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FINAL TECHNICAL REPORT

An Investigation of the Adsorption Characteristics of 5'ATP and 5'AMP onto the Surface of $CaSO_4 \cdot 2H_2O$

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(NASA-CR-177101) AN INVESTIGATION OF THE ADSORPTION CHARACTERISTICS OF 5 PRIME ATP AND 5 PRIME AMP ONTO THE SURFACE OF CaSO SUB 4 X 2H SUB 2 O Final Technical Report (Santa Clara Univ.) 13 p CSCL 07D G3/25

N86-29005

Unclas 43234

NCC 2-170

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SUMMARY

A model has been proposed (Lahav and Chang, 1982) in which solid surfaces can act as a site for catalytic activity of condensation reactions for certain biomolecules. From this model, the adsorption characteristics of 5'ATP and 5'AMP onto the surface of $CaSO_4 \cdot 2H_2O$ was chosen for study. It has been proven that 5'ATP and 5'AMP do adsorb onto the surface of $CaSO_4$. Studies were then made to determine the dependance of adsorption versus time, concentration, ionic strength and pH. It was found that the adsorption of the nucleotides is highly pH dependent, primarily determined by the phosphate acid groups of the nucleic acid molecule. From this investigation, the data obtained is discussed in relation to the the model for the prebiotic earth.

Introduction

The possible role of soluble salts in chemical evolution was recently discussed in relation to fluctuating prebiotic environments (Lahav and Chang, 1982). In this environment, a model was proposed in which a hydration-dehydration system could have been responsible for reactions of biomolecules.

During the dehydration stage of such a model, some biomolecules tend to leave the solution and concentrate at certain microenvironments, such as micelles and aggregates, at the liquid-gas interface, and at the emerging solid surfaces. In addition to inorganic clays, crystals of soluble salts may have acted as sites for biomolecules to leave the solution.

In a fluctuating environment, such as a tidepool, lake, or pond, some of the dissolved salts begin to leave solution and form solid crystals upon dehydration. It is on these

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solid crystals that biomolecules can adsorb and possibly react with one another to form organic complexes. Then, upon rehydration, both the biomolecules and metal salts dissolve back into solution to repeat the process.

In one investigation (Rishpon, O'Hara, Lahav, Lawless, 1982), it was found that 5'ATP catalyzes the formation of Glycine oligomers. It was also found that addition of $MgCl_2$ or $ZnCl_2$ increased the yield by enhancing the thermal stability of 5'ATP. It was thought that somehow the Mg^{++} and Zn^{++} ions retarded the pyrolysis of the of the ATP, which would allow a greater concentration of ATP to react with the glycine.

It was then a subject of curiosity as to the means by which the soluble salt acted in retarding this breakdown. From the aforementioned model, one can conceive of a primordial lake or pond containing soluble salts, nucleic acids, and amino acids. During dehydration, the concentration of the soluble salt soon reaches a point at which the salt is at its saturation level. Any further dehydration will cause the salt to form its hydrated crystals, on which the adsorption of biomolecules can take place, 5'ATP being one of them. Once adsorbed onto the surface of the salt, condensation reactions can become more favorable due to the lower entropy of the system and enhanced thermal stability of the nucleotide.

It is with this scenario in mind that experiments in a laboratory were conceived. Since it was thought that nucleic acids and amino acids were adsorbed onto the surface of the

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metal salts, experiments were devised to measure the amount and characteristics of such adsorption, if any. The two biomolecules chosen for this investigation were 5'ATP and 5'AMP. Original studies of adsorption using the soluble salt $MgCl_2$ showed no measurable adsorption. It was then decided to use the less soluble salt $CaSO_4$, in hopes that a less soluble salt will provide a more stable surface area.

Experimental

The nucleic acid 5'ATP was obtained from P.&L. Biochemicals. The 5'AMP was obtained from Boehringer Mannheim Pharmaceuticals. All salts were analytical quality reagents and obtained from either Fisher, J. T. Baker, or Mallinckrodt. All other chemicals were of the purest commercial quality available. All solutions were prepared with filtered, ion-exchanged high purity water.

Saturated solutions of $CaSO_4$ were prepared at 22° C by adding excess salt (> 0.24 g/100 mL) to the volume of water desired. The solutions of liquid were allowed to equilibrate overnight to ensure saturation. All solutions of 5'ATP and 5'AMP were prepared volumetrically using the saturated $CaSO_4$ as dilutent.

