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THE SELF-ASSOCIATION OF ADENOSINE-5′-TRIPHOSPHATE STUDIED BY CIRCULAR DICHROISM AT LOW IONIC STRENGTHS

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The self-association of adenosine-5′-triphosphate (ATP) was studied as a function of pH, additional counterions, concentration and temperature. Circular dichroism measurements were employed as a measure of the base-stacking. The self-association of ATP is pH dependent with the protonation of the adenine ring helping stabilize the association. Highly charged counterions alter this aggregation. At pH 2.8 and 20°C, a dimerization constant of 88 M^{-1} is obtained, while an isodesmic model leads to an equilibrium constant of 158 M^{-1}. With increasing pH, the association constants decrease. At pH 2.8 there is a very strong temperature dependence of the CD amplitude. These results indicate the existence of additional electrostatic stabilization for the stacking of the adenine rings. At acidic pHs, models are proposed to explain this high degree of stability and a calculation of the approximate electrostatic contribution to the aggregation shows it to be of the proper magnitude.

1. Introduction

In recent publications [1,2] the self-association of adenosine-5′-triphosphate (ATP) has been studied. This interaction has been characterized as base-stacking, like that of other purines and pyrimidines as shown by Ts'o [3]. Such interactions of ATP are important in the storage of some neurotransmitters [4–6] where it is thought the base-stacking plays an important role in stabilizing the aggregates. The importance of the base-stacking in other biological systems is well established. Heyn and Bretz [2] used circular dichroism (CD) plus UV measurements to determine an association constant and an enthalpy change for ATP aggregation. Their studies were done largely at one pH (8.7 in a 1 M Tris/HCl buffer) with a large excess of magnesium chloride (0.5 M). Ferguson et al. [1] used sedimentation equilibrium experiments in 0.154 M NaCl solution, where the pH varied from 4.6 to 3.3 as the ATP concentrations varied. The results of Heyn and Bretz [2] are fitted to an isodesmic association model, yielding an association constant of 9 M^{-1} at 20°C, while the results of Ferguson et al. [1] are fitted best by a monomer–dimer, monomer–trimer association, where K_D is 101 M^{-1} and K_T is 1.71×10^5 M^{-2} at 20°C. Studies of AMP indicate that it associates beyond the dimer stage [7–9].

This variation in association constant of ATP, especially the strong association found in acidic solutions [1] where the adenine ring is protonated, indicates an unusual pH dependence. Studies with purine bases show a decrease rather than an increase in the self-association when the base is protonated [10,11]. This decrease results from electrostatic repulsion of the charged bases decreasing the amount overlap. Increasing the ionic strength can reduce this repulsion through screening [12]. However in this study, no additional salt is present. For ATP to show this strong self-association at acidic pHs, this electrostatic repulsion must be reduced.

We find that the CD spectra of ATP do exhibit a very marked pH dependence. At acidic pHs, the results can be interpreted in terms of a monomer–dimer model, with the formation of a rather rigid complex where the charged phosphate of one ATP interacts electrostaticly with the charged adenine ring of the other ATP. This binding of one phosphate to the other adenine ring could preclude the formation of aggregates larger than dimer. From the concentration depen-

dence of the CD spectra it is possible to obtain an association constant, assuming only monomers and dimers contribute to the signal. At basic pHs, the dimer model appears no longer to be valid and the results are fitted to an isodesmic model. The influence of counterions indicates a decrease in association with increasing ionic strength which likely results from ion binding destabilizing the base stacks more than electrostatic screening helps stabilize them.

2. Methods

The adenosine-5'-triphosphate was purchased from Merck as the disodium salt and dissolved in doubly distilled water. The concentration was determined by the UV absorbance at 259 nm in a dilute pH 7.5 solution (ϵ_{max} = 15 400) [13]. The concentrations of AMP and ADP in the ATP were determined by an enzymatic method [14] and their total concentration was much less than 1%. The pH was varied by adding either HCl or NaOH and was measured using a Polymetron pH meter.

