A Model for the Binding of Adenosine to Polyuridylic Acid

by

John G. Burr, Terence L. McDowell and Sherril D. Christian

Communication from the Department of Chemistry, The University of Oklahoma, Norman, Oklahoma 73069

Received November 13,1973

SUMMARY

We have developed a simple thermodynamic model to represent the entire binding isotherm for cooperative binding systems such as the triple helix formed by adenosine and polyuridylic acid (poly-U). An equation is derived to express θ (the fraction of poly-U sites occupied by bound molecules) as a function of C_a (the concentration of free adenosine) and two parameters, K_1 and n. K_1 is related to the total free energy of binding of a monomer unit in the complex and n is the minimum number of monomer units which must interact cooperatively with the polymer to form a stable complex. Accurate values of both C_a and $d\theta/dC_a$ at $\theta = \frac{1}{2}$ can be calculated from the analytical expression for $\theta(C_a)$. For a sample of unmodified poly-U we found that $K_1 = 432M^{-1}$ and n = 5 were the optimum values of the parameters. The mathematical form of the binding isotherm and computational procedures also apply to binding data for adenosine on modified poly-U.

INTRODUCTION:

The cooperative binding of adenosine to polyuridylic acid (1,2) has been studied by several groups (1-6). In connection with similar studies of the effects of chemical modification of the uracil portion of poly-U on the binding of adenosine to poly-U (7), we have developed a simple thermodynamic model to represent the entire binding isotherm for such systems. Equations based on the model provide an excellent fit of binding data for both unmodified and modified poly-U. Parameters determined by the method are related to the total free energy of binding of a monomer unit in the complex and to the minimum number of monomer units which must interact cooperatively with the polymer to form a stable complex.

THEORY:

We assume first that each adenosine unit (A) which binds to poly-U bridges between two U units to form a segment of a triple-helix complex. The known (1-3,6,7) cooperativity of the binding indicates that several A units must attach contiguously in order to stabilize the binding; Figure 1 shows schematically how this binding may occur. Stacking interactions between adjacent A molecules and hydrogen-bonding between A and U units can lead to a free energy of formation of the complex which is sufficiently negative to overcome the positive free energy change which should accompany the disruption of interactions between U units on opposite strands. We assume next that appreciable amounts of bound A exist only in complexes of the form poly-U$\cdot A_m$, where the integer m must equal or exceed a critical value (n). Complexes for which $m<n$ are assumed to be unstable with respect to the formation of monomers. In the case of complexes for which $m \geq n$, we assume that for the reaction mA + poly-U = poly-U·Am the standard free energy change per unit of bound A is independent of m. That is, once the critical value m = n is reached or exceeded, the average binding free energy per mole of adenosine is assumed to be constant. If the value of n is relatively large, or in regions of the binding isotherm where the average value of m is large, this should be a good assumption.

Using mass action relations, the total concentration of bound adenosine, A_b, can be written in terms of equilibrium constants for formation of the various poly-U$\cdot A_m$ complexes and the monomer concentration of A in the bulk solution, C_a. Thus,

$$A_b = [A]_o - [A]_{\text{bulk solution}}$$

$$= nK_n C_a^n([S] - A_b) + (n+1)K_{n+1}C_a^{n+1}([S] - A_b) + \ldots \quad (1)$$

where $[S]$ is the total concentration of binding sites, $[A]_o$ is the total concentration of A (in solution and bound to poly-U), and where each of

the K_i's (K_n,K_{n+1}, . .) is a formation constant for the complex containing i units of A cooperatively bound to poly-U. The term $[S] - A_b$ is the concentration of available binding sites. In writing equation 1, it is assumed that the concentrations of species and binding sites may be used in place of the thermodynamic activities. By utilizing the assumption that the average free energy of binding per mole of A is constant, one may now relate the K_i values to K_1, the equilibrium constant for the reaction

$$A + \frac{1}{m} (\text{poly-U}) = \frac{1}{m} (\text{poly-U}\cdot A_m).$$

Thus, in general $K_m = K_1^m$ and equation 1 may be rewritten

$$A_b = \left[n(K_1C_a)^n + (n+1)(K_1C_a)^{n+1} + \ldots \right] ([S] - A_b)$$

$$A_b = (K_1C_a)^n([S] - A_b) \sum_{j=0}^{\infty} (n + j) (K_1C_a)^j$$

$$= (K_1C_a)^n([S] - A_b)\left[n/(1 - K_1C_a) + K_1C_a/(1 - K_1C_a)^2 \right] \quad \ldots \quad (2)$$

