Chelation of Uranyl Ions by Adenine Nucleotides[1]

Isaac Feldman, Joann Jones, and Richard Cross

Contribution from the Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York. *Received July 20, 1966*

Abstract: Potentiometric titrations of equimolar mixtures of uranyl nitrate and the 5′-adenine nucleotides, H_2ATP^{2-} $HADP^{-}$, and AMP have been performed. From the initial pH values and r values (*i.e.*, moles of added base : moles of total nucleotide) at the first inflections, we conclude that the number of protons which dissociate upon formation of the U–ATP and U–AMP complexes are one and two, respectively, and that an average of about 1.3 protons dissociate upon formation of the U–ADP complex. From a comparison of the U–ATP titration curve with that of Na_2H_2ATP, it appears that the first proton which dissociates upon formation of the U–ATP complex comes from a protonated adenine group; *i.e.*, the terminal phosphate proton does not start to dissociate until sometime after the first inflection (pH 4.5). Both the dissolution and dephosphorylation of the precipitated complex began just after the first inflection. There was complete dissolution at about $r = 2$, at which point there was also the maximum rate of dephosphorylation. Titrations of U–adenosine, U–glycerophosphate, and U–(adenosine + glycerophosphate) mixtures and their comparison with the U–AMP curve showed that even though the uranyl ion does not react with adenosine it does attach to both the adenine and phosphate groups of AMP to form an intramolecular U–AMP chelate. All these facts are consistent with a U–adenine nucleotide chelate structure in which uranium is attached to (i) an O atom of the α-phosphate group, (ii) the ribose ring oxygen, and (iii) the N_3 atom of the adenine ring, these ligands plus one water oxygen lying close to the equatorial plane of the uranyl group.

The physiological importance of the uranyl–adenosine triphosphate (U–ATP) complex[2] was shown by Hurwitz,[3] who proposed that the inhibition of cellular metabolism by uranium is due to its replacement of Mg^{2+} from the metabolically active ATP–Mg^{2+}–hexokinase complex to form an ATP–UO_2^{2+}–hexokinase complex which does not phosphorylate glucose. In their study on inhibition of myosin ATPase activity by UO_2Cl_2, Bowen and Kerwin[4] found that UO_2Cl_2 partially dephosphorylates ATP at pH 7.4.

We have therefore begun an investigation of the reactions of UO_2^{2+} with nucleotides. The present paper is primarily concerned with pH titrations of various mixtures of uranyl nitrate and adenine nucleotides.

Experimental Section

Materials. Adenine, adenosine, and the nucleotides used were all Calbiochem, grade A. β-Glycerophosphate was Eastman White Label grade. The ADP and ATP used were initially about 1% hydrolyzed, as indicated by an inorganic phosphate phosphorus content of $\sim$0.5 μg/mg. The apparent molecular weights were obtained by titration with tetramethylammonium hydroxide (TMNOH) or hydrochloric acid. All other chemicals, including uranyl nitrate hexahydrate, were analytical reagent grade. All mixtures were prepared under nitrogen with CO_2-free, deionized water.

Titrations. Each pH titration referred to as a pointwise titration was carried out by adding varying increments of TMNOH to a series of indicated mixtures. After proper dilution and vortex mixing, the mixtures were stored in the dark at 25° in a constant-temperature bath for about 24 hr, unless specified otherwise, after which pH readings were taken with a Beckman Research Model pH meter. CO_2-free, constant-temperature pH measurements were made with the aid of a water-jacketed cell with fittings for nitrogen atmosphere.

Pointwise titrations were resorted to because there was continual drift in the pH measurements taken during a conventional continuous titration. However, the drift was less than 0.01 pH unit in 10 min for all supernatants and slurries of all mixtures aged 24 hr.