The circular dichroism spectra were measured on a Cary 61 spectrophotometer using a 0.1 mm cell for the concentrated solutions and 5.0 cm for the dilute solutions. Since the optical density should not exceed 2, this narrow path length is necessary for the concentrated solutions. All the cells can be thermostatted and the temperature measured using a thermistor. Since the Cary 61 is a single beam instrument it is necessary to measure four different solutions before a usable spectrum can be obtained. A base line of water must be measured in both cells and then the concentrated solution and the dilute solution are measured. After subtracting the base lines from the concentrated and dilute solutions, one can subtract the dilute solution, which has the same number of ATP molecules in the light path, from the concentrated solution leaving a spectrum that is a function of the dimer concentration and a spectral constant for the dimer model.

The salts used were all the best grade available from Merck and were weighed into doubly distilled water to obtain the necessary concentrations.

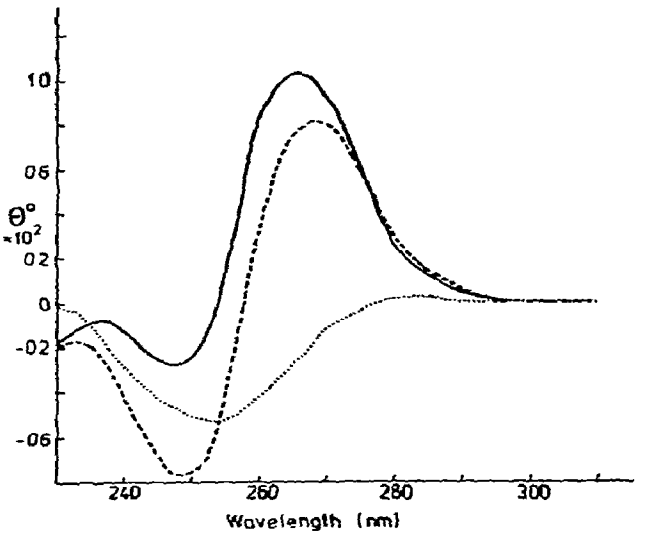

Fig. 1. The CD spectra of ATP at pH 2.83 and 2°C. (---) 10 mM ATP in a 0.1 mm cell, (···) 0.1 mM ATP in a 1.0 cm cell and (——) resultant spectrum for 10 mM ATP after 0.1 mM ATP is subtracted from 10 mM ATP. All spectra have been corrected for solvent baseline.

3. Results

The CD spectra of ATP in aqueous solutions show a signal at neutral and basic pHs quite similar to that of the monomer. As the pH is lowered, a positive band appears at about 268 nm and the negative band of the monomer at about 255 nm increases in amplitude and shifts to lower wavelengths having a maximum amplitude at about 249 nm. These uncorrected spectra for the concentrated solutions are very similar in shape to those of ApA [15] and of ATP aggregates at high ionic strength and pH 8.7 [2]. With changing pH one obtains an isosbestic point at 254 nm. Fig. 1 gives the spectra for 10.0 mM ATP at pH 2.83 and 2°C in the 0.1 mm cell and 0.1 mM ATP at the same pH and temperature in the 1.0 cm cell. The change in the monomer spectra with pH is very slight, reflecting changes in the adenine moiety with protonation. However the change in the signal of the concentrated solution with pH is very large. Fig. 2 gives the intensity of a 10 mM solution of ATP at 268 nm as a function of pH at 2°C. Above pH 7.0, the signal is constant. As the solution becomes more acidic the intensity increases to a broad maximum between pH 3.0 and 2.5

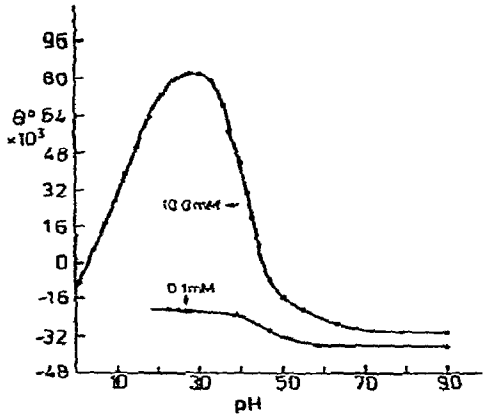

Fig. 2. The pH dependence of the CD signal of 10 mM ATP and 0.1 mM ATP at 2°C, measured at 268 nm and corrected for solvent baseline.

and then decreases again. The inflection point for the increasing signal coincides very closely with the pK_a of 4.2 for the adenine ring protonation [16]. The first phosphate protonation is at pH 6.5 and the rest are protonated at pHs of 2 or less [17]. The inflection point for the decreasing intensity is in the proper pH range for the additional protonation of the charged phosphate group.

