ROTATORY DISPERSION OF INOSINE TRIPHOSPHATE AND THE INFLUENCE OF METALS ON ITP AND ATP

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SUMMARY

The rotatory dispersions of inosine monophosphate (IMP), inosine diphosphate (IDP), and inosine triphosphate (ITP), have been determined and interpreted to indicate a folded structure of the ITP molecule, somewhat similar to that suggested for the ATP molecule. From the results obtained for the rotatory dispersion of ITP and ATP in the presence of Ca, Mg, or Zn ions, it appears that at pH 7 the combination of ATP with either Ca or Mg does not influence the optical symmetry of the molecule, but with ITP the combination with magnesium results in a product that has similar optical rotatory characteristics to ATP. These observations suggest configurational relationships that allow correlation of the physiological activities of the two molecules. The apparent alteration of the configuration of the ATP molecule in the presence of Zn ion may be used to suggest a configurational mechanism for the action of Zn ion on the 5-nucleotidase enzyme of cobra venom.

INTRODUCTION

In a previous communication[1] an assignment of a structure for the adenosine triphosphate (ATP) molecule in solution was made from a consideration of the rotatory dispersion characteristics of ATP and some of its derivatives. It was further proposed that this structure may be related to the function of the molecule. Work by SZENT-GYÖRGYI[2] with fluorescent dyes led to a similar proposal for the relation of the configuration of ATP and its function.

There are numerous questions raised by such proposals. If ATP configuration and function are causally related, it should then be possible to demonstrate similar structures for compounds known to behave in an "ATP-like" manner. One such compound is inosine triphosphate (ITP) which under appropriate conditions may react in a manner similar to ATP; but under other conditions demonstrates either reduced or negligible "ATP-like" activity.

In this communication the results of the investigation of the rotatory dispersion of inosine and its derivatives inosine phosphate (IMP), inosine diphosphate (IDP) and inosine triphosphate (ITP) are reported. Further, the effects of calcium, zinc and magnesium ions on the rotatory dispersion of both ITP and ATP are reported. Using these results, an attempt is made to correlate the proposed structures and changes in structure with known physiological function of both molecules.

METHODS

The method for the determination of rotatory dispersion has been described elsewhere[1]. Briefly, however, the optical rotation is determined by means of a Rudolph spectropolarimeter at wavelengths between 3200 Å and 6000 Å in increments of 200 Å. In each experiment the square of the wavelength of the light used (λ^2) is plotted as a function of the reciprocal of the specific rotation ($1/[\alpha]$). The resulting graphs are inspected visually to assure that there is no significant deviation from a straight line. The data are then fitted by the method of least squares to obtain the slope (K_m) and the intercept (λ_c^2). From this latter value the so-called critical frequency (λ_c) is calculated. The standard deviation of both the slope and the intercept is calculated for each determination. The data are then presented in table form with the slope (K_m) and the critical frequency (λ_c) reported for each experimental condition.

The rotatory dispersion of inosine, IMP, IDP and ITP was determined in $M/15$ phosphate buffer solution. In all experiments the final concentration of solute was 0.5 % and the temperature was 23 ± 1°. The pH of the solution was varied by the addition of concentrated HCl or NaOH. The volumes of acid or base required were not enough to affect the concentration significantly.

In the experiments in which metal ions were added to the solutions, the solute was first dissolved in water to a final concentration of 0.5 % and the metal ion was added in the form of the solid chloride salt. The final ionic strength of the solution was then made equal to that of the samples in which phosphate buffer was used. The metals were added in equimolar proportion with the ITP or ATP to give maximum opportunity for binding to take place.

Solutions of ATP and solutions of ITP were investigated in the presence of Zn, Ca, and Mg ions. Solutions of AMP and ADP were investigated in the presence of Zn ions and solutions of IMP and IDP were investigated in the presence of Mg ion. Since the ionic strength of solute-metal solutions was that of the solute-buffer solutions, comparisons were made in each case between these two sets of conditions. By this method the ionic strength of the solution could be eliminated as a variable.