Phosphate Analyses. Inorganic orthophosphate analyses were performed using the Martin–Doty[5] modification of the Berenblum–Chain method, in which phosphomolybdic acid is extracted into equivolume isobutyl alcohol–benzene solution before reduction with stannous chloride. We considered this method trustworthy since the "reagent blank" absorbance, 0.008, was increased by an insignificant amount, 0.005, when we analyzed ATP and ADP solutions having concentrations of interest to us. Neither AMP nor $Na_2P_2O_7$ changed the blank reading at all. Inorganic orthophosphate gave a perfectly linear analysis plot. Uranyl ion did not affect the analysis at the concentrations of our work. All mixtures were prepared for analysis by first adding sufficient base to dissolve any precipitate and then diluting the resulting solution to 4.5×10^{-5} M in both uranium and nucleotide, since close examination showed slight cloudiness which interfered with analysis when both UO_2^{2+} and ATP were 10^{-4} M near pH 2. This necessitated slight changes in the details of the Martin–Doty procedure. Our analyses were carried out as follows.

A 15-ml mixture of uranyl nitrate and nucleotide, either 0.01 M or 0.005 M in each, containing TMNOH sufficient to give the desired r, was prepared in a 100-ml volumetric flask. After the specified time interval, sufficient additional TMNOH was added to raise r to a value of 3. This solution was quickly diluted with water to the 100-ml mark and then immediately diluted further to 4.5×10^{-5} M. A 25-ml aliquot was pipetted into a large test tube and 15 ml of an equivolume isobutyl alcohol–benzene solution was layered. Molybdate reagent (5 ml) was then added, and the test tube was capped and rapidly inverted three times (30 sec total required). After the layers separated (2 min), 10 ml of the organic layer was pipetted into a 25-ml volumetric flask and was diluted with ethanolic 0.35 M H_2SO_4 to about 20 ml. $SnCl_2$ reagent in 0.35 M H_2SO_4 (1 ml) was added and the solution was diluted to 25 ml with the ethanolic H_2SO_4. The solution was shaken and the absorbance at 625 mμ was read immediately using a Beckman DU spectro-

(1) This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y. A preliminary report of this work was presented at the 9th Annual Meeting of the Biophysical Society, San Francisco, Calif., 1965.

(2) The symbolism used in this paper is as follows: mono- and diprotonated species of adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate are represented, respectively, by $HATP^{3-}$ and H_2ATP^{2-}, $HADP^{2-}$ and H_2ADP^{-}, and $HAMP^{-}$ and H_2AMP. When it is unnecessary to denote the degree of protonation, the more general symbols, ATP, ADP, and AMP are used. NTP refers to any nucleotide. U–ATP, U–ADP, U–AMP, and U–NTP refer to uranyl nitrate–nucleotide mixtures regardless of the pH, the extent of protonation at a given pH being evident from the text. The symbol r represents the ratio of moles of base added per mole of total NTP in a given mixture.

(3) L. Hurwitz, Ph.D. Thesis, University of Rochester, 1953.

(4) W. J. Bowen and T. D. Kerwin, *Proc. Soc. Exptl. Biol. Med.*, **88**, 515 (1965).

(5) J. B. Martin and D. M. Doty, *Anal. Chem.*, **21**, 965 (1949).

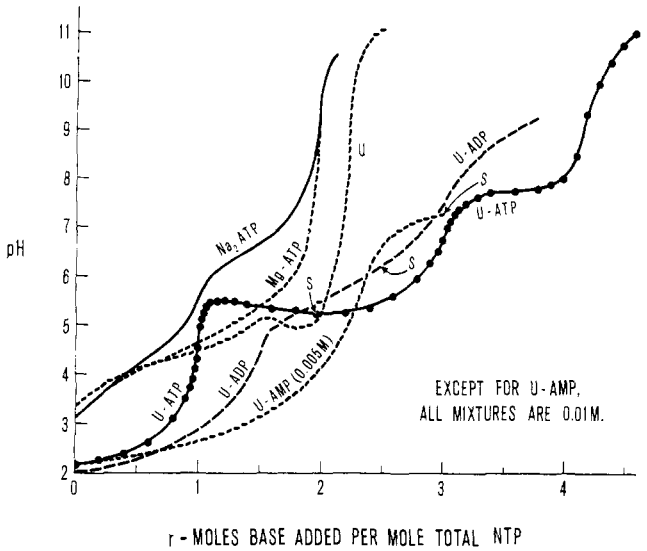

Figure 1. Pointwise titrations of Na_2H_2ATP, $UO_2(NO_3)_2$, and equimolar metal ion–adenine nucleotide mixtures (see text for experimental details). The symbol S indicates the point of titration where dissolution of precipitate seems to be complete.

