CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 36, No. 8 August 1988

Regular Articles

[Chem. Pharm. Bull. 36(8)2735—2741(1988)]

Interaction of Theophylline with L-Tryptophan. Study by Proton Nuclear Magnetic Resonance Spectroscopy

JUZIRO NISHIJO,* EMIKO IWAMOTO, IKUKO YONETANI, SATSUKI TOKURA, and MAKIKO SUGIURA

Kobe Women's College of Pharmacy, Motoyama-kitamachi, Higashinada-ku, Kobe 658, Japan

(Received October 16, 1987)

The interaction of theophylline with L-tryptophan was investigated in aqueous solution adjusted to ionic strength 0.20, at pD 6.2, by using proton nuclear magnetic resonance spectroscopy. Theophylline interacted with L-tryptophan at a molar ratio of 1 : 1 by parallel stacking. The thermodynamic parameters obtained for the complexation were as follows: $\Delta G^\circ = -1.52$ kcal/mol ($K = 13.1\ M^{-1}$), $\Delta H^\circ = -5.1$ kcal/mol, $\Delta S^\circ = -12.3$ e.u. It was found that the driving force for parallel stacking is a combination of electrostatic, polarization, and dispersion interactions, and the contribution of hydrophobic bonding is small. A possible stacking mode is postulated, based on the complexation shifts and π-electron charge distributions of theophylline and L-tryptophan.

Keywords——theophylline; L-tryptophan; ^{1}H-NMR; stability constant; thermodynamic parameter; complexation shift; parallel stacking

When various medicinal drugs are mixed, they often interact with each other and sometimes form complexes. These phenomena have important implications in pharmaceutics. For example, complex formation give rise to increases in solubility and stability,[1] and administration of drugs in a mixture is expected to show different efficacy from that obtained by single administration. Thus, it is important to know how each drug interacts with any other drug. Also, it is important for elucidating biomechanisms and modes of drug action to study the complexes formed in aqueous solution.[2]

In the present study, we investigated the interaction of theophylline with an aromatic amino acid, L-tryptophan, by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy since this amino acid is a constituent of serum albumin and was suggested to be the binding site of serum albumin for certain drugs.[3] On the other hand, theophylline is an important drug and moreover, as it is purine base, it is interesting from the standpoint of the interaction between protein and nucleic acid to study the interaction of theophylline with L-tryptophan. Although there are many reports on the interaction of xanthine derivatives with drug molecules,[4] little work has been done on the interaction of drugs with L-tryptophan.

Experimental

Materials——Reagent grade theophylline and L-tryptophan were used without further purification. Deuterium oxide (99.8%) was used as received from Merck Co. Other chemicals were of reagent grade. Na_2DPO_4 and KD_2PO_4 were obtained by treating Na_2HPO_4 and KH_2PO_4 with deuterium oxide and evaporating the solvent, and were used to adjust pD.

^{1}H-NMR Spectra——^{1}H-NMR spectra were recorded in deuterium oxide solution at pD 6.2 (10 mM phosphate buffer) adjusted to ionic strength 0.20 with NaCl, on a Varian XL-200 (200 MHz) spectrometer with tetramethylsilane (TMS) as an external reference. Bulk susceptibility corrections to chemical shifts of theophylline or amino acid were examined, but were found to be unnecessary, because the volume magnetic susceptibilities of the test solutions were equal to that of the medium having no theophylline and amino acid, owing to the low concentrations of theophylline and amino acid used. Temperatures of measurement were 0, 15, 25, and 40 °C, each controlled to ± 0.2 °C. The chemical shifts were reproducible to better than 0.003 ppm. The conditions for Fourier transfer measurements were: spectral width, 2400 Hz; pulse width, 3.0 μs (flip angle, about 40 °); acquisition time, 3 s; number of data points, 16384; number of transients, 40—800.

Ultraviolet (UV) Spectra——UV spectra were measured with a Shimadzu UV-160 spectrophotometer in aqueous solution at pH 6.5 (10 mM phosphate buffer) at 25 °C.

