

EFFECT OF POLYELECTROLYTES ON THE KINETICS OF IONIC REACTIONS. III. HYDROLYSIS OF PYROPHOSPHATE IN POLY-(ETHYLENEIMINE) SOLUTIONS

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Received 19 November 1973

The effect of the weak polyelectrolyte poly-(ethyleneimine) on the rate of hydrolysis of pyrophosphate has been studied as a function of pH at 70°C. Inhibition by the macroions was observed at pH ≥ 2 , the inhibition becomes stronger as pH increases. At pH > 7 the hydrolysis in the presence of polyions becomes undetectable. Addition of NaCl to the solutions reduces the inhibitory effect of the polyelectrolyte.

Electrostatic interactions between macroions and substrate ions cause the observed inhibition, therefore this is another manifestation of the polyelectrolyte effect due to the large charge density on the polycations.

1. Introduction

In the course of the study of the effect of polyelectrolytes on the rate of ionic reactions, we have undertaken the study of the kinetics of pyrophosphate hydrolysis in the presence of polycations. The substrate was chosen because its study extends our current work on the decomposition of organic phosphate esters in polyelectrolyte solutions [1, 2]. Furthermore, there are evidences [3] that an excess of free amino groups in hardtissue collagen bears a relation to the process of crystallization of calcium phosphate. It seemed interesting to test the possible influence of the free amino groups of a macroion on the rate of pyrophosphate hydrolysis as one possible explanation of the observed behaviour, this can be assessed employing a relatively simple synthetic polycation like poly-(ethyleneimine) (PEI), which is a weak polyelectrolyte having free amino groups in basic solutions.

In spite of previous work [4–6] on the hydrolysis of pyrophosphate in water, its mechanism is not conclusively established. The fact that the substrate may exist in five different charged states, according to the pH of the solution, complicates very much the interpretation of the kinetic data. In polyelectrolyte solu-

tions the system is even more complicated and it is not possible at present to offer a quantitative and detailed description of the effect of PEI upon the hydrolysis of pyrophosphate. It was observed that PEI inhibits the hydrolysis of the substrate, which becomes undetectable above pH 7. In the absence of macroions the hydrolysis is not observed to occur at pH > 10 (70°C). Added NaCl reduces the magnitude of the observed polyelectrolyte effect.

In more alkaline solutions (pH > 8.5) a different reaction between substrate and PEI seems to occur.

2. Experimental

The methods usually employed to determine phosphate in the presence of pyrophosphate were found to fail in solutions containing polyelectrolytes. A modification of the method described by Wirth [7] for the determination of small amounts of pyrophosphate in the presence of orthophosphate (up to a hundred times more PO_4^{3-} than $\text{P}_2\text{O}_7^{4-}$) was adopted in this work.

The method is based on the fact that pyrophosphate complexes Fe(III) ions. Hence, if pyrophosphate ions are added to solutions containing Fe(III) and SCN^- ions, there will be competition between $\text{P}_2\text{O}_7^{4-}$ and SCN^- for the Fe(III) ions and the optical absorp-

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tion at 470 nm due to the ferric-thiocyanate complex decreases.

The concentrations of the reagents employed, were standardized to the following values. Solutions having no PEI: $[\text{Fe(III)}] = 3.6 \times 10^{-5}$ molar, $[\text{SCN}^-] = 0.13$ molar; solutions containing PEI: $[\text{Fe(III)}] = 5.3 \times 10^{-5}$ molar, $[\text{SCN}^-] = 0.052$ molar. In all cases the pH of the solutions was fixed to a value of 2 with HNO_3 . Under these conditions the percentual optical transmission was found to vary linearly with the concentration of pyrophosphate in PEI solutions up to 3.8×10^{-5} molar.

Unfortunately, it was not possible to find suitable experimental conditions for the determination of pyrophosphate in solutions containing poly-(vinylbenzyltrimethylammonium chloride). Consequently the effect of this strong polycation could not be studied.