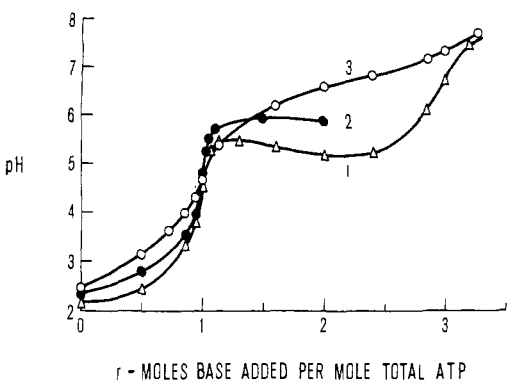

Figure 2. Pointwise titrations of equimolar 0.01 *M* $UO_2(NO_3)_2$–adenine nucleotide mixtures: curve 1, no added salt; curves 2 and 3, 1.0 *M* KNO_3 added; curves 1 and 2, pH readings taken 24 hr after mixing; curve 3, pH readings taken 2 min after mixing.

photometer. An inorganic phosphorus concentration of 1.0 μg/25 ml in the final aqueous solution analyzed corresponds to 2.2 μmoles of orthophosphate in the original 15 ml of U–ATP mixture before dilution.

Results and Discussion

Pointwise titrations of various metal ion–adenine nucleotide mixtures are represented by Figure 1. The most unique feature is the steep rise at $r = 1$ in the 1:1 U–ATP titration. As far as we are aware, this is the first report of an inflection point at $r < 2$ for the titration of a mixture of H_2ATP^{2-} and a divalent cation. For titrations of 1:1 mixtures of H_2ATP^{2-} and either Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, or Cu^{2+}, the first inflection occurs[6,7] at $r = 2$. A slight inflection occurred at $r = 2$ in our titration of a VO^{2+}–ATP mixture also. Our Fe^{3+}–ATP titration showed no clearly discernible inflection until about $r = 3.5$.

(6) H. Brintzinger, *Helv. Chim. Acta*, **44**, 1199 (1961).
(7) M. M. Taqui Khan and A. E. Martell, *J. Phys. Chem.*, **66**, 10 (1962).

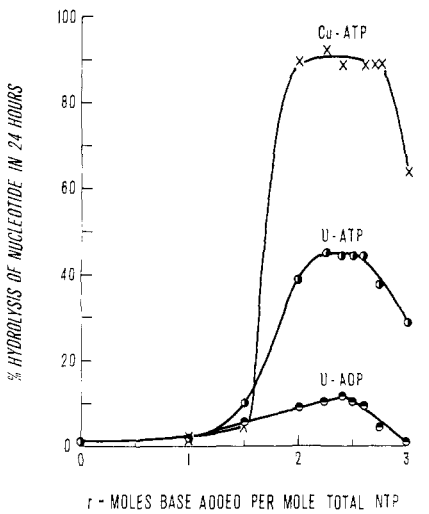

Figure 3. Rate of dephosphorylation of ATP in 0.005 *M* equimolar metal ion–Na_2H_2ATP mixtures as a function of *r*; inorganic phosphate content determined 24 hr after mixing.

In the 1:1 U–ATP mixture (0.01 *M*) there was almost complete precipitation (98%) of both reactants at the start of the titration as shown by failure to notice further precipitation in the colorless supernatant when either reactant was added and by inorganic phosphate analysis of the supernatant.[8] The initial pH in the 24-hr pointwise titration was 2.15 for both the supernatant and the slurry obtained after mixing. As base was added the yellow precipitate gradually became gelatinous until at just before the first inflection the mixture became viscous and nonsedimenting. In the very small region $r = 1.1$ to 1.3, there is a slight maximum in the titration curve which is shown below to be due to the onset of ATP hydrolysis. Here, the system formed a clear thixotropic gel upon standing overnight. From this point on in the titration, the mixture remained clear and gradually changed from a viscous solution to a nonviscous solution at about $r = 2$ (pH 5.3). A second and a third inflection occurred at $r = 3$ (pH 7) and $r = 4.3$ (pH 9.5), respectively.

