis.

EXPERIMENTAL

The cell used in the potentiometric measurements was a silver concentration cell of the type, $Ag|(Ag^+)\|(Ag^+, 0.800\ mM)|Ag$, with a polyethylene agar bridge, 3.0 M in KNO_3. For all measurements the solution in the reference cell was 0.800 mM $AgNO_3$. For each titration a series of ten solutions was placed in the measuring cell and the potential difference across the cells was measured to the nearest tenth of a

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millivolt using a Radiometer pH Meter. Both the measuring cell and the reference cell were maintained at the particular temperature employed ± 0.2° throughout the measurements. The pH during the adenosine titrations was found to be 5.6–5.8. The series of ten solutions included both a cell calibration and a titration of Ag^+ (as $AgNO_3$) with adenosine at constant volume. A typical set of data for such a series is given in Table I.

TABLE I

DATA FOR A TYPICAL SERIES OF MEASUREMENTS INCLUDING AN Ag^+ CONCENTRATION CELL CALIBRATION AND A TITRATION OF Ag^+ (AS $AgNO_3$) WITH ADENOSINE AT 10°. REFERENCE CELL $Ag|Ag^+$, 0.800 mM

Case		*Initial $AgNO_3$ (mM)*	*Initial adenosine (mM)*	*Final mV*
Calibration		0.800	—	0.0
Titration	1	1.000	4.00	8.0
Calibration		0.600	—	6.7
Titration	2	1.000	5.00	11.2
Titration	3	1.000	7.00	17.3
Calibration		0.400	—	16.1
Titration	4	1.000	9.00	23.2
Titration	5	1.000	11.25	29.5
Calibration		0.200	—	32.2
Calibration		0.800	—	0.0

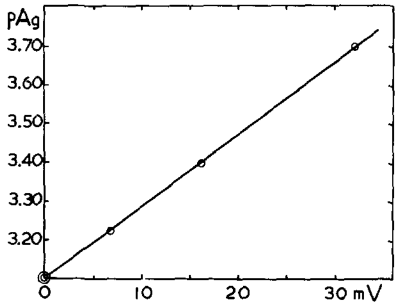


Fig. 1. Calibration curve for the silver concentration cell $Ag|(Ag^+)|(Ag^+, 0.800\ mM)|Ag$ at 10°. pAg, which is defined as the negative log of $[Ag^+]$, is taken to be 3.097 for the 0.800 mM reference solution of $AgNO_3$. The data on which this curve is based are given in Table I.

In order to obtain a calibration curve, millivolts were plotted against pAg for the five calibration solutions. In all cases the plots were linear and showed less than 0.6 mV drift in the zero point in the course of the titration. The calibration curve for the data of Table I is given in Fig. 1. With this curve the millivolt readings for the titration solutions were converted to Ag^+ concentration, thus providing free Ag^+ concentration for the equilibrium condition.

Several such titrations were carried out at 10°, 17°, 25°, 33° and 40° to provide temperature variation data.

RESULTS

Trial calculations were carried out assuming formation of only 1:1 complex. These calculations failed to yield a true constant, confirming the expectation that much of the Ag^+ is in the Ag^+(adenosine)$_2$ form.

The case is, then, one of an ML_2 complex in which the equilibrium concentration of M is followed. A method of successive approximation by graphical differentation of a dlog[M] *vs.* dlog[L] plot has been used to analyse such data[2]. However, in this study, a more convenient, and perhaps more informative computer method has been developed. This method is as follows:
The two equilibria are:

$$M + L \overset{K_1}{\rightleftharpoons} ML \tag{1}$$

$$ML + L \overset{K_2}{\rightleftharpoons} ML_2 \tag{2}$$

The overall gross equilibrium is:

$$M + 2L \overset{\beta_2}{\rightleftharpoons} ML_2 \tag{3}$$

The two conservation equations are:

$$[M_0] = [M] + [ML] + [ML_2] \tag{4}$$

$$[L_0] = [L] + [ML] + 2\,[ML_2] \tag{5}$$

Beginning with the expression for the overall equilibrium:

$$\beta_2 = \frac{[ML_2]}{[M]\,[L]^2} \tag{6}$$

and substituting $[ML_2] = [M_0] - [M] - [ML]$, $[L] = [L_0] - [ML] - 2\,[ML_2]$, and rearranging

$$\beta_2 = \frac{M_0 - M - K_1M(L_0 - 2M_0 + 2M)/(1 - K_1M)}{M(L_0 - 2M_0 + 2M + K_1M(L_0 - 2M_0 + 2M)/(1 - K_1M))^2} \tag{7}$$

