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CHROMATOGRAPHIC STUDY OF THE ACIDIC HYDROLYSIS OF CYCLIC OCTAMETAPHOSPHATE

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SUMMARY

The hydrolysis of cyclic octametaphosphate in acidic aqueous solution ($[H^+] = 0.1$) at various temperatures was investigated. Separation of octametaphosphate and linear phosphates produced by the hydrolysis was achieved by combined anion-exchange and gel chromatography. From the rate constants at 20°C, 30°C, 40°C and 50°C, the Arrhenius activation energy for scission of a P–O–P linkage was estimated as 25.2 kcal/mol.

INTRODUCTION

Extensive studies on the hydrolysis of inorganic phosphates have been made from the standpoints of applied and basic chemistry[1,2]. In particular, the hydrolysis of linear phosphates having relatively low degrees of polymerization have been investigated because of the biochemical importance of ATP, ADP and AMP.

Some data have been reported for the hydrolysis of cyclic tri-[3] and tetrametaphosphate[4], but little information is available for cyclic phosphates having higher degrees of polymerization[5]. In the present study the rates of acidic hydrolysis of octametaphosphate anion at various temperatures have been studied. This anion is one of the highest cyclic phosphate polymers and is relatively easily obtainable in large quantities from lead tetrametaphosphate tetrahydrate[6].

It has been concluded that the stability of linear phosphates to hydrolysis in aqueous solution decreases with increasing chain length[7]. In contrast, the hydrolysis rate of cyclic phosphates in alkaline aqueous solution decreases with increasing size[6].

The study of octametaphosphate hydrolysis will aid in the preparation of linear octaphosphate and in clarifying the hydrolysis kinetics of linear phosphate oligomers.

Most previous studies on cyclic phosphate hydrolysis involved the use of paper chromatography. Separation of a parent compound from the hydrolysis products and determination of the phosphate species were achieved as follows. The phosphate mixture after hydrolysis was separated by filter-paper chromatography and the sample spots were developed with a molybdenum reagent. The blue spots containing

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the components were cut off, and the heteropoly blue was extracted in a flask and determined colorimetrically using a spectrophotometer. The disadvantages of this method are the need to use a number of filter-papers to maintain high accuracy and the difficulty in achieving complete separation between high phosphate polymers having relatively lower R_F values. For these reasons, a column chromatographic technique was developed for the analysis of hydrolysis products.

In this paper, linear and cyclic phosphates are designated as P_n and P_{nm}, respectively, where n is the degree of polymerization.

EXPERIMENTAL

Materials

Pure sodium octametaphosphate hexahydrate was prepared by Schülke's method[6]. Lead tetrametaphosphate tetrahydrate, obtained from sodium tetrametaphosphate and lead nitrate, was heated at 150°C for 2 h and then at 350°C for 1 h. The product was converted into the sodium salt by treating it with sodium sulphide solution and fractionating with ethanol.

All chemicals used were commercially available reagent grade and were recrystallized when necessary.

Sephadex G-25 gel and anion-exchange dextran gel, QAE-Sephadex A-25, were purchased from Pharmacia (Uppsala, Sweden).

Hydrolysis

The final concentration of octametaphosphate and hydrochloric acid added were 0.01 *M* as phosphate and 0.1 *M*, respectively.

Each test solution was hydrolyzed in a water-bath regulated to ± 0.1°C. The starting time was assumed to be the time at which the sample solution was placed in the bath. At measured time intervals, 1 ml of test solution was pipetted into a glass bottle containing 1 ml of 0.1 *M* sodium hydroxide solution cooled to about 0°C, and this solution was stored in a refrigerator until analysis.