The rotatory dispersion values of AMP, ADP and ATP in both water and buffer solutions were found to agree with the values reported by LEVEDAHL AND JAMES[1].

RESULTS

Table I contains values of λ_c and K_m for inosine, IMP, IDP and ITP at pH values of 1, 3, 7, 8 and 9.

The interpretation of these data as attempted in this publication depend on two assumptions. These are: (1) changes in λ_c values arise from changes in the sum of the vicinal forces acting on all optical centers of the molecule or from changes in the over-all symmetry of the molecule and (2) the absolute value of K_m is to a first approximation a measure of the over-all symmetry of the molecule. Hence low absolute values of K_m indicate a high degree of optical symmetry in the molecule where the optical symmetry may result from balancing of the various vicinal forces or from changes in the gross shape of the molecule or both. Changes in the so-called characteristic frequency are undoubtedly very complex and are not subject to a simple interpretation. However, it does appear that the value of the slope (K_m) has theoretical significance[3].

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TABLE I

INFLUENCE OF pH ON λ_c AND K_m OF INOSINE AND DERIVATIVES

pH		*Inosine*	*IMP*	*IDP*	*ITP*
1	λ_c (Å)	2478 ± 33	2767 ± 23	2880 ± 164	2485 ± 121
	$K_m \cdot 10^{-8}$	−9.23 ± 0.043	−5.61 ± 0.081	−4.41 ± 0.083	−5.08 ± 0.061
3	λ_c (Å)	2363 ± 44	2373 ± 10	2144 ± 146	2278 ± 28
	$K_m \cdot 10^{-8}$	−13.75 ± 0.079	−8.99 ± 0.073	−7.41 ± 0.046	−7.46 ± 0.052
7	λ_c (Å)	2345 ± 41	2232 ± 91	2004 ± 144	2545 ± 79
	$K_m \cdot 10^{-8}$	−14.24 ± 0.073	−10.68 ± 0.098	−7.46 ± 0.044	−6.28 ± 0.065
8	λ_c (Å)	2393 ± 25	2253 ± 45	2348 ± 79	2462 ± 61
	$K_m \cdot 10^{-8}$	−15.32 ± 0.164	−10.54 ± 0.047	−7.06 ± 0.055	−7.02 ± 0.061
9	λ_c (Å)	2321 ± 22	2246 ± 62	2139 ± 47	2358 ± 74
	$K_m \cdot 10^{-8}$	−17.85 ± 0.151	−11.28 ± 0.072	−8.13 ± 0.046	−7.46 ± 0.055

In an attempt to give an over-all interpretation to the data of Table I it is immediately apparent that there are numerous complex changes occurring. To date no over-all unifying interpretation has been possible; however, conservative interpretation of the data would indicate that the principle change in the λ_c values occurs with IMP and IDP, with a much smaller change occurring with ITP, at pH 1. Such action is subject to the interpretation that the shift in λ_c at pH 1 can be explained in terms of ionization of the phosphate groups and thus there is no shift in the λ_c value for inosine. A folded structure for ITP would permit interaction of the hydroxyl group at position 6 of the molecule with the terminal phosphate group and in this way suppress, or at least alter, the shift in λ_c that would result from the phosphate ionization that is apparent with IMP and IDP.

In general, the trend of the K_m values is to show that the optical symmetry of the molecule increases as phosphate groups are added (IMP → IDP → ITP). This argument is in excellent agreement with the concept of a folded structure for the ITP molecule.

Studies of muscle contraction have shown both Ca and Mg ions are involved in the action of ATP or ITP[4]. These ions are also necessary for the ATP-ase activity of myosin while Zn ion has been implicated in the ATP augmentation of 5-nucleotidase activity of cobra venom[5]. ITP could not substitute for ATP in this latter role. It was, therefore, decided to determine the effect of these metals on the rotatory dispersion of both ATP and ITP. The data of Tables II and III represent the results of this study.

