Interactions of Adenine, Thymine, and Uracil with Epinephrine: UV Studies

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Abstract □ Based upon UV absorption studies, charge transfer complex formations by the nucleic acid bases adenine, thymine, and uracil with epinephrine were demonstrated. The pertinent equilibrium constants were calculated by assuming 1:1 complexes using the Benesi–Hildebrand equation and were found to decrease in the following order: uracil–epinephrine > thymine–epinephrine > adenine–epinephrine. The values of $\Delta G°$, $\Delta H°$, and $\Delta S°$ were calculated for the various interactions. Theoretical arguments are presented as to the possible stoichiometries of the various complexes existing in aqueous solutions. The electron-donating or electron-accepting abilities of the interacting molecular species are discussed.

Keyphrases □ Nucleic acid bases—adenine, thymine, and uracil, interaction with epinephrine, UV absorption studies □ Epinephrine—interaction with adenine, thymine, and uracil, UV absorption studies □ Complex formation—adenine, thymine, and uracil, with epinephrine, UV absorption studies □ UV spectrophotometry—absorption studies, interaction of adenine, thymine, and uracil with epinephrine

Catecholamines form a biologically important class of chemical compounds, called sympathomimetics, which mimic the action of the neurohumoral transmitters epinephrine and norepinephrine.

The receptors for catecholamines are considered to contain adenosine triphosphate (1). The common occurrence of phosphate and magnesium ions beside adenosine triphosphate at the adrenergic sites suggested that these amines form complexes with one or more of these ions during storage or the accompanying initiation of their biological actions. The existence of such compounds *in vivo* has not yet been established. Seifter *et al.* (2) synthesized the complex *in vitro*. The reaction among catecholamines, magnesium monophosphate ion, and the magnesium adenosine triphosphate complex was demonstrated by the formation of a water-soluble complex. This complex exhibits characteristic UV absorption and fluorescence spectra.

Kirshner *et al.* (3) investigated the uptake and storage of catecholamines and obtained further evidence for the storage of these biogenic amines in association with adenosine triphosphate and protein in the chromaffin granules. The presence of magnesium was not found to be essential for the uptake of catecholamines, indicating that an enzymatic system is not required to transfer these amines intracellularly.

The significance of nucleic acids for biological systems (4–6) has led to their being considered as possible targets for the action, directly or indirectly, of many drugs. The interactions between nucleic acids and some drugs were studied previously (7, 8). Indirect actions of drugs were attributed to interference with protein synthesis, either induction or inhibition (9, 10). For example, catecholamines affected the production of cyclic 3′,5′-adenosine monophosphate indirectly through their interferences with the enzymatic activity of adenyl cyclase (11). Cyclic 3′,5′-adenosine monophosphate was considered as the causative agent for the inhibitory responses.

Therefore, to gain a more basic understanding of: (*a*) the adrenergic receptors, (*b*) the mechanism of action of catecholamines at the molecular level, and (*c*) the possibility of adenosine triphosphate-like action of some other nucleoside triphosphates on the storage of catecholamines in the adrenal medulla and the splenic nerves, the nature of the complexes between nucleic acids, including synthetic polynucleotides and their derivatives, and catecholamines must be investigated.

The present work deals with the interactions of adenine, thymine, and uracil with epinephrine using a UV spectroscopic method.

EXPERIMENTAL

Epinephrine hydrogen tartrate[1], thymine[1], a standard solution of hydrochloric acid[1], adenine[2], and uracil[2] were obtained commercially.

Solutions for measurement were prepared by diluting concentrated stock solutions. All mixtures contained a fixed concentration of the nucleic acid base, 0.02 *M*, and the concentration of epinephrine was varied from 0.3 to 0.8 *M* in 0.1 *N* HCl. Solutions were freshly prepared each day, and their absorbances were measured within 12 hr.

An oxide film[3] was used to calibrate the spectrophotometer[4]. Constant temperatures were achieved throughout the measurement period by using a thermostated cell holder connected to a constant-temperature circulator and refrigerator[5]. The stoppered cells (1-cm path length) were flushed with nitrogen gas to avoid condensation of moisture at the cell surfaces when working at low temperatures.

The baseline was recorded before running each experiment, using the same solvent in the reference and sample compartments. Most work was done at 18–20° in a room with subdued light to avoid oxidations induced by light. At the beginning of the measurements of a set of solutions, the spectra of the pure components were taken at the working temperature to detect any decomposition.

