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The Influence of Metal Ions on the Hydrolysis of Polyphosphates*

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ABSTRACT: The influence of some metal ions on the hydrolytic degradation of polyphosphates has been studied. Uranyl salts catalyze the hydrolysis of polyphosphates.

A number of metal ions (*e.g.*, uranyl and nickelous ions) change the rate of hydrolysis, presumably by changing the conformation of the polyphosphate chains. The influence of metal ions on the hydrolysis of pyrophosphate is quite different from the influence of these ions on the hydrolysis of larger polyphosphates. The possible relationship between these effects and the influence of some metal ions on enzymatic reactions involving polyphosphates is briefly discussed.

Polyphosphates occur in a wide variety of microorganisms (Lohmann, 1958; Kuhl, 1960). More recently polyphosphates have been detected in liver nuclei (Penniall and Griffin, 1964) and mitochondria (Lynn and Brown, 1963). Most investigators consider these condensed phosphates as phosphate and energy reserve of the cells.

Recently it has been proposed that polyphosphates play an important role in the process of glucose transport into yeast cells (van Steveninck, 1962; van Steveninck and Booij, 1964). Polyphosphates at the surface of yeast cells probably take part in an enzymatic phosphorylating process, preceding the uptake of glucose into the cells. The binding of certain metal ions (*e.g.*, Ni^{2+} and UO_2^{2+}) to these polyphosphates inhibits glucose transport. This inhibition was ascribed to changes in the spatial arrangement of the polyphosphate chains, induced by the metal ions.

It seems to be of interest to compare the influence of metal ions on this enzymatic scission of the P–O–P linkage with the influence of these ions on the hydrolytic scission of the P–O–P bond in acidic solutions. Some experiments on the influence of metal ions on the hydrolytic degradation of polyphosphates will be presented in this communication.

Methods

The sodium salts of the polyphosphates were dissolved in distilled water and converted into the corresponding acid over an ion-exchange column (Amberlite IR-120, H^+ form). In most experiments the solution contained finally 100 μg of P/ml. The pyrophosphate used in these experiments appeared to be chemically pure. The other polyphosphates were mixtures of molecules with an average chain length of n = 8, 13, 20, 120, and 200, respectively.

Metal ions were added as the corresponding nitrates. With the low polyphosphate concentrations used in these experiments, no precipitates were formed. The pH of the solution was adjusted with HCl or tetramethylammonium hydroxide, as it has been shown that tetramethylammonium ions do not influence the hydrolysis of polyphosphates (Van Wazer *et al.*, 1952). The pH was measured again at the end of the experiment. The pH shift, caused by the hydrolysis of the polyphosphate, was always <0.02 pH unit; therefore, the velocity of the hydrolysis was not appreciably influenced by it (see Results). In most experiments the pH was between 0.60 and 2.40. The reaction mixture was kept at a constant temperature in a water bath. Samples were withdrawn at intervals, cooled rapidly in melting ice, and analyzed for liberated orthophosphate, by the method of Fiske–Subbarow, as modified by Meyerhof and Oesper (1947). In preliminary experiments it was noted that under the described experimental conditions, at 40–50°, the liberation of orthophosphate proceeds linearly with time for *ca.* 1 hr, with all polyphosphates tested. So determination of the amount of ortho-phosphate, liberated in the course of time, is a good indication for the rate of hydrolysis.

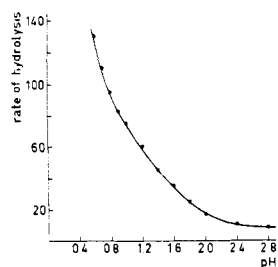The velocities of orthophosphate formation at a

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FIGURE 1: The influence of pH on the rate of hydrolysis of polyphosphate ($n = 13$) at 40°. The rate of hydrolysis is expressed in μg of ortho-phosphate P formed/30 min per 10 ml. Polyphosphate concentration, 100 μg of P/ml.