To check the influence of counterions on the self-association of ATP, the ionic strength of a 10 mM ATP solution at 2°C was varied using several different electrolytes at constant pH in the region of maximum amplitude. When sodium chloride is added in the concentrations as high as 25 mM, no appreciable alterations are observed in the spectra at 268 nm. More sensitive measurements indicate an amplitude decrease with increasing sodium chloride concentration. A 20 mM solution of ATP at 5.5°C shows a 30% amplitude reduction for a 500 mM NaCl solution. The addition of increasing amounts of magnesium chloride results in spectral changes analogous to those observed when the pH increases. 2.5 mM solutions of sodium sulfate, magnesium chloride and calcium chloride all yield amplitude decreases of 16%, while 7.5 mM solutions yield decreases of a third. The addition of 5 mM terbium chloride causes a 70% amplitude reduction. Cesium chloride at 25 mM causes a slight decrease while tetramethylammonium chloride causes no observable change at 25 mM. Thus, the presence of small amounts of divalent and trivalent ions, either cations or anions, causes appreciable decreases in the amplitude of both 268 nm and 249 nm peaks. These changes are similar to those observed for changes in the pH. The magnitude of the changes induced by these ions is dependent largely on their charge. The same concentration of terbium has a much larger effect on the amplitude than the divalent ions which in turn have a much larger effect than the monovalent ions. These amplitude changes could result from either decreases in the association constant or extinction coefficient or both.

The concentration dependence of the amplitude of the CD signal can be used to obtain thermodynamic constants. We have determined the equilibrium constant for ATP at several pHs and salt concentrations at 20°C. This was done measuring the CD spectra from 320 nm to 230 nm in the 0.1 mm cell. In a 5 cm cell, a 500-fold dilution of the concentrated solution was also measured and at this dilute concentration only monomers were present. This monomer signal was subtracted from the concentrated solution signal after baseline corrections were made and the resultant spectra were analyzed. The maximum in the peak amplitude at 265 nm will then be a function of the equilibrium constant, an extinction coefficient and the initial concentration. Fig. 1 also gives the resultant spectrum for the 10.0 mM ATP at 2°C and pH 2.8.

Since we are assuming a monomer–dimer model is sufficient to explain our results at acidic pHs (see section 4), the equilibrium constant can be obtained by the following method. The total molar concentration of ATP in the sample, C_T, is simply a sum of the monomer, C_M, and dimer, C_D, concentrations such that

$$C_T = C_M + 2C_D. \tag{1}$$

The association constant, K_a, for the formation of dimers from monomers is then

$$K_a = C_D/C_M^2. \tag{2}$$

Eq. (2) assumes ideality for the solutions. To correct for non-ideality the activities of the monomers and dimers should be used and activity coefficients must be introduced. Thus eq. (2) becomes

$$K_a = \gamma_D C_D/\gamma_M^2 C_M^2. \tag{3}$$

The activity coefficients can then be expressed by

means of a second virial coefficient (BM_1) [18]

$$\ln \gamma_i = i\, BM_1\, C_T, \qquad i = 1,2. \tag{4}$$

and then $\gamma_D = \gamma_M^2$. ΔA, the difference in absorbance between left-handed and right-handed circularly polarized light, is

$$\Delta A = d_1(C_M \Delta\epsilon_M + 2C_D \Delta\epsilon_D), \tag{5}$$

where d_1 is the path length of the cell and $\Delta\epsilon_M$ and $\Delta\epsilon_D$ are the differences between the extinction coefficients for left-and right-handed circularly polarized light of an ATP moiety in the monomer and dimer respectively. In the dilute solution,

$$\Delta A = d_2 C_S \Delta\epsilon_M = d_1 C_T \Delta\epsilon_M \tag{6}$$

because $d_2 C_S$ is made to equal $d_1 C_T$ with $d_2 \gg d_1$. If one then subtracts eq. (6) from (5) and substitutes for C_T from eq. (1), the result is