RESULTS

The formation of a complex between a nucleic acid base and epinephrine was detected by observing the shift in the UV spectrum of the mixture toward longer wavelengths when the nucleic acid base solution in 0.1 *N* HCl was used as a reference (Fig. 1). The absorbance of a complex at a given wavelength was estimated by measuring the absorbance of the entire mixture and subtracting from it the sum of the absorbances, at the same wavelength, of equimolar solutions of the pure components. The experimental data used to calculate the various quantities (Table I) were actually measured in regions where the absorption of the free components was as small as possible and that of the complex was quite appreciable. The accuracy of the measurements was ±0.005 absorbance unit.

The absorbances of complexes obtained experimentally were used to calculate equilibrium constants using the Benesi–Hildebrand equation:

$$\frac{(A_0)}{A_\lambda^{AD}} = \frac{1}{\epsilon_\lambda^{AD}} + \frac{1}{K_c^{AD}\epsilon_\lambda^{AD}(D_0)} \qquad \text{(Eq. 1)}$$

[1] BDH Biochemicals, Ltd., Poole, England.
[2] Hopkin and Williams Chemical Co., Chadwell Heath, Essex, England.
[3] Holmium.
[4] Model 402 Perkin-Elmer UV–visible spectrophotometer.
[5] Model FK2, Haake Instrument Co.

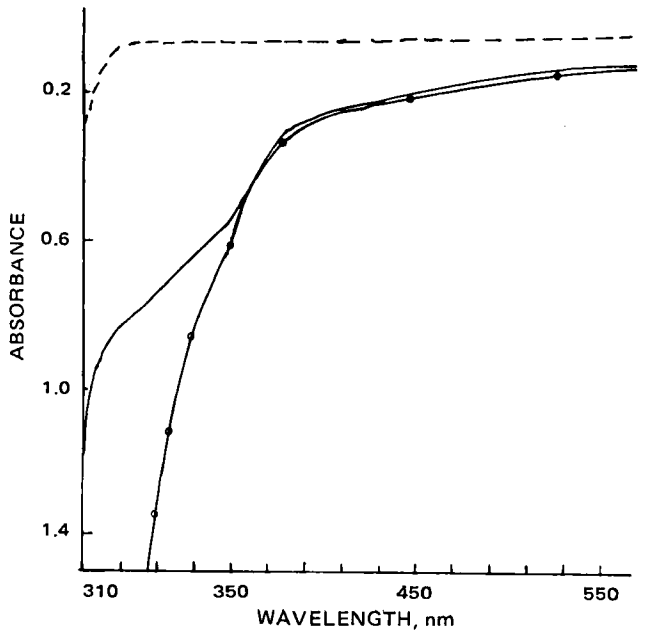

Figure 1—*Absorption spectra of 0.8 M epinephrine hydrogen tartrate (——), 2×10^{-2} M adenine (- - -), and 0.8 M epinephrine hydrogen tartrate plus 2×10^{-2} M adenine (O—O). All solutions were in 0.1 N HCl, and measurements were at 2°.*

where (A_0) and (D_0) are the total concentrations of the interacting species, A_λ^{AD} and ϵ_λ^{AD} are the absorbance and molar absorptivity of the complex at a given wavelength, and K_c^{AD} is the equilibrium constant.

The complex between cytosine and epinephrine showed a high degree of absorption in the region where the base itself absorbs. Because of this overlap, equilibrium constants could not be calculated.

Equation 1 is based on the assumptions that a 1:1 complex is formed:

$$A + D = AD \qquad \text{(Eq. 2)}$$

and that the total concentration of compound A, (A_0), is much less than the total concentration of compound D, (D_0), so that (D_0) can be approximated to the concentration of the free species, *i.e.*, $(D) \simeq (D_0)$.