Results and Discussion

First, the interaction of theophylline with L-tryptophan was studied at 25 °C. To reduce the effect of self-association of theophylline,[5] a low concentration of theophylline was used. The ^{1}H-NMR spectrum of 1×10^{-3} M theophylline shows signals due to 8-H, 3-Me, and 1-Me at 7.31, 2.86, and 2.65 ppm (from external TMS), respectively, with a relative intensity of 1 : 3 : 3. Then, various concentrations of L-tryptophan from 1.0×10^{-2} to 4.5×10^{-2} M were added to the solution of theophylline at a constant concentration 1.00×10^{-3} M and the ^{1}H-NMR signals of theophylline were examined. With an increase in L-tryptophan concentration, the signals of 1-Me, 3-Me, and 8-H of theophylline shifted upfield and the order of magnitudes of upfield shifts was 3-Me > 1-Me > 8-H. It is clear that theophylline associates with L-tryptophan by parallel stacking to form a complex, and these upfield shifts occur as a result of the ring-current magnetic anisotropy as well as local magnetic anisotropy of L-tryptophan.[6] Then, similar experiments were carried out at 0, 15, and 40 °C. The results showed that the upfield shift changes of theophylline signals caused by adding L-tryptophan become larger as the temperature is lowered. The order of magnitudes of upfield shifts was 3-Me > 1-Me > 8-H, independently of the temperature of measurement. It was found that the parallel stacking process is an exothermic reaction and the interaction of theophylline with L-tryptophan is greater at lower temperature than at higher temperature.

Next, the interaction of theophylline with L-tryptophan was investigated at a fixed concentration, 1.00×10^{-3} M, of the latter while varying the former concentration from 1.00×10^{-2} to 4.5×10^{-2} M. The interaction was examined at 25 and 15 °C. The ^{1}H-NMR spectrum of 1.00×10^{-3} M L-tryptophan at 25 °C is shown in Fig. 1. The assignment of the proton signals was undertaken on the basis of homonuclear decoupling and comparison with published data.[7] These signals shifted upfield when theophylline was added. The order of magnitudes of upfield shifts was 4-H = 7-H > 6-H $\geq$ 5-H > 2-H > β-H > α-H at both temperatures, and the upfield shift changes were larger at 15 °C than at 25 °C. It is clear that the benzene and pyrrole nuclei of L-tryptophan are located above the pyrimidine or imidazole ring of theophylline, and these upfield shifts occur as a result of the ring-current magnetic anisotropy as well as the local magnetic anisotropy of theophylline. The experimental results described above also indicate that the interaction of theophylline with L-tryptophan occurs not by perpendicular but by parallel stacking.

Then, we attempted to determine the apparent stability constant K of the stacked complex using Eq. 1 according to the procedure of Hanna and Ashbaugh assuming[8] a 1 : 1 complex

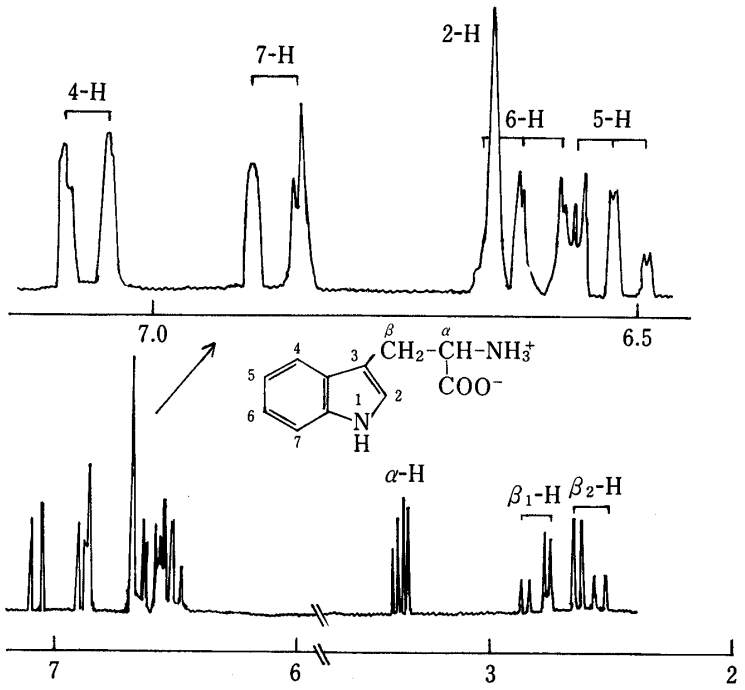

Fig. 1. The 200 MHz ^{1}H-NMR Spectrum of L-Tryptophan in D_2O

$$\frac{1}{\Delta_{obsd}} = \frac{1}{KC(\delta_c - \delta_m)} + \frac{1}{\delta_c - \delta_m} \tag{1}$$