$$\delta\Delta A = 2d_1 C_D \delta\Delta\epsilon, \tag{7}$$

where $\delta\Delta\epsilon = \Delta\epsilon_D - \Delta\epsilon_M$. Then in eq. (2), one can substitute for C_D and C_M in terms of $\delta\Delta A$, d_1, C_T and $\delta\Delta\epsilon$. The result is

$$K_a = \frac{\delta\Delta A}{2d_1\delta\Delta\epsilon}\left(C_T - \frac{\delta\Delta A}{d_1\delta\Delta\epsilon}\right)^{-2}. \tag{8}$$

Since θ^0 is measured experimentally and

$$\theta^0 = 33.0\Delta A, \tag{9}$$

we can now determine K_a in terms of the experimental quantities $\delta\theta^0$, C_T and d_1 and the unknown $\delta\Delta\epsilon$, which is a constant independent of concentration. The result then becomes

$$C_T^2 - 2C_T a + a^2 - a/2K_a = 0, \tag{10}$$

where

$$a = \delta\theta^0/33d_1\delta\Delta\epsilon.$$

Eq. (10) was fitted for the two unknowns, K_a and $\delta\Delta\epsilon$, by means of a non-linear regression analysis. The computer program employed the damped Taylor series algorithm [19] with pure steepest descent correction when the parameter vector lies outside the acceptable space at maximum damping [20]. Fig. 3 gives the experimental points for ATP at pH 2.80 ± 0.02 and 20.0° ± 0.1° in terms of $\delta\theta^0$ at 265 nm versus the UV determined concentrations. The solid line gives the computer fit for the data for the dimer model.

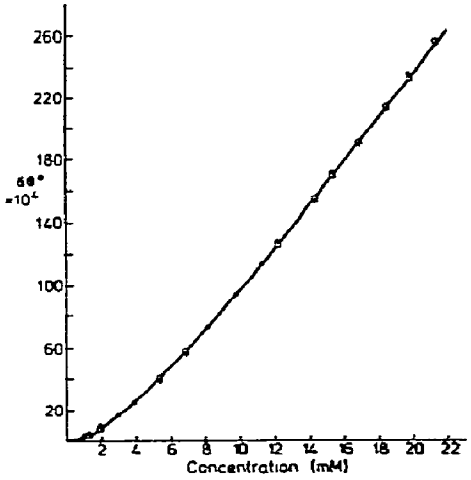

Fig. 3. The concentration dependence of the CD amplitude of the resultant spectra for ATP at pH 2.8, 20°C and 265 nm. (•) are the experimental points, (○) represent the fitted values at the same concentration. Where the fitted and experimental are the same only the experimental are indicated.

The fitted value of K_a is 87.7 ± 0.3 M^{-1} with $\delta\Delta\epsilon$ = 5.76 ± 0.28 M^{-1} cm^{-1} for 90% confidence limits.

Eq. (10) can also be written in linear form where

$$C_T\delta\Delta\epsilon/\sqrt{\alpha} - \sqrt{\alpha} = \sqrt{\delta\Delta\epsilon/2K_a}, \tag{11}$$

where

$$\alpha = \delta\theta/33d_1.$$

For the dimerization model, a plot of $\sqrt{\alpha}$ versus $C_T/\sqrt{\alpha}$ should be linear yielding $\delta\Delta\epsilon$ from the slope and K_a from the intercept. Fig. 4 shows the results for ATP at 20° as a function of pH. Note that at a pH between 5.3 and 6.5 the dimer model appears to be singular, implying an infinite association constant. The dimer model requires a negative intercept for eq. (11). The zero or positive intercepts require rejection of this model. Thus at pHs where the dimer model would be expected to fail, since there is no longer an electrostatic interaction limiting the association to the dimer, it does.