FIGURE 3: The inhibition of the rate of hydrolysis caused by Ni^{2+} ions, at different pH values; temperature, 40°, polyphosphate, $n = 13$, 100 μg of P/ml.
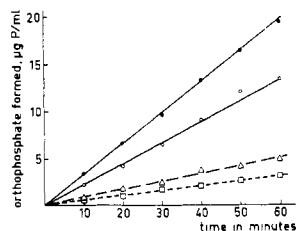

FIGURE 2: The influence of nickelous ions on the hydrolysis of polyphosphate, $n = 20$, at 40°, pH 1.00. Polyphosphate concentration: 100 μg of P/ml. ● ●: control; O O: 2 mmoles of $Ni(NO_3)_2/l.$; Δ Δ: 9 mmoles of $Ni(NO_3)_2/l.$; ☐ ☐: 15 mmoles of $Ni(NO_3)_2/l.$

FIGURE 4: ●, ■, ▲: inhibition of the rate of hydrolysis of polyphosphate by Ni^{2+} (in per cent of the maximal inhibition that can be achieved, viz., 90%); O, ☐, Δ: conversion of polyphosphoric acid into Ni^{2+} polyphosphate (e.g., the amount of Ni^{2+} complexed by the polyphosphate, in per cent of the maximal amount of Ni^{2+} that can be bound), at different pH values. Polyphosphate, $n = 13$, 100 μg of P/ml. Hydrolysis was studied at 40°.

certain temperature and pH are about equal for all condensed phosphates tested, with the exception of pyrophosphate. The hydrolysis of pyrophosphate proceeds ca. 3.5 times slower than the hydrolysis of the other phosphates. This means that in the experiments with polyphosphates with $n > 2$ the initial rates are virtually uncomplicated by additional hydrolysis from pyrophosphate, formed during the degradation of the polyphosphate.

H^+–metal ion-exchange experiments were conducted with equilibrium dialysis technique. The same reaction mixture (10 ml) as used in the experiments on hydrolysis (polyphosphate, salt, and HCl) was placed in a dialysis bag (Kalle Aktiengesellschaft, 15451402). This dialysis bag is placed in a cylinder, containing 200 ml of a solution of the same HCl concentration as the contents of the bag. This cylinder is left at 0° for 15 hr. Hydrolysis of the polyphosphate is neglectable under these circumstances. In preliminary experiments it was found that free Ni^{2+} and UO_2^{2+} ions attain the same concentration inside and outside the bag within 4 hr. The polyphosphoric acid and the polyphosphate–metal ion complex on the other hand did not diffuse out of the bag; no appreciable amounts of phosphate were found in the outer solution. After 15 hr the metal ion concentration in the outer solution was determined. Ni^{2+} was determined according to
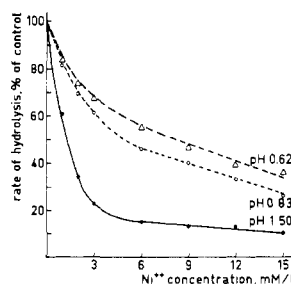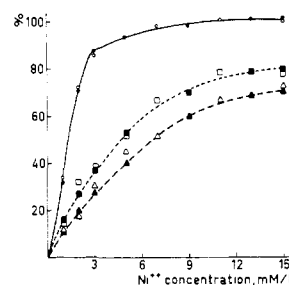
Makepeace and Craft (1944); uranyl was determined according to Francois (1958). From the concentration of free metal ions and the amount of ions added at the beginning of the experiment, the amount of metal ions complexed by the polyphosphate can be calculated.[1]

Results

The influence of pH on the rate of hydrolysis is shown in Figure 1. The shape of this curve is equal for all polyphosphates tested. From these results it could be concluded that the pH shift during the experiments (<0.02 unit) was so small that its influence on the rate of hydrolysis could be neglected (see Methods). From experiments at different temperatures (40–50°), at pH

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[1] The impact of the Donnan equilibrium on the distribution of metal ions should be considered. Calculation reveals, however, that under the described experimental conditions the ratios [polyphosphate]/[HCl] and inner/outer solute volume make the influence of Donnan equilibrium negligible, so far as determination of the amount of complexed metal ions is concerned (error <1%).
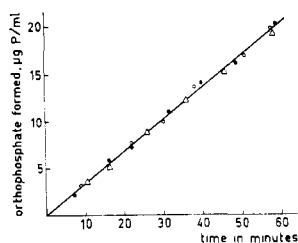
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FIGURE 5: The influence of nickelous ions on the hydrolysis of pyrophosphate at 50°, pH 0.80. Pyrophosphate concentration, 100 μg of P/ml. ● ●: control; O O: 5 mmoles of Ni (NO₃)₂/l.; Δ Δ: 40 mmoles of Ni-(NO₃)₂/l.
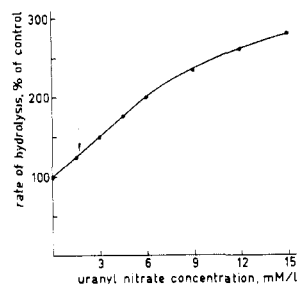