C: concentration of L-tryptophan or theophylline
δ_c: chemical shift of theophylline or L-tryptophan proton in the complex form
δ_m: chemical shift of theophylline or L-tryptophan proton in the uncomplexed form. Therefore, $\delta_c - \delta_m$ is the complexation shift
Δ_{obsd}: the difference between observed chemical shift and δ_m

Hanna–Ashbaugh plots are shown in Figs. 2 and 3. Also, K and $\delta_c - \delta_m$ are shown in Table I. The plot in the case of adding theophylline to a fixed concentration of L-tryptophan showed a slight deviation from linearity, as the theophylline concentration was increased. The deviation is considered to result from the self-association of theophylline. Accordingly, K and $\delta_c - \delta_m$ were determined by extrapolation of the straight line obtained in the range of low concentration. However, the reliability of these values may be inferior to that of the values obtained from Fig. 2. Therefore, K values obtained by adding L-tryptophan to a fixed concentration of theophylline as shown in Fig. 2, were used in the following discussion.

Thermodynamic parameters were derived by using Eqs. 2, 3, and 4,

$$\Delta G^\circ = -2.303\, \boldsymbol{R}T \log K \tag{2}$$

$$\log K = -\frac{\Delta H^\circ}{2.303\boldsymbol{R}T} + \text{constant} \tag{3}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{4}$$

where ΔG°, ΔH°, ΔS°, T, and $\boldsymbol{R}$ are standard free energy change, standard enthalpy change, standard entropy change, absolute temperature, and gas constant, respectively. The van't Hoff plots of the data based on Eq. 3 are shown in Fig. 4. The thermodynamic parameters obtained are listed in Table I. From the thermodynamic parameters, the driving force for the complexation of theophylline with L-tryptophan is the enthapy term, while the entropy term

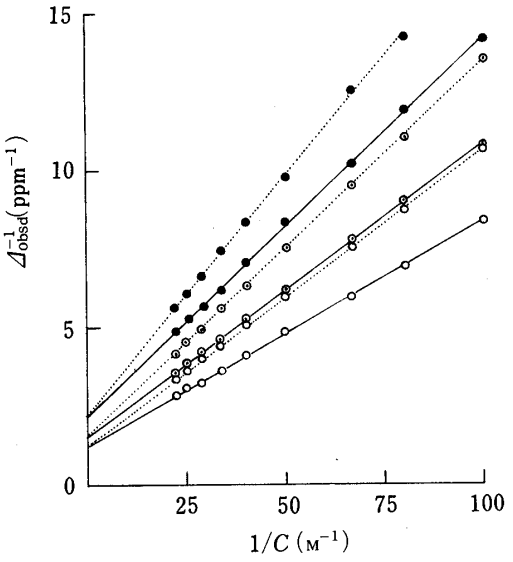

Fig. 2. Plots of Δ^{-1}_{obsd} Values of Theophylline Proton Signals *vs.* Reciprocal of L-Tryptophan Concentration

---⊙---, 1-CH_3 (25 °C); —⊙—, 1-CH_3 (15 °C); ---○---, 3-CH_3 (25 °C); —○—, 3-CH_3 (15 °C); ---●---, 8-H (25 °C); —●—, 8-H (15 °C).

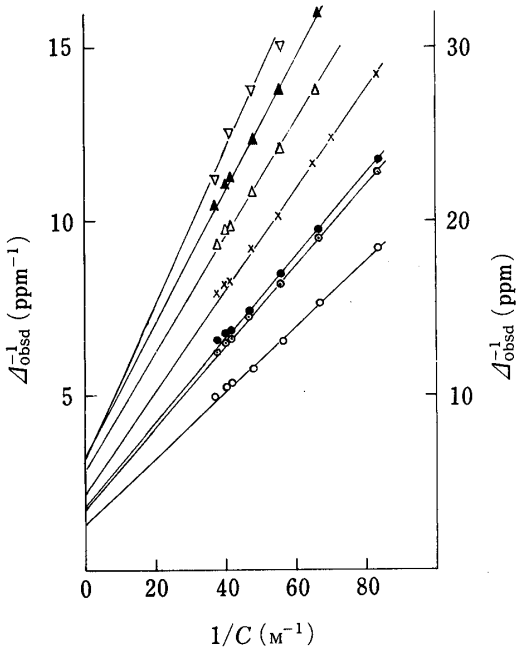

Fig. 3. Plots of Δ^{-1}_{obsd} Values of L-Tryptophan Proton Signals *vs.* Reciprocal of Theophylline Concentration at 25 °C