For comparison a 2-min pointwise titration (*i.e.*, pH readings made 2 min after mixing reagents) is presented as curve 3, Figure 2. Gelation had not yet occurred in these mixtures. The inflection at $r = 1$ is evident although it is not as sharp as in the 24-hr titration.

These observations and the U–ATP curve itself were reproducible to better than 0.05 pH unit before the first inflection and to about 0.1 pH unit thereafter for both forward and reverse pointwise titrations. Several reverse titrations were performed back to about pH 2.15 by adding increments of HCl to the system *immediately* after adding sufficient TMNOH to give various *r* values as starting points for the reverse titrations, *viz.*, $r = 0.8$, 1.5, 2.5, 3.5, and 4.5.

Clearly, if the acid for the back-titration is added *before* any ATP hydrolysis occurs, the titration is reversible and the complex, whether precipitated or

(8) The per cent precipitation was determined as follows. The mixture, after 24 hr of incubation, was centrifuged and then filtered through millipore paper (0.45-μ pore size); 2 ml of filtrate plus 2 ml of 2 *M* HCl were then boiled 0.5 hr, after which an analysis for inorganic orthophosphate was performed. The boiling procedure caused the reaction, $ATP \rightarrow AMP + 2H_2PO_4^-$, to proceed quantitatively.

suspended, may be treated theoretically as a polyelectrolyte. Hydrolysis of ATP, as indicated by orthophosphate analysis, was negligible before addition of HCl in these reverse titrations. In fact, in 4 hr only 10% hydrolysis of ATP occurred at $r = 2$ and 7% at $r = 3$.[9]

The percentage of ATP hydrolyzed as a function of r at 25° in 24 hr in the U–ATP system is shown by Figure 3. These values make it evident that hydrolysis in the U–ATP system at 25° significantly affects the 24-hr titration *only after* the first inflection. No AMP hydrolysis at all was detected in the U–AMP system at any r value up to 3 even after 48 hr of incubation at 25°. It is also apparent that hydrolysis occurs much more slowly (by about a factor of 0.25) in the U–ADP system than in the U–ATP system and that the failure to obtain an inflection at $r = 1$ in the U–ADP titration was not due to any dephosphorylation.

The validity of our interpretation (*vide infra*) of the titration curves depends upon how closely the experimental pH value for a given r measures the hydrogen ion activity at the surface of the precipitate, *i.e.*, upon the magnitude of any difference between the hydrogen ion activities in the supernatant and at the surface of the precipitate.[10] There would be close agreement only if there were not a high charge density at the particle surface. Concerning this point several experimental results give information.

First, as seen in Figure 2, the first inflection in the U–ATP curve remained at $r = 1$ as the ionic strength was varied from about 0.03 (*i.e.*, curve 1, no added salt) to 1.0 with added KNO_3 (curve 2). If the precipitate had a surface charge density sufficiently high to cause a large difference between the hydrogen ion activities of the supernatant and the particle surface, one would expect a significant drop in the measured pH of the mixture as the ionic strength increased.[10] On the contrary, the U–ATP curve was raised slightly by adding 1.0 *M* KNO_3. This small increase could be due to the effect of ionic strength on activity coefficients.

Second, at each point of the U–ATP curve up to $r = 0.9$ (above which precipitate and supernatant did not separate), there was agreement within 0.05 unit in the pH values of (i) the supernatant, (ii) the slurry obtained by slight mixing, and (iii) the very fine suspension obtained by vortexing the mixture for 2 min. One would not expect such agreement if the precipitated particles had high surface charge density, for in such a case the surface charge density should depend on the surface/volume ratio of the particulate matter.