It is evident from the data of Table II that at the pH value of most interest to the physiologist, namely pH 7, the addition of either calcium or magnesium to ATP does not influence the rotatory dispersion. There is, however, a significant change in the symmetry of the molecule when zinc is added to the solution. In a similar consideration of the data of Table III it is apparent that ITP interacts at pH 7 with both Ca and Mg ion in such a manner as to influence the optical symmetry of the molecule. The interaction with calcium leads to greater optical symmetry while the interaction with Mg leads to an apparent relaxation of the molecule and decreased optical symmetry. The result of this relaxation is a combination that possesses rotatory dispersion characteristics that are the same as those of the ATP molecule within the statistical significance of the data. The data at pH's 5 and 9 are given simply to bracket the pH value of interest and will not be discussed further except to say that the apparent configurational changes that occur would probably have to be explained on the basis of the salt type bonds that would be formed at these non-physiological pH values.

Since interactions of interest were observed between Zn with ATP and Mg with ITP, the nature of the interaction was studied further by investigating the abilities of IMP and IDP to interact with Mg and those of AMP and ADP to interact with Zn. The results of these studies are given in Table IV.

It appears that the optical symmetry of the diphosphate compounds as well as

TABLE II

INFLUENCE OF pH AND METALS ON λ_c AND K_m OF ATP

pH		*Zn*	*Ca*	*Mg*	*Buffer*
5	λ_c (Å)	2530 ± 36	2483 ± 123	2482 ± 88	2328 ± 18
	$K_m \cdot 10^{-8}$	—6.17 ± 0.064	—6,89 ± 0,107	—6.71 ± 0,075	—7.58 ± 0.04
7	λ_c (Å)	2574 ± 121	2434 ± 72	2350 ± 101	2332 ± 63
	$K_m \cdot 10^{-8}$	—5.84 ± 0,098	—7.51 ± 0.059	—7.78 ± 0.055	—7.97 ± 0.32
9	λ_c (Å)	2547 ± 114	2278 ± 57	2521 ± 147	2364 ± 54
	$K_m \cdot 10^{-8}$	—6,43 ± 0.099	—8,12 ± 0,052	—6.45 ± 0.063	—8,28 ± 0,14

TABLE III

INFLUENCE OF pH AND METALS ON λ_c AND K_m OF ITP

pH		*Zn*	*Ca*	*Mg*	*Buffer*
5	λ_c (Å)	2369 ± 86	2434 ± 96	2333 ± 341	2174 ± 35
	$K_m \cdot 10^{-8}$	—6,65 ± 0,056	—5.84 ± 0,059	—6.33 ± 0,024	—7.75 ± 0.029
7	λ_c (Å)	2399 ± 36	2367 ± 47	2043 ± 122	2412 ± 65
	$K_m \cdot 10^{-8}$	—6.96 ± 0.05	—6.43 ± 0,056	—7.54 ± 0.003	—7,06 ± 0,06
9	λ_c (Å)	2905 ± 75	2437 ± 74	2195 ± 115	2358 ± 74
	$K_m \cdot 10^{-8}$	—3.91 ± 0.084	—7.19 ± 0.059	—7.83 ± 0.002	—7.46 ± 0,055

TABLE IV

THE INFLUENCE OF METALS ON λ_c AND K_m OF MONO AND DI PHOSPHATE COMPOUNDS AT pH 7

		IMP	*IDP*	*AMP*	*ADP*
Buffer	λ_c (Å)	2232 ± 91	2049 ± 144	2500 ± 69	2346 ± 52
	$K_m \cdot 10^{-8}$	—10,68 ± 0.098	—7.46 ± 0.044	—11,64 ± 0.22	—9.06 ± 0.11
Metals added	λ_c (Å)	2092 ± 120*	1992 ± 85*	2301 ± 56**	2581 ± 64**
	$K_m \cdot 10^{-8}$	—10.61 ± 0.092	—8,77 ± 0,059	—12.23 ± 0,24	—7.89 ± 0,081

* Mg ion added.
** Zn ion added.