It is thus necessary to employ another model to fit the results at basic pHs. An isodesmic model requires the fewest fitted parameters and has been used successfully at basic pHs for ATP [2]. For this model [2,21, 22] the association constant for each step is the same, thus

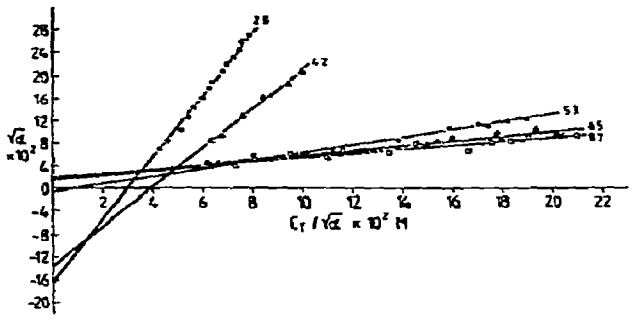

Fig. 4. The concentration dependence of the CD signal for ATP at 20°C and varying pHs employing eq. (11) for the dimer model. The slope $\delta\Delta\epsilon_D$ and the intercept yields K_a. For the pHs shown, $\delta\Delta\epsilon_D$ is: 5.5 for pH 2.8 (•), 3.5 for pH 4.2 (△), 0.70 for pH 5.3 (○), 0.43 for pH 6.5 (▲) and 0.37 for pH 8.7 (□).

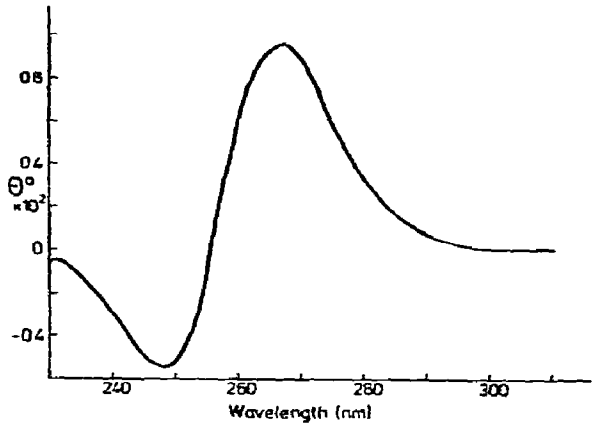

Fig. 5. The CD spectrum for the ATP dimer at 12.9 mM, pH 2.97, and 20°C.

$$C_T = \sum_{i=1}^{\infty} iC_i \quad \text{and} \quad C_i = K^{i-1}C_M^i, \quad \text{for } i \geqslant 1. \quad (12)$$

Assuming nearest neighbor interactions alone influence the optical parameters, then

$$i\Delta\epsilon_i = 2\Delta\epsilon_D + (i-2)\Delta\epsilon_{int}, \quad i \geqslant 2$$

$$\text{and} \quad \Delta\epsilon_1 = \Delta\epsilon_M, \quad (13)$$

where $\Delta\epsilon_{int}$ is for bases in the interior of stacks while $\Delta\epsilon_D$ is for the bases on the end of the stacks.

If we proceed as in the dimer model, defining

$$b = \delta\Delta\epsilon_{int}/\delta\Delta\epsilon, \quad (14)$$

then

$$\delta\theta = 33.0 d_1 \delta\Delta\epsilon C_T \left(\frac{2}{C_T} \sum_{i=2}^{\infty} C_i + \frac{b}{C_T} \sum_{i=2}^{\infty} (i-2)C_i \right). \quad (15)$$

Eq. (15) may be expressed in terms of total concentration with $L = K_a C_T$. Using eq. (12) and letting $s = K_a C_M$, then $L = s/(1-s)^2$ and

$$\delta\theta = (33.0 d_1 \delta\Delta\epsilon/K_a) \quad (16)$$
$$\times \left\{ b(2+L) - 3 + \frac{b-2}{2L} + \left(1 - b + \frac{2-b}{2L}\right)\sqrt{4L+1} \right\}.$$

This can be fitted by the same non-linear regression analysis used for the dimer. The fit for each pH is done by allowing $\delta\Delta\epsilon$ and K_a to vary for a fixed b. The b is then varied until the sum of squares is a minimum. This minimum in the sum of squares is a very sensitive function of b. Table 1 gives the results for both the isodesmic and dimer models obtained from the non-linear regression analysis. Also included is ATP at pH 2.7 and 20°C with 20 mM magnesium chloride.