Third, since neither the U–ADP nor the U–AMP curves (Figure 1) show a steep rise near $r = 1$, it seems completely unreasonable to consider the first inflection in the U–ATP curve to be an artifact occurring exactly at $r = 1$.

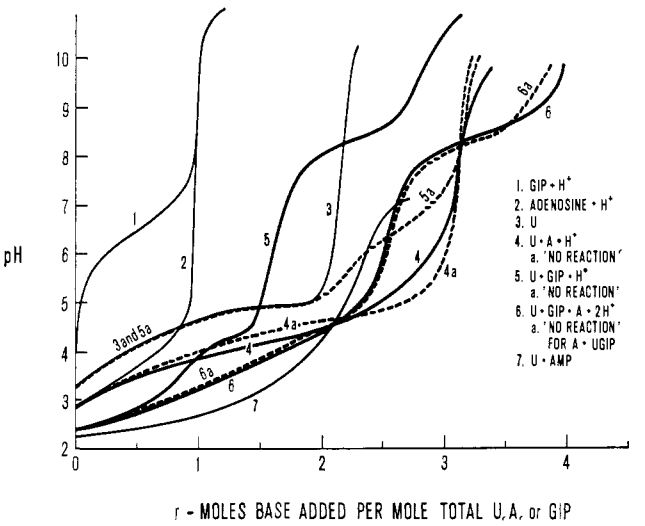

Figure 4. Pointwise titrations of solutions and mixtures indicated. (All reagents 0.005 *M*). Symbols: U, $UO_2(NO_3)_2$; GIP, β-glycerophosphate; A, adenosine. pH readings taken 24 hr after mixing.

The first inflection point in the U–ATP titration must represent removal of a proton *from the adenine group*. This conclusion results from a comparison of the H_2ATP^{2-} and U–ATP curves in Figure 1. In the latter curve the measured pH at $r = 1$ is 4.5. In the H_2ATP^{2-} curve this pH corresponds to $r = 0.68$. Hence, the adenine group of H_2ATP^{2-} is 68% deprotonated[12] at pH 4.5 in absence of uranium. Therefore, if the first H_2ATP^{-2} proton released in the U–ATP titration were the terminal phosphate proton, the adenine deprotonation would have shifted the inflection from $r = 1$ to about $r = 1.68$.

It seems inconceivable that the steep rise at exactly $r = 1$ in the U–ATP titration and the large decrease in apparent pK of H_2ATP^{2-} from 4.1 to 2.5 (*i.e.*, pH measured at half-neutralization in absence and in presence of UO_2^{2+}, respectively) could arise from a field effect on the adenine proton from a uranyl ion attached only to the phosphate chain.[15] Such an explanation was suggested by Khalil and Brown[14] for the relatively small lowering of the pK of the H_2ATP^{2-} adenine group caused by *monatomic* cations, *e.g.*, 0.15 log unit in the presence of Mg^{2+}, 0.5 log unit in the presence of Cu^{2+}. Actual bonding of the uranium to an adenine nitrogen atom therefore seems evident.

Uranyl ion lowers the pK of protonated adenosine to only a slight extent. This is shown by Figure 4 in which curve 4, the U–adenosine curve, below pH 4.5 lies only slightly under the "no reaction" curve 4a. The latter

(9) This result is at variance with that of Bowen and Kerwin,[4] who reported 10% hydrolysis at pH 7.4 in only 1 hr for an ATP concentration of only 0.001 *M*. However, their analyses were performed by the Fiske–Subbarow method, which, we find, gives too large an ATP blank reading to allow an accurate determination of inorganic orthophosphate in presence of a large excess of ATP.

(10) This problem is well discussed in I. Michaeli and A. Katchalsky, *J. Polymer Sci.*, **23**, 683 (1957). These authors found that, when the volume ratio of gel to external solution was high (as is the case on both sides of the first inflection in our U–ATP titration), increasing the ionic strength of the external phase of a polymethacrylate gel–NaCl system markedly lowered the pH of the external solution. Also, L. Goldstein, *et al.*,[11] calculated that the pH difference between the external solution and a polyanionic trypsin derivative gel was decreased from 2.4 to 0.4 when the ionic strength was increased from 0.06 to 1.0.