Clearly, this expression involves only the three experimentally known quantities M_0, L_0, and M, and the two unknown constants β_2 and K_1. In Table I there are listed data for five different sets of M_0, L_0 and M values. If the proper value of K_1 were substituted into Eqn. 7 and the equation solved with the five different sets of M_0, L_0, and L, the same value within experimental error for β_2 would be obtained each time. As a first step towards obtaining the proper value of K_1, calculations were carried out on the five sets of data assuming only 1:1 complex formation. These calculations gave five different values of K_1 which were plotted against L_0, and extrapolated to $L_0 = 0$. For the case at 25° the extrapolation indicated K_1 about 100. A computer program was then written which would keep solving Eqn. 7, with the five sets of M_0, L_0 and M while varying K_1 from 80 to 120 in increments of 5. It was found in all cases that the calculated values of β_2 showed a definite trend with a wide spread of values at one extreme of K_1—the values converged to a single β_2 at one given value of K_1 and then diverged to a wide spread of values with the trend in the opposite direction to the initial one at the other extreme of K_1. For the final refined calculations the increments in K_1 were reduced to 1. Selected values for the convergence of the case at 25° are given in Table II as an illustration of how β_2 behaves as K_1 is varied.

Each of the sets of titration data was analysed in this way. In all cases it was found that log β_2 converged to a single value ± 0.03 log units. The log K_1 and log K_2 values thus obtained were plotted against $1/T$ as indicated in Fig. 2. A least-squares straight line was drawn through the points in each case and the slope was used to calculate an average ΔH over the temperature range 10–40° by application of the Van 't Hoff isochore.

The equations $\Delta G = -RT\ln K$ and $\Delta S = (\Delta H - \Delta G)/T$ were then used to calculate ΔG and ΔS, respectively, for the two equilibria. Since this work was carried out at the very low ionic strength of $I = 10^{-3}$, and since, theoretically, this reaction is independent of ionic strength in the Debye–Hückel region, the values obtained theoretically may be written as the values at $I = 0$. Also, several experiments carried out with $[M_0] = 0.100$ mM, *i.e.* $I = 10^{-4}$ confirmed that the reaction has little or no ionic strength dependence at $I < 10^{-3}$. The values given in Table III are, therefore, presented as values at $I = 0$, *i.e.* as $K°$, $\Delta G°$, $\Delta H°$, and $\Delta S°$.

TABLE II

SELECTED VALUES OF LOGβ_2 ILLUSTRATING CONVERGENCE TO A SINGLE VALUE AS K_1 IS VARIED

Data for the titration of Ag^+ (as $AgNO_3$) with adenosine at 25°. The best value of log K_1 and the values of logβ_2 calculated from it are given in bold faced type.

M_0 (*mM*)	L_0 (*mM*)	M (*mM*)	*Log* K_1 *1.954*	*Log* K_1 *1.973*	*Log* K_1 *1.991*	*Log* K_1 **2.013**	*Log* K_1 *2.033*	*Log* K_1 *2.053*	*Log* K_1 *2.072*
1.000	4.00	0.681	4.056	4.006	3.949	**3.866**	3.764	3.632	3.443
1.000	5.00	0.622	4.002	3.957	3.907	**3.835**	3.750	3.644	3.506
1.000	7.00	0.515	3.969	3.935	3.899	**3.848**	3.791	3.726	3.650
1.000	9.00	0.429	3.950	3.923	3.894	**3.855**	3.813	3.766	3.714
1.000	11.25	0.353	3.934	3.911	3.888	**3.857**	3.824	3.789	3.750