In all experiments, 0.500 + .001 g of solid $CaSO_4 \cdot 2H_2O$ was weighed into 13x100mm screw-top test tubes. To this, five millilitters of saturated $CaSO_4$ solution with the desired concentration of the dissolved biomolecule (0.05 mM) was added. If pH adjustment were necessary, they were made at this time using 1.0M (or less when necessary) HCl or NaOH

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solutions. The test tubes were then sealed with a teflon coated screw-cap and allowed to equilibrate. The pH was measured using a Beckman pH meter with combination electrode.

All experimental solutions were shaken for at least three hours at 22°C (room temperature) on a standard horizontal shaker. Every 30-40 minutes (except for samples that were allowed to equilibrate overnight), each sample was vortexed to ensure efficient mixing of solute biomolecule and solid salt, and to minimize occlusion of the biomolecule as an impurity in the salt crystal lattice. After the appropriate equilibration time, each sample was centrifused for five minutes on a standard desk-top analytical centrifuge to separate the supernatant liquid from the solid salt. Low speeds were used as this affected the adsorption of the biomolecule and the pH of the solution.

An aliquot was withdrawn and filtered through 0.45 um filters (Millipore Corp.) to remove any suspended salt particles. The concentration of the supernatant liquid and the original stock solution was measured by ultraviolet absorption (λ_{max} = 259 nm for 5' ATP @ pH 7.2) using a Cary 14 double-beam recording spectrophotometer. The amount of biomolecule adsorbed onto the solid salt was determined as the difference in UV absorption between the initial stock solution and the measured sample solution after shaking.

Ionic strength measurements were made using NaCl to vary the concentration of ions over the solid salt. The salt NaCl was added to the original saturated $CaSO_4$ solution before the

overnight equilibration to ensure that the solution was saturated. PH measurements were made of all samples after the supernatant liquid was filtered and measured for absorbance. In determining the surface area of the salt, a 0.242 mM solution of methylene blue indicator in saturated $CaSO_4$ was used. The absorbance of the methylene blue solution (λ_{max} = 662 nm) that was treated by the same procedure as in the preceding paragraph was compared to the stock methylene blue solution at the same PH value and the difference in concentration determined.

Data and Discussion

In the first set of experiments, 10.00 ml of a 45.8 mM 5'ATP saturated $CaSO_4$ solution was added to 2.000 g of $CaSO_4 \cdot 2H_2O$. The final 5'ATP concentration was measured to be 10.5 nM, which indicated an adsorption of 176 nmoles 5'ATP adsorbed per gram of solid $CaSO_4 \cdot 2H_2O$. It was this experiment that first showed that the adsorption of 5'ATP could be measured. It was then decided to do several series of experiments to characterize this adsorption.

The next set of experiments were to determine wether or not this measured adsorption followed a Langmuirian curve. Experiments were done to determine the adsorption of 5'ATP as a function of final concentration. The results are shown in fig. 1. As the graph indicates, the adsorption is linear at low concentrations, and begins to level off as the concentration over the solid salt increases. In fig. 2, a plot of a Langmuir adsorption isotherm shows that the adsorption fol-

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lows a Langmuirian adsorption, except at very low concentrations. The binding constant for the adsorption is Kb = 1.30 x 100, which indicates a very weak interaction.

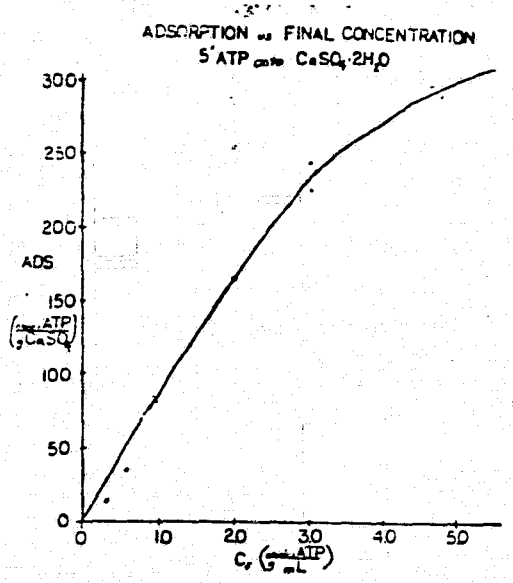

Figure 1.