FIGURE 6: The influence of uranyl nitrate on the hydrolysis of polyphosphate (n = 13), at pH 1.50, 40°. Polyphosphate concentration: 100 μg of P/ml. The arrow indicates the point where virtually all polyphosphoric acid is converted into uranyl polyphosphate (see Discussion).
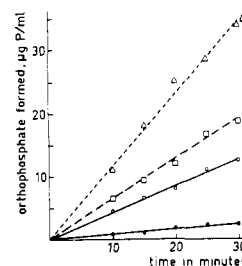

FIGURE 7: The influence of uranyl nitrate on the hydrolysis of polyphosphate (n = 13), at 40°. Polyphosphate concentration, 100 μg of P/ml. 1 = control, pH 0.70; 2 = control, pH 1.80; 3 = 40 mmoles of uranyl nitrate, pH 0.70; 4 = 40 mmoles of uranyl nitrate, pH 1.80.
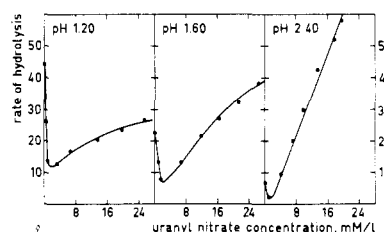

FIGURE 8: The influence of uranyl nitrate on the hydrolysis of pyrophosphate at 50°, at different pH values. Pyrophosphate concentration, 100 μg of P/ml. The rate of hydrolysis is expressed in μg of ortho-phosphate P formed/30 min per 10 ml.

0.8, the energy of activation was calculated. A value of *ca.* 22.3 kcal/mole was found for all the polyphosphates studied. This figure is in agreement with results published by other investigators (Friess, 1952; Abbott, 1909). The presence of metal ions (Ni^{2+}, Co^{2+}, Mg^{2+}, UO_2^{2+}, and Zn^{2+}) in concentrations of 0.01 to 0.1 M did not change the energy of activation.

Nickelous ions inhibit the hydrolysis of polyphosphates with $n > 2$ (Figure 2). The maximal inhibition that can be achieved is *ca.* 90%. The percentage of inhibition depends on the ratio Ni^{2+}/H^+ and on the polyphosphate concentration. The influence of nickelous ions on the velocity of hydrolysis of polyphosphate (n = 13) at different pH values is depicted in Figure 3. H^+–Ni^{2+} exchange experiments show that at each pH value the increasing inhibition with increasing Ni^{2+} concentrations parallels the conversion of polyphosphoric acid to Ni^{2+} polyphosphate. Plotting the amount of Ni^{2+} complexed by the polyphosphate (in per cent of the maximal amount of Ni^{2+} that can be complexed) and the inhibition of hydrolysis (in per cent of the maximal inhibition that can be achieved with Ni^{2+}, *viz.*, 90%) *vs.* the amount of $Ni(NO_3)_2$ added, both curves coincide (Figure 4).

A number of other bivalent metal ions (Mg^{2+}, Zn^{2+},

and Co^{2+}) also inhibit the hydrolysis of polyphosphates. The mechanism of these inhibitions is presumably comparable with the Ni^{2+} inhibition. However, this was not studied in detail. The hydrolysis of pyrophosphate on the other hand is not inhibited at all by nickelous ions (Figure 5).

The influence of uranyl salts on the hydrolysis of polyphosphates is quite different from the Ni^{2+} influence. The hydrolysis of polyphosphoric acids with $n > 2$ is stimulated by uranyl nitrate. At low uranyl concentrations this stimulation is proportional to the amount of the uranyl salt added to the solution. At higher concentrations the curve flattens out (Figure 6). This stimulation increases not only relatively but also absolutely with increasing pH (Figure 7).