The equilibrium constant and the molar absorptivity of Eq. 1 can

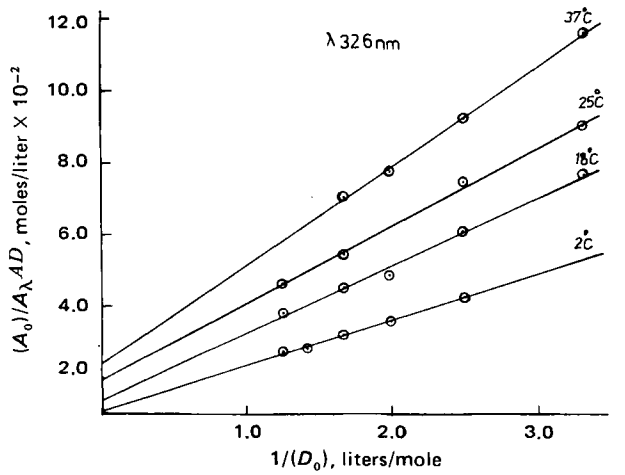

Figure 2—*Plots of $(A_0)/A_\lambda^{AD}$ versus $1/(D_0)$ of epinephrine hydrogen tartrate solutions containing adenine in 0.1 N HCl at different temperatures. The adenine total concentration, (A_0), = 2×10^{-2} M, and the epinephrine hydrogen tartrate total concentration, (D_0), = 0.3–0.8 M; A_λ^{AD} represents the absorbance of the complex at the wavelength. Curves have the same intercepts as that of 2°. They were separated to avoid crowding.*

Table I—Equilibrium Constants (K), Standard Free Energy ($\Delta G°$), Standard Enthalpy ($\Delta H°$), and Standard Entropy ($\Delta S°$) Changes Associated with the Interactions of Nucleic Acid Bases with Epinephrine Hydrogen Tartrate in Aqueous Solutions Containing 0.1 N HCl together with Molar Aborptivities (ϵ) at Different Wavelengths (λ)

Temperature ±0.5°	K, M^{-1} ±0.01	$\Delta G°$, kcal/mole	$\Delta H°$, kcal/mole	$\Delta S°$, cal/deg/mole ±0.1
		Adenine–Epinephrine		
2	0.79	0.129		
18	0.52	0.376		
			−3.857	−14.5
25	0.45	0.468		
37	0.35	0.635		
λ = 326 nm; ϵ = 103.1				
2	0.78	0.131		
18	0.55	0.348	−3.569	−13.5
25	0.48	0.438		
λ = 328 nm; ϵ = 79.4				
		Thymine–Epinephrine		
2	0.97	0.017		
18	0.73	0.183	−2.541	−9.3
37	0.57	0.347		
λ = 314 nm; ϵ = 51.8				
2	0.63	0.248		
18	0.53	0.369		
			−3.048	−11.8
25	0.44	0.487		
37	0.40	0.564		
λ = 316 nm; ϵ = 50.0				
		Uracil–Epinephrine		
3	1.04	−0.023		
18	0.69	0.209	−6.936	−24.5
37	0.49	0.433		
λ = 316 nm; ϵ = 31.3				

be obtained from the slope and intercept of a curve plotting $(A_0)/A_\lambda^{AD}$ *versus* $1/(D_0)$ when keeping (A_0) constant. Representative plots are shown in Fig. 2.

The standard enthalpy change, ΔH^0, of an interaction can be obtained graphically by plotting $\log K_c^{AD}$ *versus* $1/T$ of the following well-known equation:

$$\log K_c^{AD} = -\frac{\Delta H^0}{2.303RT} + \text{constant} \qquad \text{(Eq. 3)}$$

A plot of Eq. 3 is shown in Fig. 3. Linear slopes were also obtained for the other combinations.

The standard free energy change, ΔG^0, is calculated from:

$$\Delta G^0 = -RT \ln K_c^{AD} \qquad \text{(Eq. 4)}$$

The standard entropy change, ΔS^0, is calculated from:

$$\Delta G^0 = \Delta H^0 - T\,\Delta S^0 \qquad \text{(Eq. 5)}$$

All slopes and intercepts used to calculate the quantities in Table I and the figures were determined statistically by the method of least squares. The thermodynamic parameters together with the molar extinction coefficients are presented in Table I.

DISCUSSION

It has been demonstrated that catechols have strong tendencies for electron donation in forming complexes with many substances (12, 13), as evidenced by the appearance of new absorbing species.

Nucleic acid bases also have been shown to form charge transfer complexes with many compounds (14). Recently, the authors (15) demonstrated that catechol has the ability to form charge transfer complexes with nucleic acid bases (adenine, cytosine, thymine, and uracil) in aqueous solutions containing 0.1 N HCl. In that study,