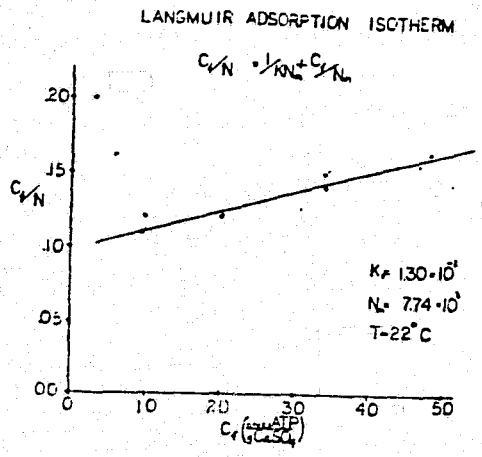

Figure 2.

The next interaction that was looked at was the adsorption as a function of ionic strength (fig. 3). As expected,

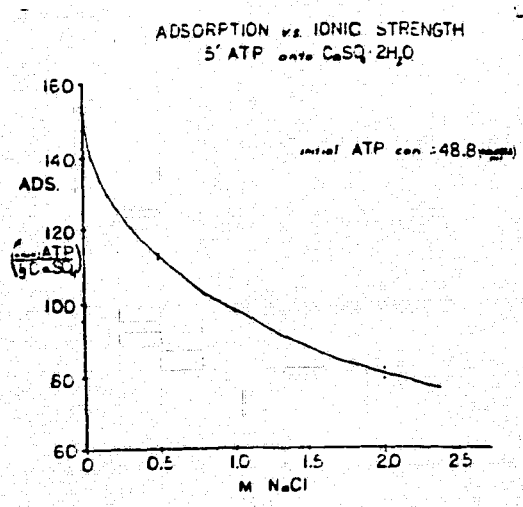

Figure 3.

the adsorption goes down as the solubility of 5'ATP goes up due to the increase of ionic activity in the solution above

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the solid salt.

The next parameter looked at was the adsorption versus time. PH versus time was also measured from this experiment. It was at this point in the investigation that we decided to also experiment with 5'AMP, since this nucleotide was also present on the primordial earth. The results of the the study are shown in fig. 4 and Fig. 5.

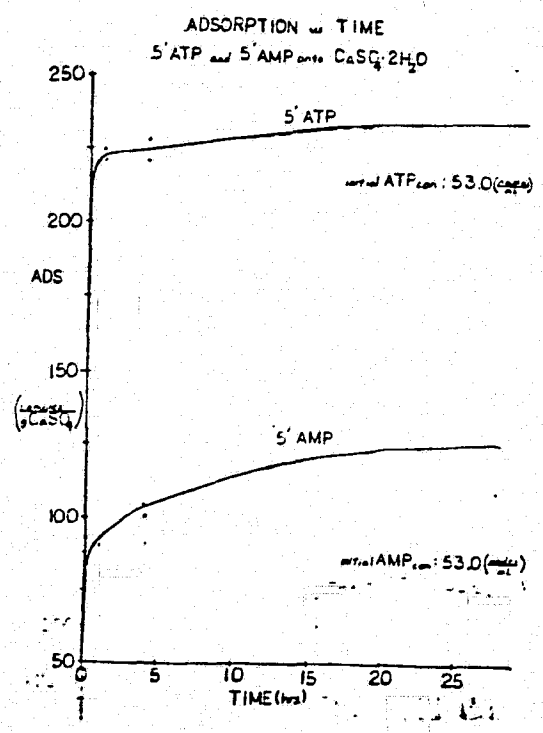

Figure 4.

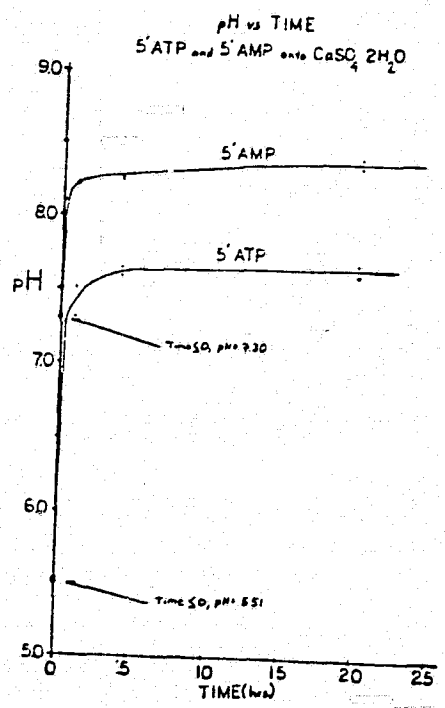

Figure 5.