Having a value of K_a from the dimer model it is possible to determine the concentration of the monomers in the concentrated solutions. The contribution of the monomer to the signal can then be subtracted leaving the dimer spectrum. Fig. 5 shows the result for an ATP concentration of 12.9 mM at pH 2.97 and 20°C. This spectrum is not totally conservative, whereas those found for ATP [2] and oligonucleotides of polyA [15] are. Spectra of the latter have symmetric couplets and the interpretation of these spectra has been in terms of a degenerate exciton coupling model [23], but such a straightforward interpretation is not possible for ATP under these conditions. The $\Delta\epsilon$ found for the dimer at 265 nm is 6.6 M^{-1} cm^{-1} which is somewhat smaller than that for the base-stacked ApA.

The amplitude of the CD signal for ATP shows a very marked temperature dependence. Ferguson et al. [1] observe a very slight temperature dependence of the pH at these concentrations which we also find. However at 10 mM the pH changes by less than 0.1 pH unit from 1°C to 32°C. This pH change would have a minimal effect on the amplitude as can be seen from fig. 2. It is possible to solve eq. (10) for the K_as at each temperature, assuming $\delta\Delta\epsilon$ is temperature independent and thus obtain ΔH. The best fit for several concentrations at pH 2.8 and temperature from 10°C to 36°C gives a ΔH of −8 kcal/mole for the dimer model. This is not an accurate value for the en-

Table 1
Non-linear regression fit for the self-association of ATP at 20°C. Extinction coefficients at 268 nm

Model	pH	$\Delta\epsilon_M$ (M^{-1} cm^{-1})	K_a (M^{-1})	$\delta\Delta\epsilon$ (M^{-1} cm^{-1})	b
Isodesmic	2.8	0.84	158	2.98	2.001
	4.2	1.02	166	1.53	2.002
	5.3	1.22	60	0.85	2.120
	6.5	1.25	58	0.67	2.170
	8.7	1.26	51	0.60	2.180
	2.7, 20 mM $MgCl_2$	0.85	22	3.26	2.017
Dimer	2.8	0.84	88	5.76	
	4.2	1.02	102	3.39	

thalpy change on dimerization, but it does give an estimation of the ΔH involved. The K_a and $\delta\Delta\epsilon$ should be determined at each temperature to obtain a more reliable result.

4. Discussion

It is well established from NMR studies [24–28] that there exists an interaction between the triphosphate group, a divalent metal ion and the adenine ring, in particular the N(7) nitrogen. This macrochelate supposedly facilitates the dephosphorylation of ATP. There is also NMR evidence that such interactions can occur in ADP [24] and to a lesser extent in AMP [9,29].

Naumann et al. [25] noted the influence of protonation of ATP's γ-phosphate on the chemical shift of the H(8) proton and attribute it to the interaction of N(7) with the γ-phosphate through a hydrogen bond. They also find the H(8) signal broadened at pH 3 again indicating an interaction with N(7). Sundaralingam [30] gives a detailed description of the orientation of the ATP-divalent metal ion complex where the base is in an anti configuration. The protonation site is the N(1) nitrogen of the adenine ring.

X-ray studies [31] on the disodium salt of ATP show the base in an anti configuration and the triphosphate group folded back toward the base. In this protonated salt, the bases stack head-to-tail with the ribose groups on the same side of the aggregate. For 5'AMP, the X-ray structure [32] indicates clearly an intermolecular interaction between the phosphate oxygen of one AMP and the protonated N(1) nitrogen of another AMP. For ATP this also appears true with the formation of an extended network. Solution studies of AMP at neutral pH [33] indicate a head-to-tail stacking geometry with the anti conformation preferred by 5'AMP.