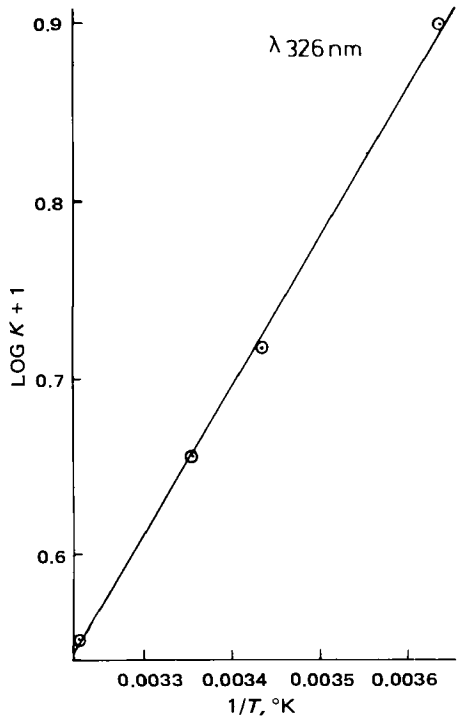

Figure 3—*Log* K *of the adenine–epinephrine interaction* versus *1*/T.

catechol was considered to be the electron donor and nucleic acid base the electron acceptor.

A simple general quantum mechanical theory of a 1:1 or *n*:1 interaction of the electron donor with the electron acceptor was proposed (16). In the ground state, the two molecules are held together when they are in close proximity by such forces as van der Waals, dispersion, London, and hydrogen bond forces. Additional forces are contributed by the transfer of a small amount of charge from the donor molecule to the acceptor one.

When a complex absorbs light of a suitable energy, it is raised from the ground to the excited state. In this state, an electron that was only slightly shifted toward the acceptor is almost wholly transferred. The transitions involved are called charge transfer transitions. Therefore, the transfer of an electron by light absorption is responsible for the characteristic colors of these complexes.

Based upon the reasoning previously pointed out for nucleic acid bases–catechol interactions, epinephrine is assumed to act as an electron donor while adenine, thymine, and uracil act as electron acceptors.

The molar absorptivities were discovered to be temperature independent; the slight change observed is attributable to experimental error. Therefore, the absorptivities presented in Table I represent essentially an average estimated from working at different temperatures. Furthermore, the molar absorptivity of the adenine–epinephrine complex changed rapidly with changes in wavelength.

The equilibrium constants of the reaction between adenine and epinephrine were wavelength independent (within the limits of accuracy), while the one between thymine and epinephrine was very sensitive to changes in the wavelengths used (Table I). Almost all equilibrium constants were less than unity. Hence, ΔG^0 values of all interactions were positive and small, even though the ΔH^0 values were generally high. The highest ΔH^0 values were those of the uracil–epinephrine interaction (approximately 7 kcal/mole); the lowest was for the reaction between thymine and epinephrine.

Another characteristic feature of the nucleic acid bases–epinephrine interactions was a high negative entropy change, which can account for the fact that ΔG^0 was small and positive even though the heat of formation of the complexes was high. A high $-\Delta S^0$ indicates a high degree of specificity of these reactions as well as a high degree of change in the structure of the solvent when the complex is formed. The order of decreasing $-\Delta S^0$ follows the same sequence as ΔH^0 (Table I).

Uracil–epinephrine interaction showed higher ΔH^0, ΔS^0, and even ΔG^0 values compared to those of other interactions (Table I). The uracil molecule is small and planar and has four centers forming hydrogen bonds with epinephrine in addition to the charge transfer forces arising from their π-system interactions. Although thymine has a similar structure to uracil, the structure of its mixed aggregate with epinephrine seems to be less ordered. For example, the number of molecules in these aggregates could be less than the corresponding uracil mixed aggregate. The presence of a methyl group in the thymine molecule changes both the electronic and steric properties. Changes in the electronic properties of a molecule obviously will change its electron-accepting or donating ability. It was found that the electron affinity of uracil is the same as that of thymine, while their ionization potentials differ considerably (17).

Recent quantum mechanical calculations on catechol derivatives (18) demonstrated that epinephrine has binding energies of -0.316 β and -1.114 β units for the highest occupied and the lowest empty molecular orbitals, respectively. These calculations also revealed high electron density at the aliphatic carbon carrying the hydroxyl group (1.148). A low binding energy of the highest occupied molecular orbital indicates a low ionization potential and a high electron-donating ability. Also, the fact that the binding energy of the lowest empty orbital is relatively low is in itself an indication of a low electron-accepting ability.

The apparent ΔH^0 values determined by experiments are actually functions of ΔH^0 values of different associated forms (19). This is evident in the case of thymine–epinephrine interaction and indicates the formation of more than one type of complex in solution.