○, 4-H and 7-H; ⊙, 6-H; ●, 5-H; ×, 2-H; △, β_1-H; ▲, β_2-H; ▽, α-H. The right scale is used for ▽ and the left scale for the others.

TABLE I. Complexation Shift, Apparent Stability Constant, and Thermodynamic Parameters for Complex Formation of Theophylline with L-Tryptophan at pD 6.2

	$\delta_c-\delta_m$ [a] (ppm)				K [a] (M^{-1})				$\Delta G°$ (kcal/mol)				$\Delta H°$ [a]	$\Delta S°$ [a]
	0	15	25	40	0	15	25	40	0	15	25	40	(kcal/mol)	(e.u.)
	(°C)				(°C)				(°C)					
Theophylline														
8-H	0.52	0.49	0.50	0.51	24.4	16.8	12.8	9.0						
3-CH_3	0.81	0.77	0.80	0.81	25.3	17.2	13.2	8.7	−1.75	−1.62	−1.52	−1.33	−5.1	−12.3
1-CH_3	0.62	0.64	0.63	0.65	25.5	17.0	13.4	8.4					±0.2	±0.7
				Mean	25.1	17.0	13.1	8.5						
L-Tryptophan														
7-H		0.70	0.73			20.3	14.7							
6-H		0.53	0.56			21.9	14.9							
5-H		0.53	0.54			20.7	15.4							
4-H		0.70	0.73			20.3	14.7							
2-H		0.42	0.45			20.7	16.0							
β_1-H		0.37	0.37			22.8	16.2							
β_2-H		0.29	0.29			24.7	18.3							
α-H		0.16	0.15			20.0	14.8							

a) These values were determined by the least-squares method. Average probable errors; ±7% for $\delta_c-\delta_m$ and ±6% for K.

decreased. As possible interactions acting between molecules having such thermodynamic parameters, there are charge transfer force, hydrogen bonding, and a combination of electrostatic, polarization, and dispersion interactions. Judging from the ^{1}H-NMR spectra, the driving force of parallel stacking is not hydrogen bonding. To examine whether or not

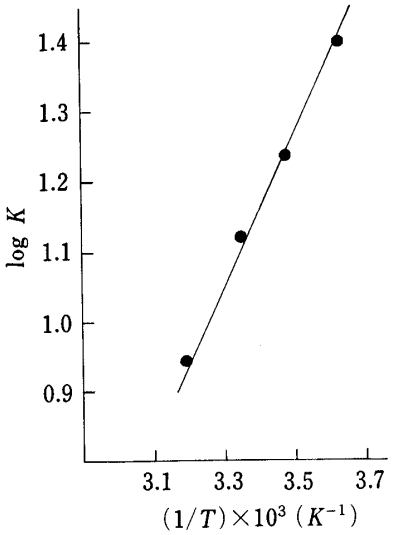

Fig. 4. The van't Hoff Plot of the Data in Table I

The K values obtained from the induced chemical shift changes for theophylline protons were used as explained in the text.

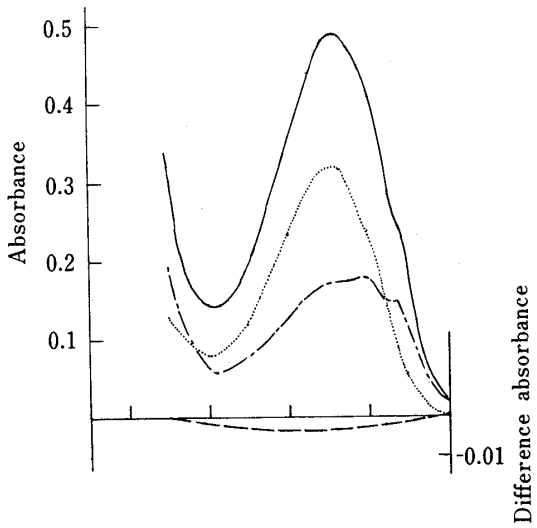

Fig. 5. UV Spectra of Theophylline (------), L-Tryptophan (– – –), an Equimolar Mixture of the Two (——) and the Difference Spectrum (— —)