Mallinckrodt (A.R.) $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ was employed as the substrate, PEI was Polymine P (B.D.H.) having a molecular weight of 40000. In the kinetic runs the concentration of pyrophosphate was 1.88×10^{-3} molar and the concentration of PEI was 4.8×10^{-2} monomoles/l (the molecular weight of PEI monomer was taken to be 59 g [2]).

The kinetic runs were carried out at 70°C ($\pm 0.1^\circ\text{C}$) and the pH was fixed by adding the necessary amount of HCl to the PEI solutions [2] since the weak polyelectrolyte has buffer capacity. In the absence of PEI appropriate buffers were employed. The hydrolysis of pyrophosphate was followed by withdrawing aliquots at various times and keeping them in alkaline solutions having such a pH that no further decomposition of pyrophosphate was observed to occur within a month. The optical density of the aliquots corresponding to a given run were all measured within a period of one hour to reduce experimental error. The reactions followed up to three half-lives were strictly first-order in pyrophosphate.

The solutions corresponding to kinetic runs at $\text{pH} > 8.5$ became yellowish after a week. In these solutions the analytical method of pyrophosphate determination did not work. The optical absorption of the solutions at 400 nm was found to increase with pH until $\text{pH} = 9.6$ and to increase with PEI concentration. It may be suspected that this behaviour involves a reaction between the substrate and the free amino groups of PEI, because a 10% of the primary amino groups of PEI appeared blocked according to the test with ninhydrine.

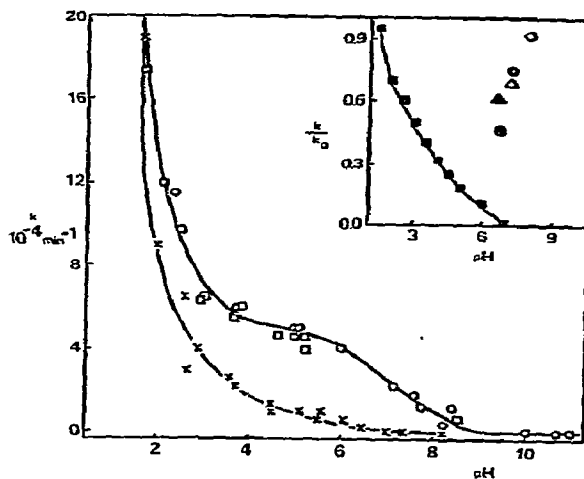


Fig. 1. Rate of hydrolysis of sodium pyrophosphate at 70°C in aqueous solutions of PEI (x), and in water: (o) present work, (□) results from ref. [5].

Insert: k/k_0 against pH at various NaCl concentrations. $[\text{NaCl}] = 0.0$ molar (■); 0.1 molar (●); 0.3 molar (▲); 0.5 molar (△); 0.7 molar (◊); and 1.0 molar (◐). The limit of precision of the measured rate constants is approximately $5 \times 10^{-6} \text{ min}^{-1}$.

Furthermore, dialysis indicated that approximately 5–6% of PO_4^{3-} corresponding to complete hydrolysis of the substrate was retained by the polyion.

3. Results and discussion

Fig. 1 shows the pH profile of the first-order rate constant for the hydrolysis of pyrophosphate at 70°C in the absence and in the presence of PEI in the solutions. Some of the results obtained by Campbell and Kilpatrick [5] are also represented in this figure. They may be seen to agree in general with our results, considering their measurements were carried out at 69.4°C and in solutions having a different ionic strength.

Our results also confirm the observation of Campbell and Kilpatrick [5] that the rate of hydrolysis decreases more slowly with pH in the range 3 to 5. The data of Mitra et al. [6] also agree with this observation but their rate constants are significantly smaller than those plotted in fig. 1.

It is quite clear from the data in fig. 1 that PEI strongly inhibits the hydrolysis of pyrophosphate at

pH > 2. The insert in fig. 1 depicts the change in the ratio k/k_0 (k_0 = first-order rate constant without polyelectrolyte) with pH and reproduces the data of five runs carried out with PEI and various concentrations of NaCl.