A linear relationship between ΔH^0 and ΔS^0 was observed for many complexes between a given acceptor and related donors (20). Even though the situation was reversed in the present study, a similar relationship existed between ΔH^0 and ΔS^0.

Another interesting observation is that an inverse relationship seems to exist between molar absorptivities of thymine and uracil (at wavelength of 316 nm) and ΔH^0, ΔS^0, and ΔG^0. This finding is not in conflict with previous work (21) because the absorptivity does not always give an indication about the energy of the complex. The value of ΔH^0 is actually related to the total oscillator strength (22, 23). Moreover, the situation in the present study was more complicated because completely isolated bands were not formed.

The exact stoichiometry of the various complexes formed between nucleic acid bases and epinephrine is difficult to determine from UV absorption studies alone. Parallel stacking interactions between the nucleic acid bases and the aromatic nucleus of epinephrine can be invoked as they have been for similar systems (24–28). The presence of many sites on the interacting molecules that can be hydrogen bonded (whether acting as hydrogen bond donors or acceptors) could play a role in determining the size of mixed aggregates as well as their mode of aggregation (29–33).

The wavelength independence of association constants of weak interactions was interpreted (34) as being due to formations of 1:1 charge transfer complexes, isomeric forms (trimers in which one molecule of a compound is sandwiched between two molecules of a different compound), or contact charge transfer complexes.

Schellman (35) determined the heat of formation of the process of self-association of urea in aqueous solution and obtained a value of 1.5 kcal/mole/hydrogen bond. Although it is difficult to extrapolate these findings to the present case, using different systems in which hydrophobic as well as other types of interactions are operating, the concern is also with weakly bonded complexes and Schellman's findings could indicate a stoichiometry other than 1:1.

Johnson and Bowen (34) point out that linear slopes are obtained from the application of the Benesi–Hildebrand plots when 1:1 complexes are assumed to be the main contributors, while the wavelength dependence of the association constants indicates the presence of more than one molecular species. However, this is not always the case.

The dependence of ΔH^0 and the total oscillator strength on temperature was also demonstrated (34). This dependence does not always exist since straight lines were obtained in some cases when plotting log K *versus* $1/T$, log $K\epsilon_\lambda{}^{AD}$ *versus* $1/T$, and even log $\epsilon_\lambda{}^{AD}$ *versus* $1/T$ when the temperature range was not too high (36).

Person (37) proposed that the most accurate values for equilibrium constants of complexes may be obtained when the equilibrium concentrations of the complexes are of the same order of magnitude as the equilibrium concentrations of the more dilute pure components. Measurement of equilibrium constants of complexes by the Benesi–Hildebrand method or one of its modifications requires that the donor concentrations in the most concentrated solutions be greater than $0.1(1/K)$. It also was shown that K and $\epsilon_\lambda{}^{AD}$ of a complex depend on

concentration ratios (38). This dependence is significant only when the ratio of $(D_0)/(A_0)$ ranges from 1 to 10. In our studies, the ratios were between 15 and 40 and the donor concentrations were much greater than $0.1(1/K)$. All these facts indicate the validity of the results derived from the Benesi–Hildebrand equation.

The association constants of the interactions between nucleic acid bases and catechol were calculated and found to be higher generally than those for the interactions between nucleic acid bases and epinephrine. This was due to the facts that the amino group of the side chain of epinephrine is protonated and that epinephrine hydrogen tartrate was used instead of epinephrine free base.

CONCLUSION

Evidence was presented here for the formation of charge transfer complexes between nucleic acid bases (adenine, thymine, and uracil) and epinephrine in aqueous solutions containing 0.1 *N* HCl. The evidence was the appearance, in the UV region and at longer wavelengths from the absorptions of the pure components, of new absorption bands which can only be attributed to charge transfer transitions.

It was possible to calculate various thermodynamic parameters as well as molar extinction coefficients at different wavelengths. In general, equilibrium constants were found to be less than unity and heats of formation were relatively high. Wavelength dependence of equilibrium constants was observed in some cases.

Compared to catechol interactions with nucleic acid bases, the presence of an alkyl side chain in epinephrine leads to an additional heat of complex formation as well as to large changes in the specificity of these interactions, as reflected by changes in the entropy associated with the formation of these complexes. The presence of a protonated cationic group as well as an increase in ionic strength decreased the equilibrium constants.

It is proposed that parallel stacking interactions between the presumed donor molecule, epinephrine, and the acceptors, adenine, thymine, and uracil, exist in aqueous solutions. Hydrogen bonding possibilities were not excluded.

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