(It is assumed here that the value of m can increase without limit, although of course for a finite molecular weight of polymer the upper value of m will be large but finite. The identity $\sum_{j=0}^{\infty} (n+j)(K_1C_a)^j = n/(1 - K_1C_a) + K_1C_a/(1 - K_1C_a)^2$ can be established, for $K_1C_a < 1$, by expanding the last two terms by long division.) It is apparent that equation 2 will apply only if $K_1C_a < 1$, and that as K_1C_a approaches 1, A_b approaches $[S]$. For $K_1C_a > 1$, it is necessary that $A_b = [S]$. Equation 2 can be rearranged to obtain a relation between θ (the fraction of the poly-U sites to which A molecules are bound) and C_a. Thus,

$$\theta = \frac{A_b}{S} = \frac{(K_1C_a)^n \left[\frac{n}{(1 - K_1C_a)} + \frac{K_1C_a}{(1 - K_1C_a)^2} \right]}{1 + (K_1C_a)^n \left[\frac{n}{(1 - K_1C_a)} + \frac{K_1C_a}{(1 - K_1C_a)^2} \right]} \quad \ldots \quad (3)$$

Equation (3) expresses the dependence of θ on C_a in terms of only two

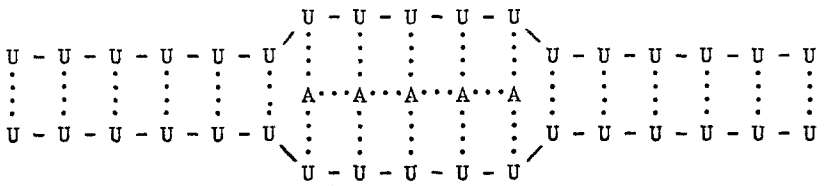

Figure 1. Cooperative binding of adenosine to polyuridylic acid.

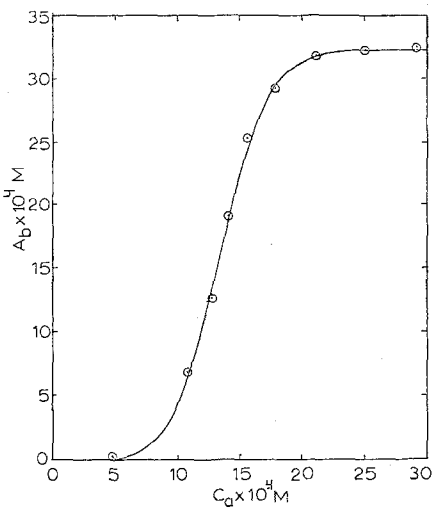

Figure 2. Binding isotherm for adenosine vs poly-U (9.06 mM). Experimental points, 0; calculated curve for n = 5, K = 432 M^{-1} and S = 32.4×10^{-4}M, ————.

adjustable parameters, K_1 and n. We have developed a computer method to obtain least squares values of K_1 and n from sets of θ, C_a data. If $[S]$ is also taken as an unknown, the program can be used to infer all three constants (k_1, n and $[S]$) from sets of values of A_b, C_a. The application of equation (3) to binding data is considered in the results section.

EXPERIMENTAL:

Adenosine was obtained from Calbiochem (A grade), adenosine-8-^{14}C from International Chemical & Nuclear Corp., and polyuridylic acid from Miles Labs (M.W. > 100,000; ε=9,800). Visking dialysis tubing was treated as described by Huang and Ts'o (1).

All solutions contained 0.10 M sodium chloride, 25 mM potassium dihydrogen phosphate, 25 mM sodium hydrogen phosphate, and 1 mM magnesium chloride. The poly-U concentration was 9.06 mM (A_{260}) and the adenosine concentrations were from 1.0 to 10.8 mM (A_{260}; ε = 15,400). The dialysis cells were loaded with 180 µl poly-U solution on one side of the membrane and 180 µl adenosine solution on the other side. The cells were equilibrated for 72 hours at 0-2° C. In this temperature range, the poly-U (melting temperature 6°) is in the double helix conformation(1,2,3).

The final concentration of free adenosine, C_a, was found by removing 50 µl solution from the monomer side, diluting it to 5.00 ml, and analyzing by uv at 260 nm. The bound adenosine, A_b, is equal to the initial concentration of adenosine minus $2C_a$.