The concentrations of theophylline, L-tryptophan, and the equimolar mixture were all 3×10^{-4} M. The spectrophotometric cell had a pathlength of 1 mm for UV spectral measurements.

charge transfer force participates in the interaction, UV spectra of the complex and its components were measured (Fig. 5). The difference absorption spectra show that no absorption due to charge transfer complex formation is observable, but a weak negative band is seen due to the hypochromic effect of the stacking interaction. Therefore, it is considered that the stacking interaction of theophylline with L-tryptophan primarily involves a combination of electrostatic, polarization, and dispersion interactions. Judging from the upfield shifts of proton signal of the amino acid, $-CH_2-CH(NH_3^+)-COO^-$ may not contribute much to the parallel stacking. Also, the interaction of theophylline with L-valine was examined by using ^{1}H-NMR spectroscopy in the same way as with L-tryptophan and it was found that no interaction occurs between them. Consequently, it is considered that the stacking interaction occurs by π–π interactions of aromatic rings.[9] Since the interaction of theophylline with L-tryptophan is accompanied with negative standard enthalpy and negative standard entropy changes, the contribution of hydrophobic bonding to the interaction is small.

The stacking mode of theophylline and L-tryptophan will be discussed taking into account the magnitudes of the upfield shift (complexation shift) of each proton signal of theophylline and L-tryptophan. It was considered that parallel stacking occurs with the maximal overlapping.[10] Also, it was assumed that the sum of ring-current and local anisotropy effects of the benzene ring is stronger than that of the pyrrole ring in tryptophan.[6] The signal of 3-Me showed the largest upfield shift, which suggests that the pyrimidine nucleus is arranged in such a way as to place 3-Me nearer to the benzene and pyrrole rings than 1-Me. Therefore, 8-H may be located above the pyrrole ring. On the other hand, the signals of benzene protons showed larger upfield shifts than that of the pyrrole proton (2-H), which suggests that the benzene ring is located above the pyrimidine ring rather than the imidazole one, and the pyrrole ring is located above the imidazole one. Since theophylline interacts with the aromatic amino acid, L-tryptophan, but not L-valine, it seems reasonable to consider that π-electrons of the rings play important role. To consider the stacking mode in detail, π-

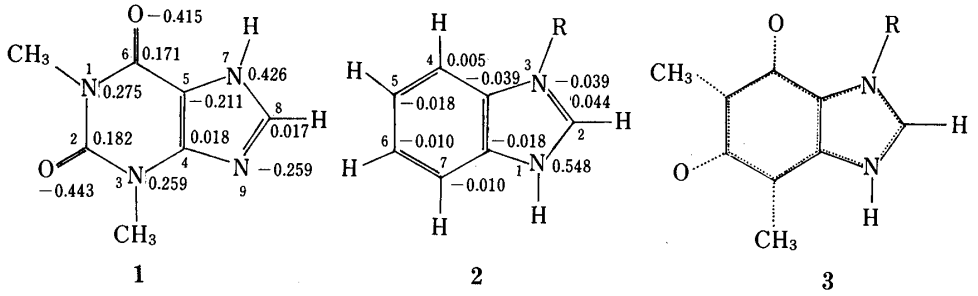

Fig. 6. π-Electron Charge Distributions of Theophylline (**1**) and L-Tryptophan (**2**) and a Possible Mode of Parallel Stacking (**3**)