Analysis

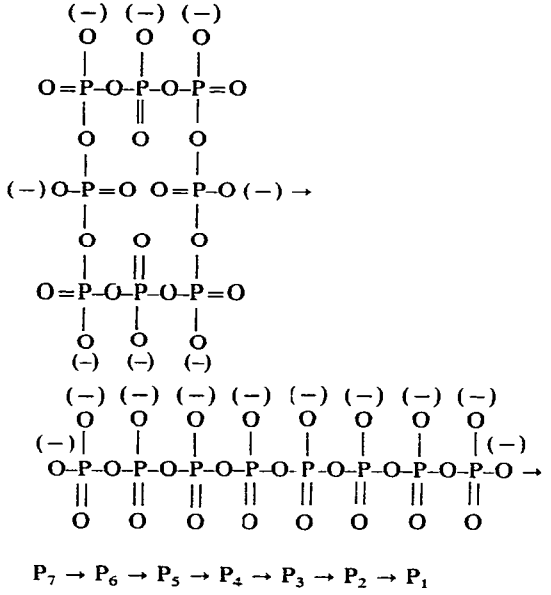
The analyses were carried out using column chromatography. Anion-exchange chromatography and gel chromatography were combined for the separation of the octametaphosphate and its hydrolysis products. Anion-exchange chromatography was carried out using a dextran gel, QAE-Sephadex A-25[8], and a glass column (60 × 1.0 cm). For gel chromatography[9], a Sephadex G-25 column (97 × 1.5 cm) was used. Conditioning and packing of the gels were done by the usual methods.

The eluting agents were potassium chloride and EDTA (disodium salt) which was added as a masking reagent. The sample volume delivered to the columns was 0.5 or 1 ml. Volumes of 1–3 ml of the effluent solution were collected by a fraction collector.

The phosphate concentration in the effluents was determined spectrophotometrically using a Mo(V)–Mo(VI) reagent at 830 nm. The concentration of Blue Dextran 2000 (Pharmacia), was determined by direct spectrophotometry at 610 nm.

RESULTS AND DISCUSSION

A serious problem in determining the hydrolysis rate of relatively highly polymeric phosphates is the separation of the parent phosphates from various phosphates formed as hydrolysis products. For instance, when octametaphosphate is hydrolyzed, a linear octaphosphate should be produced initially:



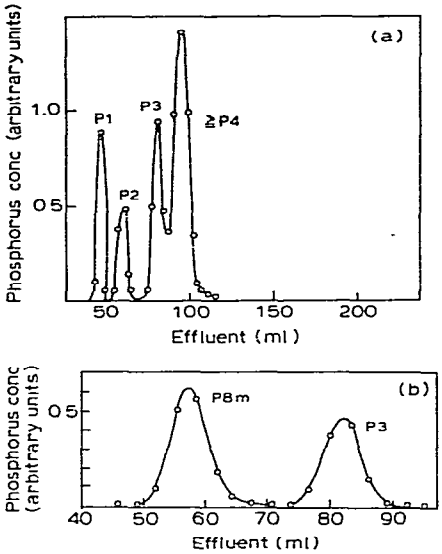
$$P_7 \rightarrow P_6 \rightarrow P_5 \rightarrow P_4 \rightarrow P_3 \rightarrow P_2 \rightarrow P_1 \qquad (1)$$

The linear octaphosphate is then hydrolyzed to lower linear phosphates and to cyclic trimetaphosphate. Thus, the number of phosphate species produced by octametaphosphate hydrolysis should be nine.

In previous studies of hydrolysis the analytical method used was paper chromatography. However, the separation of the nine species shown above is practically impossible by this method. An anion-exchange chromatographic method using dextran gel was therefore developed for the separation of cyclic phosphates.

By the use of a QAE-Sephadex A-25 gel column (60 × 1 cm), a mixture of linear phosphates containing P_1, P_2, P_3, P_4 and higher oligomers was chromatographed with 0.4 *M* potassium chloride solution as eluent (Fig. 1a). A mixture of P_{8m} and P_3 was also separated under the same conditions as shown in Fig. 1b. The elution volume of P_{8m} was the same as those of P_1 and P_2. All attempts to separate P_{8m} from other species by changing the elution conditions failed. From the chromatographic results, it is seen that fractionation of P_{8m} and linear phosphates larger than the trimer, and of P_{8m} and P_{3m}, is possible by this method.