In our CD studies, there are no divalent cations present in the solutions that give the largest signals. The pH dependence of the 268 nm peak (fig. 2) indicates that the protonation of the adenine ring is important in increasing the signal and that the second protonation of the phosphate group is important in decreasing the amplitude. It is apparent from the pH dependence of the K_as that the charge on the adenine ring stabilizes the base-stacking. This seems at first contradictory since two positively charged adenine rings will repel one another. If, however, there is an electrostatic interaction between the positively charged adenine ring and the triply negatively charged triphosphate, then the positive charge could be neutralized. This would then constrain the doubly charged phosphates, shielding them from one another and reducing their electrostatic repulsion. When more acid is added to a pH 2.8 solution, the strength of the electrostatic interaction between the ring and the phosphates will be reduced. The phosphate tails will not be so tightly bound to the adenine ring and the charge on the ring not so effectively neutralized. When the amount of charge is reduced on the adenine ring by adding base, the result will be a more loosely bound triphosphate tail, thereby reducing the stability of the stacked nucleotides and altering the conformation of the bases. K_a and $\delta\Delta\epsilon$ do decrease with increasing pH as would be expected from the arguments above.

The results from the addition of salts to the ATP

solutions can be explained in terms of preferential binding of the divalent and trivalent cations to the triphosphate groups, thereby reducing the charge neutralization of the adenine ring and making the triphosphate tail more mobile. The divalent anion has the same effect through preferential binding to the charged adenine. The monovalent ions only bind appreciably when they are in large excess and presumably more than one cation will bind to the triphosphate at such concentrations. Since sodium and chloride ions will have lower affinities for the triphosphate and the adenine ring respectively than the latter have for each other, low NaCl concentrations do not effectively alter the aggregated ATPs. More highly charged ions are capable of disrupting this species at much lower concentrations, either by decreasing the amount of stacking or by changing the conformation. The results using 20 mM $MgCl_2$ indicate that decreases in K_a are the most influential at acidic pHs. The influence of the magnesium counterion on the ATP aggregation could then account for the large K_a and smaller $\delta\Delta\epsilon$ we find at pH 8.7 and 20°C, as opposed to those found previously [2].

The aggregation constant determined for the formation of an ATP dimer from its monomers is larger than that seen for other adenine systems [21,34]. The highest association constants are found for the methyl substituted adenines, with N^6,N^9-dimethyladenine being the largest with a K_a of about 50 M^{-1} at 25°C [11, 21]. ATP at acidic pHs in a low ionic strength solution did show a very strong self-association with dimerization constant of 101 M^{-1} at 20°C and a trimerization constant of 1.71×10^5 M^{-2} [1]. This value of the dimerization constant is close to our result. Unfortunately it is not possible to compare their result with ours since the pH in their experiments varied from 3.3 to 4.6 as the concentration was varied. Fig. 2 indicates a strong pH dependence of the amplitude at constant concentration in this region. The strong association they observe probably does result partly from the type of interaction we postulate at pH 2.8 and their result if done at constant pH should be comparable to ours. As the pH increases, other modes of association are likely to dominate. Ferguson et al. [1] also found a ΔH of −15 kcal/mole for dimerization where we find approximately −8 kcal/mole. The former value is substantially higher than those found for other base-stacking interactions [11,21,22], while the latter is rather typical.

To develop a model which will fit these experimental results it is necessary to account for the pH and counterion dependence, the high association constant and the large ΔH. Since charge neutralization of the adenine ring is important in the stacking at acidic pHs, one must then postulate an electrostatic interaction of the phosphate tail with the adenine ring. This charge interaction of the ATP molecules should yield an additional decrease in the free energy relative to the simple adenine bases and nucleosides. This charge neutralization could occur in two ways, intramolecularly or intermolecularly. Although sterically feasible, the intramolecular interaction between the phosphate tail and its own adenine ring should not result in base-stacking that is more stable than the base-stacking of the uncharged bases. This intramolecular interaction still leaves the monomer with two negative charges and base-stacking as the only means of aggregation.

The intermolecular charge neutralization could occur in two ways. The first (shown in fig. 6) would be