(11) L. Goldstein, V. Levin, and E. Katchalsky, *Biochemistry*, **3**, 1913 (1964).

(12) Since the titrimetric work of Alberty, *et al.*,[13] in 1951, it has been the universal belief that the protonated adenine group is the most acidic group in H_2ATP^{2-}. Khalil and Brown[14] have proven this point conclusively by their recent infrared studies of D_2O solutions of ATP.

(13) R. A. Alberty, R. M. Smith, and R. M. Bock, *J. Biol. Chem.*, **193**, 425 (1951).

(14) F. L. Khalil and T. L. Brown, *J. Am. Chem. Soc.*, **86**, 5113 (1964).

(15) We have, in an earlier paper,[16] postulated a significant field effect on the α-carboxyl proton of aspartic and glutamic acids by a UO_2^{2+} ion chelated by the β- (or γ-) carboxyl group, but in those cases the proton affected is quite acidic, pK = 2.15, whereas the pK of the adenine proton in ATP is 4.1.

(16) I. Feldman and L. Koval, *Inorg. Chem.*, **2**, 145 (1963).

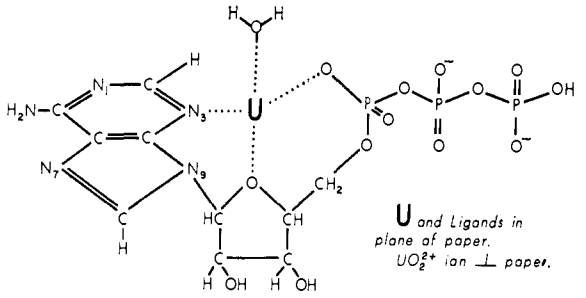

Figure 5. Postulated structure of U–ATP complex before first inflection point, *i.e.*, below about pH 4.5.

curve was calculated by adding the abscissas of the uranyl nitrate curve and the adenosine curve at various pH values. One might therefore question our conclusion that the uranium is ligated by an adenine N atom in the adenine nucleotide. Such a critical view, however, completely ignores the beneficial chelate effect which should be operative when the uranyl ion is chelated simultaneously by both the adenosine and the phosphate moieties of the nucleotide.

Evidence for the chelate effect and for the intramolecular nature of the chelate was obtained by comparing the U–AMP titration with titrations of mixtures containing the adenosine and phosphate moieties in separate molecules. In Figure 4, before its first inflection, the experimental U–GlP (glycerophosphate) titration (curve 5) lies considerably below the U–GlP "no reaction" curve (curve 5a) but significantly above the experimental U–AMP curve (curve 7) even though the concentrations in the U–AMP mixtures were only half those for the U–GlP system (*i.e.*, 0.005 *M vs.* 0.01 *M*). It is evident that GlP reacts fairly strongly with UO_2^{2+} ions, but not as strongly as does AMP. Curve 6, which represents titration of a mixture 0.01 *M* in UO_2^{2+}, GlP, and adenosine (and 0.02 *M* HNO_3 to protonate GlP and adenosine initially) almost coincides with curve 6a, which represents "no reaction" between adenosine and a mixture of UO_2^{2+} and GlP.

Hence, the UO_2^{2+} ion reacts significantly with adenosine only when adenosine and phosphate groups are parts of the same molecule and when these groups are in a geometrical arrangement favorable for intramolecular chelation simultaneously to both moieties. One can sum up by saying that most of the ΔF causing complexation of the uranyl ion by an adenine nucleotide is associated with the reaction of uranium with the phosphate group and that chelation of uranium to the adenine group is a secondary reaction resulting from the beneficial chelate effect.