The influence of uranyl nitrate on the hydrolysis of pyrophosphoric acid is more complicated. At low concentrations uranyl nitrate inhibits the hydrolysis. The maximal inhibition measured was 66%. At higher concentrations there is a reversal of the inhibitory effect, marked by a sharp inflection of the curve (Figure 8). The increase of the rate of hydrolysis beyond the inflection point is the larger, the higher the pH of the solution. At relatively high pH the velocity of hydrolysis increases to values much higher than the control. H^+–uranyl exchange studies reveal that the inhibition

with low, increasing uranyl concentrations parallels the conversion of pyrophosphoric acid to uranylpyrophosphate (Figure 9). The inflection points of the curves in Figure 8 thus coincide with the points that all pyrophosphoric acid has been converted into the corresponding uranyl salt.

Discussion

The influence of metal ions on the H^+-catalyzed hydrolysis of polyphosphates appears to be complicated. A number of modes of action of metal ions must be distinguished. (1) Metal ions have an influence on the conformation of the polyphosphate chain. This has been demonstrated by Jost (1958), Thilo (1959), and Corbridge (1955, 1956) by crystallographic methods. Thilo found comparable conformation changes of polyphosphates in solution. As it is recognized that conformation frequently influences reaction rate (Fieser and Fieser, 1956), metal ions can change the kinetics of polyphosphate hydrolysis in this way. (2) The metal ions themselves may catalyze the hydrolytic degradation of the polyphosphate (see, e.g., Thilo, 1962). (3) A swamping electrolyte influences the catalytic activity of the H^+ ions, either by a direct competition with H^+ ions at the formation of the activated complex, or according to the Brønsted–Bjerrum rule (Van Wazer, 1958; Brønsted, 1922, 1925; Bjerrum, 1924).

The following conclusions are indicated by the experimental results. The inhibitory effect of nickelous ions on the hydrolysis of polyphosphates with $n > 2$ is apparently caused by the influence of Ni^{2+} on the polyphosphate chain conformation, as the observed inhibition is exactly proportional to the conversion of polyphosphoric acid to Ni^{2+} polyphosphate, if both the inhibition and the conversion are expressed in per cent of the maximum (Figure 4). Apparently the conformation change brought about by nickelous ions increases the stability of the P–O–P linkage vs. hydrolytic scission in acidic solutions.

At higher Ni^{2+} concentrations the inhibition still increases slowly, even though the polyphosphate is completely converted to Ni^{2+} polyphosphate already. This small increase must be explained on the basis of the Brønsted–Bjerrum rule.

The hydrolysis of pyrophosphate is not altered by nickelous ions. This indicates that the influence of Ni^{2+} ions on the conformation of pyrophosphate is not directly comparable with the influence of these ions on the conformation of longer polyphosphate chains.

The rate of hydrolysis of polyphosphoric acids with $n = 2$ is increased by uranyl nitrate. Obviously uranyl salts act as strong catalysts on the P–O–P bond scission. This catalytic activity is superimposed on the H^+-catalyzed hydrolysis. The fact that the absolute catalytic activity of uranyl nitrate increases strongly with increasing pH indicates that not $UO_2{}^{2+}$, but $(UO_2OH)^+$, is the catalyst. Analysis of results at different pH values actually reveals that the catalytic activity of uranyl parallels the hydrolysis of the uranyl salt (van Niekerk, 1961) at increasing pH values.
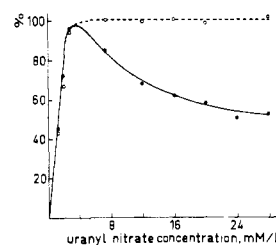

FIGURE 9: The inhibition of the rate of hydrolysis of pyrophosphate by uranyl nitrate (● ●) (expressed in per cent of the maximal inhibition that can be achieved, viz., 66%) and the conversion of pyrophosphoric acid into uranyl pyrophosphate (O O) (e.g., the amount of uranyl complexed by the pyrophosphate, in per cent of the maximal amount of uranyl that can be bound), at pH 1.20. Pyrophosphate concentration, 100 μg of P/ml; hydrolysis studied at 50°.