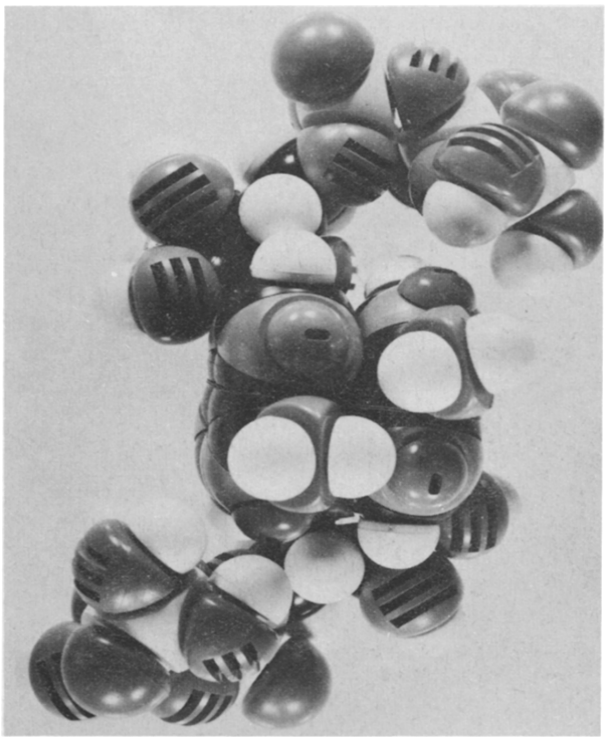

Fig. 6. Proposed configuration of ATP dimer at acidic pHs.

where the faces of the two adenine rings are stacked head to tail and the phosphate tail from one would interact with the adenine ring of the other. Here the ATP could maintain its preferred anti configuration [30,31] and the sites of protonation would be as far apart as possible. This dimer could then have double ion-pair formation giving additional stabilization to the base-stacking. The remaining charges on the phosphate tails would be well separated. Such an interaction could account for the observed experimental results. The second possibility is that the inter-molecular electrostatic interaction creates chains where the bases are stacked head to tail or head to head, but the phosphate tail of one stacked base does not interact with the adenine ring of the other stacked base. It interacts with a different adenine base. The network formed would be analogous to the hydrogen bonding of alcohols and similar to that found in the crystalline state. However because of the interaction of the phosphate tail with the backside of the stacked adenine rings, it is not likely that more than two bases stack in either case. In the latter case the extra electrostatic stabilization would be less than in the former. Both models would yield only dimerization constants because the CD method is only sensitive to base-stacking and is not influenced by possible electrostatic interactions that could cause larger aggregates.

It is possible to estimate the contribution of this electrostatic stabilization to the dimerization constant of ATP. First

$$RT \ln K_a = -\Delta G_D^0, \tag{17}$$

where ΔG_D^0 can be split into a contribution from base stacking, ΔG_{st}^0, and from the electrostatic interaction, ΔG_{el}^0. With $RT \ln q_{el} = -\Delta G_{el}^0$, then

$$K_a = q_{el} K_{st}. \tag{18}$$

We can then evaluate ΔG_{el}^0 from

$$\Delta G_{el}^0 = \frac{N_A e_0^2}{4\pi\epsilon_0 \sigma} \Delta \sum_{i>j} \frac{z_i z_j}{r_{ij}} \tag{19}$$

where σ is a screening factor, i.e., the effective dielectric constant between the aggregating species including screening by the electrolyte [12]. N_A is Avogadro's number, e_0 the elementary charge and ϵ_0 the absolute permittivity of a vacuum. The sum gives the electrostatic forces in two monomers and in the dimer. Using a σ of 160, assuming there is no change in orientation between the monomer and dimer and measuring the distances from the CPK models used for fig. 6, we find a ΔG_{el} of -1.5 kcal/mole. This gives a q_{el} of 13 and a K_{st} of 6.8 M^{-1}. This value of K_{st} compares favorably with the 9 M^{-1} found when any electrostatic contribution should be eliminated [2]. Although the value of σ is merely an estimate, it is comparable to values used in other base stacking systems [12]. The actual conformation in solution will be dynamic and the charge separations in the monomer and dimer can be somewhat different. Thus this is not rigorous but does give an estimate if the electrostatic interaction postulated can account for the large equilibrium constant encountered at acidic pHs.

At neutral and basic pHs, the triphosphate tail still seems to give additional stabilization to the stacked bases. The orientation of the bases probably changes appreciably since $\delta\Delta\epsilon$ changes substantially from acidic to basic solutions. The electrostatic interaction is no longer possible, so there must be other forces stabilizing the base stacks which can be influenced by counterions. Since the bases would be no longer obstructed by the electrostatic interaction, higher aggregates could be formed.

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