Using equimolar concentrations of 5'ATP and 5'AMP, the adsorption plot shows a greater (almost twice as much) adsorption for ATP than AMP. This seems to indicate that the adsorption is somehow linked to the extra two phosphate groups on the ATP molecule. Further, the equilibration time for 5'ATP is much faster than that of 5'AMP. This was important only to give information about how long samples should

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be equilibrated for on the shaker when doing further adsorption studies.

From Fig 5, one sees that equilibration time for pH is about the same for ATP and AMP. The shape of the curves however seem to follow the shape of the adsorption curves. With the thought that the adsorption is dependent on the phosphate groups and the match of the shape of the adsorption and pH curves, it was thought that the adsorption is highly pH dependent.

This hypothesis was tested in the next (and the most difficult to control) set of experiments: the adsorption as a function of pH. Due to the weak buffering capacity, the data points between pH 4-6 were hard to stabilize, and as a result, only had two hours of equilibration. The result of the 5'ATP adsorption versus pH are shown in fig. 6.

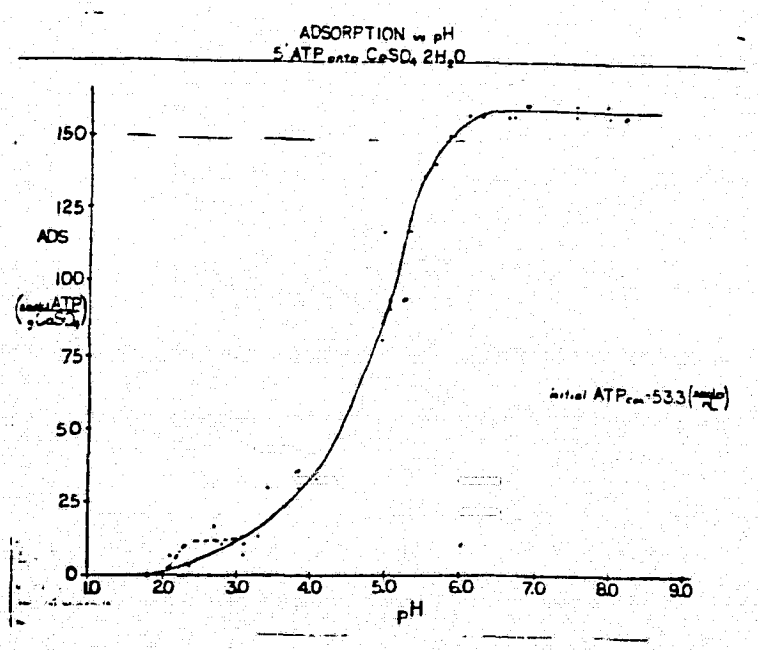

Figure 6.

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As one can see, the adsorption curve follows that of a weak acid titration. The curve has an endpoint of -5.2, which is quite different from the 5'ATP pK_a2 of 6.2. The dotted line shows what would have been expected if the adsorption followed the deprotonation of the first phosphate proton. However, this curve does lend credence to the hypothesis that the adsorption is linked to the phosphate groups.

In contrast to 5'ATP, 5'AMP shows a curve that clearly demonstrates an adsorption that follows the dissociation of the phosphate protons. The curve shows endpoints at the expected range of the pK_a's of 5'AMP, which are pK_a1 = 2.3 and pK_a2 = 6.3 (fig. 7). From the pH of 1-2, there is no

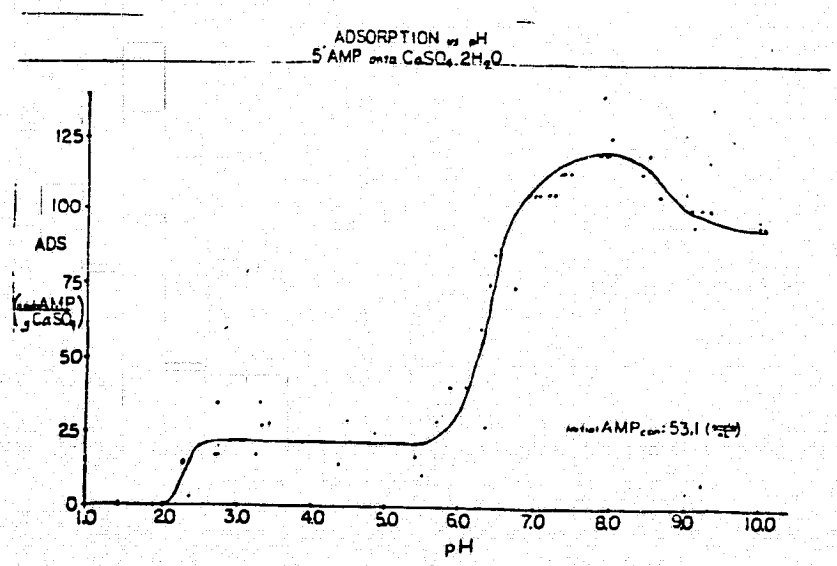

Figure 7.