The point where virtually all the polyphosphoric acid is converted into uranyl polyphosphate is indicated in Figure 5 with an arrow. In our experiments the slope of the curve never changed at or near this point. This suggests that the conformation change of the polyphosphate chain, caused by uranyl salts, has little or no impact on the stability of the P–O–P linkage vs. hydrolytic scission in acidic solution. This is more clearly demonstrated by experiments at lower pH. At pH 0.40 the catalytic activity of the uranyl salt itself is very low. At this pH value uranyl nitrate has virtually no influence on the rate of hydrolysis whereas the polyphosphoric acid is converted into uranyl polyphosphate, just as at higher pH values. A small increase of the rate of hydrolysis is observed only at rather high uranyl concentrations.

The hydrolysis of pyrophosphate is inhibited by very low uranyl concentrations. With increasing uranyl concentrations the inhibition increases proportionally with the conversion of pyrophosphoric acid into uranyl pyrophosphate, up to the point that the acid is completely converted to the uranyl salt (corresponding to the inflection point of the curves in Figure 8). This indicates that the conformation change of the pyrophosphate molecule, induced by the uranyl salt, increases the stability of the P–O–P linkage vs. acid hydrolysis. The increase of the rate of hydrolysis at higher uranyl concentrations is apparently caused by the catalytic activity of the uranyl salt itself. Studies at different pH values show that again $(UO_2OH)^+$ rather than $UO_2{}^{2+}$ is the catalyst.

Rainey et al. (1964) have shown that acid hydrolysis of pyrophosphate is not influenced by salts of Mg^{2+}, Ca^{2+}, K^+, Na^+, Mn^{2+}, and Al^{3+}. Cr^{3+} on the other hand inhibits acid hydrolysis of pyrophosphate completely. The authors ascribe these results to different types of coordination: most metal ions (Mg^{2+}, etc.) are bound to one or two oxygen atoms on a single phosphorus atom, whereas the Cr^{3+}–pyrophosphate complex would be chelation of the usual sort. The experiments dis-

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cussed in this paper indicate that Ni^{2+} behaves like Mg^{2+}, etc., with respect to acid hydrolysis of pyrophosphate, whereas uranyl ions behave more or less like Cr^{3+}. Unlike Cr^{3+}, however, uranyl salts have a catalytic effect on pyrophosphate hydrolysis, which dominates the initial inhibition (Figure 8), at higher uranyl concentrations.

The relationship between many results in the literature, which show catalysis of hydrolysis of polyphosphates ($n > 2$) by metal ions (see, e.g., Thilo, 1962), and the inhibition of hydrolysis caused by Ni^{2+}, as described in this paper, is complicated. An important factor is that metal ion catalysis is studied at pH values around 8. As pointed out by Rainey et al. (1964) under these circumstances the catalytically active metal ions are at least partially hydrolyzed. The significance of metal ion hydrolysis is demonstrated in this paper for the case of uranyl salts (hydrolyzing already at relatively low pH values).[2]

Enzymatic reactions involving ATP[3] require the presence of a divalent cation. It has been suggested that the role of the metal ions in these reactions may be involved in bond breaking (Hammes and Levison, 1964). In some microorganisms inorganic polyphosphates instead of ATP are involved in certain phosphorylating reactions (Szymona and Ostrowski, 1964; Dirheimer and Ebel, 1962; van Steveninck and Booij, 1964). The influence of metal ions on one of these processes has been investigated in some detail and ascribed to metal ion induced changes in the conformation of the polyphosphates involved (van Steveninck and Booij, 1964). It is quite obvious that the mechanism of enzymatic scission of the P–O–P linkage cannot be directly compared with the H^+-catalyzed hydrolysis of polyphosphates. Therefore, it cannot be expected that the influence of metal ions on these two reactions will be the same. But the experimental results described in this paper emphasize the fact that metal ions have a profound influence on reactions involving polyphosphates. In enzymatic reactions involving polyphosphates the influence of metal ions may possibly be carried back on the influence of the polyphosphates on bond breaking, as in the case of ATP-involving reactions.

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[2] Moreover, at low pH a possible catalytic activity of metal ions may be dominated by inhibition of H^+-catalyzed hydrolysis. The influence of uranyl on acid hydrolysis of pyrophosphate demonstrates such dual action. At pH 8 only the actual catalytic activity of the metal ion would manifest itself.

[3] Abbreviations used: ATP, adenosine triphosphate.

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