As mentioned previously, in Figure 1 there is no inflection near $r = 1$ for either the U–ADP or the U–AMP titrations. Rather, an inflection occurs in the U–ADP curve near $r = 1.5$ and in the U–AMP curve near $r = 2.3$. Since AMP has only two ionizable protons, it is evident that some hydrolysis of the uranium must occur in the U–AMP system before the first inflection. This does not happen in the U–ATP system. It seems probable that this difference in hydrolytic tendencies of uranium in U–ATP and U–AMP complexes is simply a reflection of their total charges which are initially -1 and 0, respectively.

Any proposed structures for the initial uranyl–nucleotide chelates must also explain the fact that the initial pH, 2.2, of the 0.005 *M* U–AMP curve is close to the initial pH, 2.15, of the 0.01 *M* U–ATP curve. That is, in the presence of an equimolar uranyl ion concentration AMP releases almost completely both its adenine proton and its phosphate proton even before any base is added, whereas H_2ATP^{2-} releases only its adenine proton before the first inflection and retains the phosphate proton until sometime after the first inflection. The initial pH, 2.03, in the 0.01 *M* U–ADP titration implies that the initial hydrogen ion concentration is about 0.013 *M* if we assume that the hydrogen ion activity coefficient in the U–ADP system is the same as it appears in the U–AMP and U–ATP systems, *i.e.*, antilog -0.15. Thus, it would appear that the average number of protons released from $HADP^-$ upon formation of the initial U–ADP complex is about 1.3. Some hydrolysis of the uranyl group may occur before the first inflection, as in the U–AMP system.

Although there is no obvious *a priori* reason for postulating the same chelate structure in each of the three systems (U–AMP, U–ADP, U–ATP), it is possible to explain all the above experimental results by doing so. In Figure 5, the uranium atom is considered to be simultaneously attached to (i) the negatively charged O atom of the α-phosphate group, (ii) the ribose ring O atom, (iii) the N_3 atom of the adenine ring, and (iv) one water oxygen. Geometric calculations, using crystallographic data for the separate groups, adenine,[17,18] ribose,[18] and triphosphate ion[19] in $Na_5P_3O_{10}$, plus the atomic models, show that these four ligand atoms can lie in a slightly puckered ring about the equator of the colinear UO_2^{2+} group.[20]

In this structure both the inductive effect and the electrostatic field effect of the uranyl ion on the proton of the H_2ATP^{2-} terminal phosphate group should be quite small and should have only slight effect, if any, on the p*K* of this group. On the other hand, both protons of AMP would be removed by uranyl ion since the uranium would be attached to the same (*i.e.*, the only one) phosphate group of which the proton is originally a part.

The curves in Figure 1 imply that $HADP^-$ behaves in a manner intermediate between that of AMP and H_2ATP^{2-} when reacting with the uranyl ion. In our proposed structure for the chelate, the distance between the uranium atom and the terminal phosphate proton is roughly 3 A shorter in U–ADP than in U–ATP. It is quite reasonable then to expect the electrostatic repulsion between uranium and the phosphate proton in U–ADP to be large enough to shift the inflection to a slightly larger r value than unity owing to a small lowering of the p*K* of the ADP phosphate group even though this effect is negligible in U–ATP.

Our postulation that the uranium atom is attached to the N_3 nitrogen atom does not necessarily suggest that

(17) W. Cochran, *Acta Cryst.*, 4, 81 (1951).
(18) M. Spencer, *ibid.*, 12, 59 (1959).
(19) D. R. Davies and D. E. C. Corbridge, *ibid.*, 11, 315 (1958).
(20) The colinearity of the uranyl group and the tendency of ligands to lie as close as possible to the equatorial plane have been shown by Zacharaisen and Plettinger.[21] Its unique effect on the coordination chemistry of the uranyl ion has been discussed by Feldman.[16,22]
(21) W. H. Zachariasen and H. H. Plettinger, *Acta Cryst.*, 12, 526 (1959).
(22) I. Feldman, C. A. North, and H. B. Hunter, *J. Phys. Chem.*, 64, 1224 (1960).

the ionizable adenine proton is originally on N_3. Because of the π-electron mobility in the adenine group, the inductive effect of the positive uranium atom would be transmitted to whichever nitrogen atom originally holds the ionizable proton.[23] We envision here an effect somewhat similar to the "local" charge-transfer concept of Szent-Gyorgyi and Isenberg,[26] *i.e.*, whichever atom of the adenine ring the acceptor uranium atom is attached to can act as a local donor draining on the π-electron pool of the whole conjugated system for the donated electron.