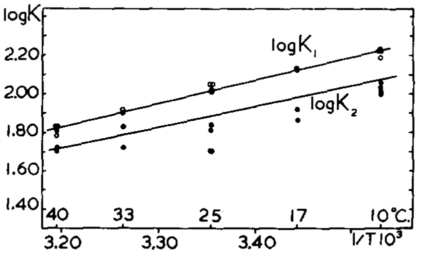


Fig. 2. Plot of log K values for the formation of $Ag^+(\text{adenosine})_2$ against the reciprocal of temperature. The lines are least-squares straight lines which give an average ΔH over the range 10–40°.

TABLE III

THERMODYNAMIC DATA FOR SILVER–ADENOSINE BINDING AT 25°

$\Delta H°$ is an average ΔH for the temperature range 10–40°.

Reaction	*LogK°*	$\Delta G°$ (*kcal/mole*)	$\Delta H°$ (*kcal/mole*)	$\Delta S°$ (*e.u.*)
$M + L \rightleftharpoons ML$	2.02 ± 0.02	−2.76 ± 0.03	−5.49 ± 0.5	−9.1 ± 1.8
$ML + L \rightleftharpoons ML_2$	1.84 ± 0.06	−2.52 ± 0.08	−3.66 ± 1.8	−3.8 ± 6.3

DISCUSSION

1. The extent of Ag–adenosine binding

According to the data of Table III $\log K_1$, and $\log K_2$ for the binding of adenosine to Ag are 2.02 and 1.84, respectively. Previous work has shown that $\log K_1$, and $\log K_2$ for the binding of ring nitrogen compounds to Ag are 1.99 are 2.25 for pyridine[3,4] and 1.38 and 1.50 for aniline[5]. These data suggest that the possibility of five-membered ring formation involving both N_7 and the amino-N in Ag–adenosine complex formation does not greatly increase the overall binding.

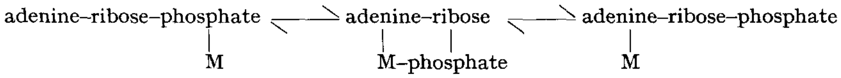
However, the fact that $\log K_2$ is slightly smaller than $\log K_1$ in adenosine does suggest that some of the Ag–adenosine complex is in a five-membered ring structure—thereby slightly inhibiting addition of a second adenosine.

2. The entropy of Ag–adenosine binding

The entropy changes for both steps of Ag–adenosine complex formation are seen to be negative as expected for complex formation in which there is no change cancellation. This is in contrast to all cases of ATP and triphosphate–metal complex formation in which ΔS is positive[1,6]. Therefore, in the case of all metal ions which bind to the ring of ATP to a considerable extent, the net ΔS for metal–ATP complex formation must be a composite of these two opposing entropy contributions.

3. Ag–ATP binding

A theory has been advanced in an earlier paper that ATP–metal complexes exist in the following equilibrium[1]:



$$\text{adenine–ribose–}\underset{\substack{|\\ \text{M}}}{\text{phosphate}} \rightleftharpoons \underset{\substack{|\qquad |\\ \text{M–phosphate}}}{\text{adenine–ribose}} \rightleftharpoons \underset{\substack{|\\ \text{M}}}{\text{adenine}}\text{–ribose–phosphate}$$

Previously the ion that placed the equilibrium furthest to the right was Cu^{2+} which was reported to have a $\log \beta_2$ value for Cu^{2+}–adenosine binding of 0.84 (ref. 7), while the present study reports $\log \beta_2$ for Ag^+–adenosine binding as 3.86. Since it can be safely assumed that Ag–triphosphate binding would be less than the very strong Cu^{2+}–triphosphate binding, it follows that the equilibrium for Ag^+–ATP binding lies far to the right of that for Cu^{2+}–ATP binding. Therefore, structural studies of ATP in which metal–bridge and metal–ring complexes are required can best be obtained by the use of Ag^+.

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