[illegible] range, the molecule is totally protonated, with a positive charge on the basic nitrogen of the purine

base. Around pH 2.3, the first deprotonation occurs, which results in the formation of a Zwitter ion. This allows the oxygen of the phosphate to form a weak interaction with the salt crystal.

From pH 3-6, no further increase in adsorption is shown. This is consistent with the fact that no further deprotonation is taking place. From pH 6-8, a large adsorption is found. This is probably due to the extraction of the second proton which changes the 5'AMP molecule from a Zwitter ion to an ion with a negative net charge. This formation of the negative charge is important, for it not only increases the adsorption, but it leads one to postulate that the oxygens on the phosphate are somehow interacting with the Ca ions in the salt lattice.

In order to determine if the surface area did not change over the pH range studied, Methylene Blue indicator was used to measure the surface area adsorption versus pH (Fig. 8).

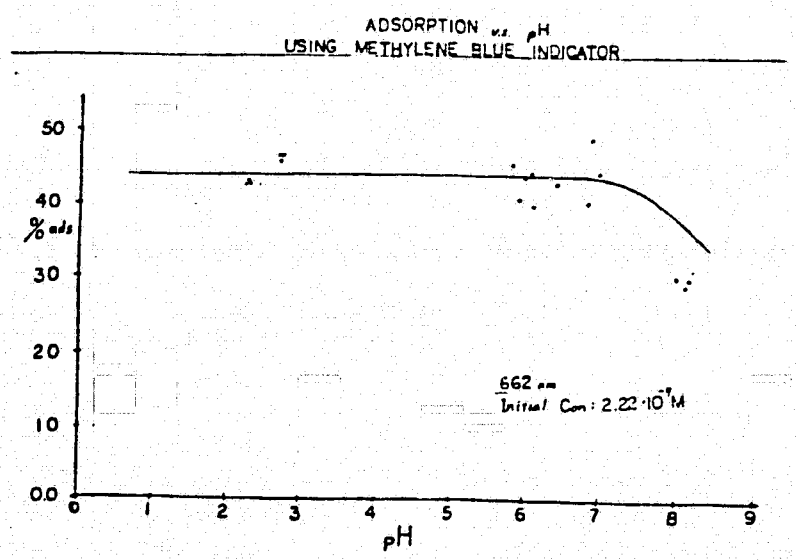

Figure 8.

As shown, the surface area did not change over the pH range. This helps to insure that the adsorption curves of both 5'ATP and 5'AMP are not in error due to changing surface area.

Conclusion

As the data indicates, 5'ATP and 5'AMP do adsorb onto the surface of $CaSO_4 \cdot 2H_2O$. The adsorption is highly dependent on ionic strength. This would account for the lack of measurable adsorption onto $MgCl_2$--the high solubility of $MgCl_2$ would increase the ionic strength to a point that the nucleotide would be almost totally soluble. This is a consideration that one must take into account when postulating a possible mechanism of adsorption in a cycling reaction.

The adsorption of the nucleotides is highly pH dependent. This is another parameter that must be taken into account when doing cycling reactions. The pH of the hydration water, the buffering capacity of the metal salts used, dissolved CO_2 amount of water left in the dehydration test tube, and other factors can change the pH of the solution above the metal salt and thus change adsorptivity.

The adsorption is probably due to the interaction of the oxygen-phosphate moiety interacting with the Ca^{++} ions in the crystal lattice. The greater adsorption of ATP at equimolar concentrations and the pH dependence of the adsorption support this. While this interaction seems to be ionic, the low binding constant (Kb = 1.3.102) would indicate that the bond formation is very weak, and thus not totally ionic.

Since it was the purpose of this investigation to determine if 5'ATP and/or 5'AMP adsorb, and if so, under what conditions, this line of experimenting is deemed concluded. The direction that the experiments will now take is to continue where cycling experiments were last examined (Rishpon, et. al.), and continue to explore the conditions that give the best yield of glycine oligomers. Further directions also include using other nucleotides, metal salts, and amino acids to find out what combinations yield the best results of complex biomolecules.