The fact that NaCl reduces the effect of PEI indicates that electrostatic interactions between macroion and substrate cause the inhibition observed in the presence of PEI. In very acid solutions (pH < 2), k/k_0 is close to unity because at such pH the added HCl acts in the same way as NaCl, that is, it shields the charges on the macroion; furthermore, at these pH values pyrophosphate has a very small charge (between -1 and 0), reducing considerably the possibility of close electrostatic interaction between substrate and macroion. From pH > 2.0, k/k_0 decreases until pH 7, where the hydrolysis was undetectable even after seven days (the maximum value that k could have at that pH is $5 \times 10^{-6} \text{ min}^{-1}$).

Previous work on the decomposition of *p*-nitrophenyl phosphate [1,2] in the presence of PEI showed that the free amino groups in the polyion acted at pH > 6 as nucleophilic reagents upon the substrate concentrated in its vicinity. This is apparently not the case in pyrophosphate solutions containing PEI up to pH 7; nucleophilic attack by the ionogenic groups of the polyelectrolyte would increase the rate of pyrophosphate decomposition, this was not observed when pH ≤ 7. However, the yellow colour appearing at pH > 8.5 may be due to nucleophilic attack of the substrate by PEI as suggested above. Thus, it may be concluded that the effect of polycations with free amino groups upon the hydrolysis of pyrophosphate does not explain the crystallization of calcium phosphate in hard-tissue collagen at least in solutions having a pH around 7.

The mechanisms usually proposed [4-6] to explain the hydrolysis of pyrophosphate in water are bimolecular attacks on $\text{H}_{4-i}\text{P}_2\text{O}_7^{-i}$ ($i = 0, 1, 2, 3, 4$) by H_3O^+ and by water. It is very difficult to distinguish between them because as the pH changes the fraction of the various $\text{H}_{4-i}\text{P}_2\text{O}_7^{-i}$ species also changes. Consequently both reaction mechanisms will show a pH dependent first-order rate constant. We do not consider that it has been proved so far that one of these bimolecular mechanisms does not contribute to the hydrolysis of the substrate. The possible contribution to the observed rate of hydrolysis of a truly unimolecular

mechanism similar to that operating in the hydrolysis of organic phosphate esters [8] cannot be completely ruled out on the same grounds.

The observed reduction of the rate of hydrolysis of pyrophosphate by PEI may be qualitatively explained taking account of the electrostatic interactions between macroions and mobile ions. The H_3O^+ ions will be repelled from the neighbourhood of the chain. This microscopic heterogeneity affects the apparent dissociation constants of the successive $\text{H}_{4-i}\text{P}_2\text{O}_7^{-i}$ acids, this type of effect has been observed with organic acids in polyelectrolyte solutions [9]. Thus, at any measured pH value, the equilibrium concentration of the more basic $\text{H}_{4-i}\text{P}_2\text{O}_7^{-i}$ species will increase near the chain of PEI, while the more acidic species will have a smaller concentration in those regions. Since according to fig. 1 in the absence of polyions the more basic species are less reactive, the presence of PEI will appear to inhibit the hydrolysis of pyrophosphate.

According to the idea of counterion condensation of the polyions postulated by Manning [10], the species having $i = 1$ will compete with the Cl^- counterions for positions close to the charged chain (in fact at finite concentrations there will be a continuous distribution of counterions). When $i \geq 2$, multicharged substrate species will condense on the macroion preferentially to Cl^- counterions, and the effect of PEI would reach a maximum.

It would have been interesting to extend our observations to solutions of a strong cationic polyelectrolyte like poly-(vinylbenzyltrimethyl ammonium chloride) in which case there is no possibility of reaction between the substrate and free amino groups. The comparison of the pH-rate constant profiles for pyrophosphate in PEI solutions to that in strong polyelectrolyte solutions would have allowed us to verify whether the inhibition is of electrostatic nature. Unfortunately, this was not possible because the analytical method employed in PEI solutions is not applicable to solutions containing that polycation, as already mentioned.

Acknowledgement

The authors thank the Consejo Nacional de Investigaciones Científicas y Técnicas (Rep. Argentina) and the Universidad de Buenos Aires for financial support.

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