electron charge distributions were calculated by the CNDO/2 method (Fig. 6). The geometry was obtained from the literature.[11] Electrostatic, polarization and dispersion forces were considered to be the driving force for the parallel stacking. It has been reported[12,13] that for distances which are not significantly larger than the dimensions of the molecules, the dipole approximation is inadequate and the monopole one, *i.e.*, considering all the negative and positive charges in the system as interacting in a single coulombic fashion, is adequate. It has been shown[14] that only the electrostatic interaction depends strongly upon the orientation of the molecules. That is, overlapping between a position of more positive charge and one of more negative charge or between a position of less positive charge and one of less negative charge may occur. Therefore, the orientation of the molecules in the parallel stacking structure may be fixed. In view of the charge distributions of π-electrons and the order of upfield shift of each proton signal (complexation shift), a possible stacking mode of theophylline with L-tryptophan postulated (Fig. 6). This stacking mode qualitatively accounts for the fact that the order of the magnitudes of upfield shifts of theophylline protons is 3-Me > 1-Me > 8-H; 3-Me is located above the benzene and pyrrole rings, 1-Me is located above the benzene ring, and 8-H is located above the pyrrole ring. Also, the order of magnitudes of upfield shifts of L-tryptophan protons, 4-H = 7-H > 6-H $\geq$ 5-H > 2-H > β-H > α-H is explicable in the same way; 4-H and 7-H are located above the pyrimidine and imidazole rings, 5-H and 6-H are located above the pyrimidine ring, 2-H is located above the imidazole ring, β-H is located somewhat away from both rings, and α-H is further away from them.

The authors have reported[3b] that theophylline binds to bovine serum albumin, albeit weakly, and a tryptophan residue is presumed to be the binding site. In the present studies, it was found that theophylline interacts with L-tryptophan, even when it exists as the free amino acid. The standard enthalpy change ($\Delta H_1^\circ = -9.72$ kcal/mol) and standard entropy change ($\Delta S_1^\circ = -19$ e.u.) obtained for the primary binding site of albumin are similar to the present results.

References and Notes

1) *a*) T. Higuchi and K. Connors, *Adv. Anal. Chem. Instrum.*, **4**, 150 (1965); *b*) M. Yoshida and M. Samezima, *Yakugaku Zasshi*, **93**, 149 (1973); *c*) N. I. Al-Ani and H. N. Borgan, *J. Pharm. Sci.*, **67**, 1381 (1978); *d*) T. Higuchi and J. L. Lack, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 351 (1954).
2) I. Horman and B. Dreux, *Helv. Chim. Acta*, **67**, 760 (1984).
3) *a*) T. P. King and M. Spencer, *J. Biol. Chem.*, **22**, 6134 (1970); *b*) J. Nishijo, N. Morita, S. Asada, H. Nakae, and E. Iwamoto, *Chem. Pharm. Bull.*, **33**, 2648 (1985).
4) *a*) T. Okano and K. Aita, *Yakugaku Zasshi*, **86**, 1343 (1967); *b*) K. A. Connors, M. H. Infeld, and B. J. Kline, *J. Am. Chem. Soc.*, **92**, 3597 (1969); *c*) D. E. Guttman and M. Y. Athaye, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 687 (1969).
5) A. L. Thakker, L. G. Tensmeyer, and W. L. Wilham, *J. Pharm. Sci.*, **60**, 1269 (1971).
6) C. Giessner-Prettre and B. Pullman, *Biochem. Biophys. Res. Commun.*, **101**, 921 (1981).

7) G. C. K. Roberts and C. D. Jardetzky, *J. Biol. Chem.*, **24**, 447 (1970).
8) M. W. Hanna and A. L. Ashbaugh, *J. Phys. Chem.*, **68**, 811 (1964).
9) *a*) J. A. Plalzier and R. E. De Nerve, *J. Pharm. Sci.*, **71**, 552 (1982); *b*) M. D. Johnston, F. P. Gasparro, and I. D. Kuntz, *J. Am. Chem. Soc.*, **91**, 5715 (1969).
10) K. A. Connors, M. H. Infeld, and B. J. Kline, *J. Am. Chem. Soc.*, **91**, 3597 (1969).
11) T. Takigawa, T. Ashida, Y. Sasada, and M. Kakudo, *Bull. Chem. Soc. Jpn.*, **39**, 2369 (1966).
12) B. Pullman, P. Claverie, and J. Caillet, *Biochemistry*, **5**, 905 (1966).
13) J. Bertran, *J. Theor. Biol.*, **34**, 353 (1966).
14) J. N. Kikkert, G. R. Kelly, and Y. Kurucsev, *Biopolymers*, **12**, 1459 (1973).