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that of the triphosphate compounds is influenced by the presence of the metal ions. The optical symmetry of the monophosphate compounds, however, is not influenced by the presence of the metal ions.

DISCUSSION

The fact that both inosine and adenosine phosphated compounds form stable complexes with metal ions is well known[6]. The use of rotatory dispersion to investigate these complexes is not for the purpose of identifying the complexes, but rather is to elucidate if possible the influence of the added metals upon the configuration of the complex.

Toward this end it should be noted that while ITP is less effective than ATP in producing muscle contraction, the addition of Mg ion to the preparation results in ATP and ITP both being about equally effective[1]. Considering the results of the present researches in this regard, it is of interest that the addition of Mg ion to ITP solutions results in a K_m of the dispersion pattern that is the same as that of ATP within the statistical reliability of the method. This implies that the two molecules have similar optical symmetry and permits the possibility of a similar configuration under conditions where the physiological activities are similar.

The extension of the rotatory dispersion studies to metal interaction with the mono- and diphosphate compounds permits some speculation as to the configuration of the complexes formed. Construction of molecular models of the compounds under investigation shows that the folded configuration of both the di- and triphosphated structures of either inosine or adenosine would permit a structure that could be stabilized by incorporation of a metal ion between the terminal phosphate group, and the substituted group at position 6 of the molecule. With the monophosphate compound the distance between the phosphate and the substituted group at position 6 is so great that the metal ion could not bridge the gap. Such a model is in excellent agreement with the data from the rotatory dispersion studies.

It is of course obvious that the present studies have no bearing on the possible interactions between metal ions and proteins. This latter type of action may well be the important factor for stabilization of a protein-metal-ATP structure. The present researches would conform to a model in which once the proper "ATP-like" structure is achieved, it would then bind to the protein perhaps through another metal bridge to produce a physiologically active compound.

Recently KAYE[5] has described a situation in which removal of Zn ion from cobra venom resulted in a two-fold increase in 5-nucleotidase activity. Readdition of the Zn ion reversed this effect. From his results he deduced that an ATP-Zn complex was formed and the ATP was removed from the system by this process. The removal of the ATP, which was necessary for the enhancement of the 5-nucleotidase activity, then resulted in a decreased enzyme activity. ITP could not substitute for ATP in this system.

The results presented in this communication support the idea of the Zn-ATP complex at all pH values studied. The complex formed is obviously quite different configurationally than the original ATP, and if the integrity of the ATP structure is necessary in the 5-nucleotidase system, the ATP could be "removed" simply by the configurational changes that occur in the interaction with the Zn ion. Our results would be compatible with those of KAYE for the ITP also since no Zn-ITP complex is observed and it could not compete for the zinc present thereby releasing the ATP, nor is ITP configurationally satisfactory, with or without Zn ion, to substitute directly for the ATP.

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AN IMPROVED METHOD FOR THE DETECTION OF N-ACETYLAMINO SUGARS ON PAPER CHROMATOGRAMS

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SUMMARY

The detection of N-acetylamino sugars on paper chromatograms has been made more sensitive by the use of an ethanol–borate spray, followed by steaming and spraying with a modified DMAB spray. As little as 1 μg of N-acetylglucosamine can be detected on paper. By extracting the coloured complexes from the paper, the method can be adapted for quantitative studies. The method was applied to the detection of acetylamino sugar compounds in partial acid hydrolysates and lysozyme digests of bacterial cell walls.

INTRODUCTION

PARTRIDGE[1] demonstrated that the reagents used in the MORGAN AND ELSON[2] reaction for N-acetylamino sugars could be adapted for the detection of these compounds on paper chromatograms. The stability and yield of the coloured complex formed under the conditions of the N-acetylamino sugar reactions is susceptible to such factors as the duration of heating with alkali, the presence of salts, the concentration of hydrochloric acid in the *p*-dimethylamino benzaldehyde reagent[2–4]. It is therefore not