RESULTS:

The results from the equilibrium dialysis experiment are shown as a plot of A_b vs C_a in Figure 2. The experimental points are circles, and the solid line is the calculated curve for n = 5. The optimum values of K_1 and $[S]$ for a few values of n are listed in Table 1. The values of 432 for K_1 and 32.4×10^{-4} for $[S]$ (for n = 5) have the lowest root mean square deviation and, therefore, are taken as the optimum values. However, the parameters for n = 6 are almost as good. The calculated value for $[S]$ is always close to the maximum experimental value of A_b. K_1 always increases and $[S]$ always decreases slightly as n is increased. Data from modified polyuridylic acids will be presented in a later paper.

DISCUSSION:

Binding isotherms obtained from equilibrium dialysis measurements have been analyzed by various methods to obtain thermodynamic information about the binding equilibria. Huang and Ts'o (1) and Davies and Davidson (3) have used an equation ($d\theta/d\ln C_a = \frac{1}{2}e^{-w/2RT}$) to infer the stacking energy, w, of adenosine in the triple-helix complex. The interpretation of data requires the determination of the slope of the binding isotherm

Table 1. Least squares results for selected n's.

n	K_1 [a]	S x10^4 [a,b]	RMSD x10^4 [c]
4	406(5)	32.7(.5)	1.0
5	432(3)	32.4(.2)	.5
6	454(3)	32.1(.3)	.7
7	472(5)	31.8(.5)	1.1

[a] Numbers in () are standard errors.

[b] Molar units.

[c] Root mean square deviation of A_b (Calc.) from A_b (Exp.).

at the midpoint ($\theta = 0.5$). Schmitz and Schurr (8) used a grand partition function Ising model, similar to that employed by Rice and Nagasawa (9) in treating polyelectrolyte solutions; they obtained the same relation between the slope of the binding isotherm and stacking energy as that inferred from earlier lattice treatments. In addition, they were able to relate the total free energy of formation of the complex to the concentration of free adenosine at $\theta = 0.5$. They were unable to obtain a good fit of the entire binding curve, although this may have been the result of experimental errors in the binding data.

Our treatment of the data provides a fit of the complete binding isotherm and allows both the slope and the free adenosine concentration at $\theta = 0.5$ to be determined accurately by mathematical analysis. These derived values for the slope and C_a can then be used in the treatments listed above to obtain more meaningful results. For example, our analysis of data published by Huang and Ts'o (1) gives a slope of 2.9 compared to

Table 2. Summary of thermodynamic data.

Source of data	$\frac{d\theta}{d\ln C_a}$	w (kcal/mol)	$-RT\ln K_1$ (kcal/mol)
Huang and Ts'o (ref.1)	30-60	-5 to -6	
Our Calc.	2.88	-2.65	-2.91
Pitha and Ts'o (ref.2)			-3.5
Our Calc.	1.65	-2.05	-3.13
Davies and Davidson (ref.3)	1.43±.27	-1.0±.1	
Our Calc.	1.26	1.76	-3.10
This Paper	1.64	-2.04	-3.29

their estimate 30-60. We calculate a stacking energy of -2.7 kcal/mole compared to their value of -5 to -6 kcal/mole of adenosine. When $[S]$ ($=A_b$max) is taken as an experimental value, the average of four runs (in which $[S]$ varied from 32.28 - 40.24) gives average values of K_1 = 398 ± 36 and n = 4.83 ± 0.22. This average value of K_1 corresponds to ΔG = -3.3 kcal. mole^{-1} for the free energy of binding of one mole of adenosine in the complex. A comparison of our data with those of prior investigators is shown in Table II.

ACKNOWLEDGEMENT:

This work was supported by Grant GM 19362 from the Department of Health, Education and Welfare and Grant No. GP-33519X from the National Science Foundation. We also acknowledge the help of Mr. Edwin Lane, who wrote the computer program for fitting the binding data.

REFERENCES

1. W. M. Huang and P. O. P. Ts'o, J. Mol. Biol., 16, 523 (1965).

2. P. M. Pitha and P. O. P. Ts'o, Biochem., 8, 5206 (1969).
3. R. J. H. Davies and N. Davidson, Biopolymers, 10, 1455 (1971).
4. F. B. Howard, J. Frazier, M. F. Singer and H. T. Miles, J. Mol. Biol., 16, 415 (1966).
5. B. W. Bangerter and S. I. Chan, Proc. Nat. Acad. Sci. U. S., 60, 1144 (1968).
6. V. N. Damle, Biopolymers, 11, 1789 (1972).
7. J. G. Burr, T. L. McDowell and S. D. Christian, Radiation Research, 55, 568 (1973).
8. K. S. Schmitz and J. M. Schurr, Biopolymers, 9, 697 (1970).
9. S. A. Rice and M. Nagasawa, Polyelectrolyte Solutions, Academic Press, New York, 1961, pp. 314 et. seq.