With the structure of Figure 5 one can also explain the fact that the particulate matter in the U-adenine nucleotide systems does not seem to have sufficiently high surface charge density to significantly raise the titration curve (*vide supra*). Since adenine has a very low solubility in water it seems reasonable to conclude that the precipitation which occurs in the U–nucleotide systems may be due to agglomeration of the adenine groups of several chelates into a central ball, aided by the weight of the uranium atoms, with the various phosphate chains pointing outward in different directions. The phosphate chains in the same agglomerate would then be quite far from each other and there would be no buildup of a surface charge density. This would also be compatible with the fact that no flocculation occurs in the 0.01 *M* U–GlP system, but only a slight cloudiness starting at about pH 4.5, although flocculation occurs in the U–adenine nucleotide systems at the start of the titration (in 0.02 *M* U–ATP system one gets immediate gel formation).

(23) The location of the ionizable proton on adenine is known only for the solid state[17] in which it is on N_1. It is, however, quite possible, as pointed out by Cochran himself, that packing considerations may play a major role in determining the location of the proton in the solid state. One cannot, therefore, assume the same location for the proton in solutions. The basicity of N_3 is almost as great as that of N_1, and N_7 is also slightly basic.[24] Despite the fact that there is some evidence favoring N_1 as the protonated site in solution, it seems likely that there would be some proton density on all three of these N atoms in solution.[25]

(24) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, Inc., New York, N. Y., 1963, p 232.

(25) C. A. Dekker, *Ann. Rev. Biochem.*, **29**, 464 (1960).

(26) A. E. Szent-Gyorgyi and I. Isenberg, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1334 (1960).

Undoubtedly, the great difference in the reactions of uranyl ions and monatomic ions with ATP is due largely to the linear triatomic structure of the uranyl ion. For instance, monatomic cations preferentially attach ligand atoms in an octahedral or tetrahedral fashion, whereas the uranyl oxygen atoms tend to force ligand atoms into the equatorial plane. As a result, whereas Mn^{2+} and Co^{2+} bind simultaneously to an oxygen of each of the three phosphates,[27] it is impossible to have such tridentate binding of UO_2^{2+} because of steric hindrance between a uranyl oxygen atom and part of the phosphate chain backbone, specifically P_β–O–P_γ. At present our explanation as to why ATP should not chelate UO_2^{2+} bidentately, as in the Cu^{2+} and Mg^{2+} chelates,[27,28] or why an α-phosphate oxygen should be the preferred ligand if the phosphate chain does indeed act monodentately in the U–ATP complex would be only speculative.

The region of the U–ATP titration between the first and second inflections, $r = 1$ and 3, respectively, is quite complex since undoubtedly several reactions are occurring in this region. It seems probable that in addition to deprotonation of the terminal phosphate group there is some hydrolysis of the uranyl ion and some proton liberation during dephosphorylation, but it is not known whether these reactions are simultaneous or consecutive. The latter seems more likely since the dissolution of the precipitate starts at $r = 1$ and is complete at about $r = 2$ and since the dephosphorylation of ATP starts at $r = 1$ and reaches a maximum near $r = 2$. This implies to us that the deprotonation of the terminal phosphate occurs primarily in the $r = 2$ to 3 region. We are at present carrying out kinetic studies with the hope of elucidating this region of the titration.

Acknowledgment. We wish to acknowledge the technical assistance of Mr. William Hsiong and Mrs. Elinor Kiel.

(27) H. Sternlicht, R. G. Shulman, and E. W. Anderson, *J. Chem. Phys.*, **43**, 3123 (1965).

(28) M. Cohn and T. R. Hughes, Jr., *J. Biol. Chem.*, **237**, 176 (1962).