Gel chromatography involving the difference in sizes of molecules to be separated has been successfully applied to the separation of condensed inorganic phosphates. The gel chromatographic separation of the hydrolysis products of P_{8m} was therefore investigated on a Sephadex G-25 column (97 × 1.5 cm). Blue Dextran 2000, P_1, P_2, P_3 and P_{8m} were eluted with 0.1 *M* potassium chloride solution. From the



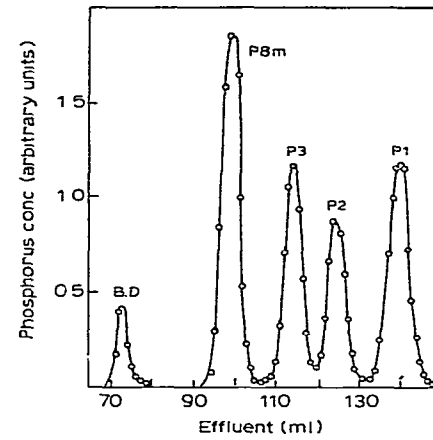


Fig. 1. Anion-exchange chromatograms of octametaphosphate and linear phosphates on a QAE-Sephadex A-25 column.

Fig. 2. Gel chromatogram of a mixture of Blue Dextran 2000 (B.D.), octameta-, tri-, di- and monophosphate on a Sephadex G-25 column.

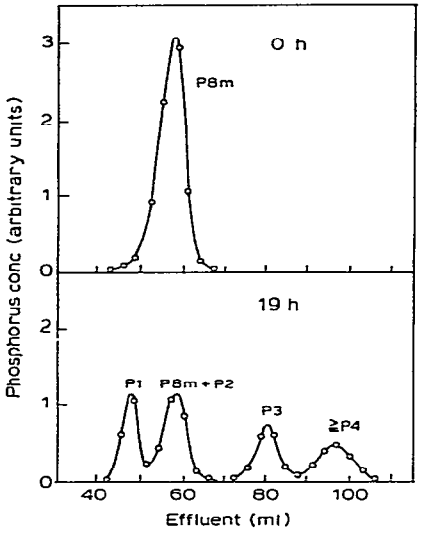
chromatogram in Fig. 2, it is evident that P_{8m} can be completely separated from P_1, P_2 and P_3. In particular, P_{8m} and P_1 or P_2, which are eluted together by anion-exchange chromatography, are completely separated by gel chromatography.

The hydrolysis products were then subjected to anion-exchange chromatography under the same conditions as in Fig. 1. The fractions containing P_{8m} and P_1 are collected and an aliquot was chromatographed on the Sephadex G-25 column. P_{8m} could be separated from other phosphate species and quantitated. As an example, an anion-exchange chromatogram of the hydrolysis products in 0.1 *M* HCl at 50°C is shown in Fig. 3.

After hydrolysis for 19 h various products had formed. Fractions of volume from 40 to 75 ml were collected and part of this solution was subjected to gel chromatography. Fig. 4 shows that the amount of octametaphosphate decreased with time. By use of these analytical procedures, the reaction kinetics can be followed; however, this method is complicated and time-consuming. To simplify the procedure, the anion-exchange column and gel chromatographic column were connected directly as in Fig. 5.

A mixture of P_{8m}, P_1, P_2, P_3 and linear oligophosphates ($\bar{n} = 5$) was chromatographed with this column as shown in Fig. 6. Complete separation of P_{8m} and other hydrolysis products was achieved. The hydrolysis reaction of P_{8m} was followed by this analytical system. Fig. 7 shows the chromatograms of the reaction products after 0, 5 and 10 h in 0.1 *M* HCl at 40°C. The concentration of P_{8m} decreases with time.

Letting the concentration of octametaphosphate at time 0 and t be $[P]_0$ and $[P]_t$, respectively, linear plots of log ($[P]_t/[P]_0$) *versus* time were obtained at 50°C, 40°C, 30°C and 20°C as shown in Fig. 8.



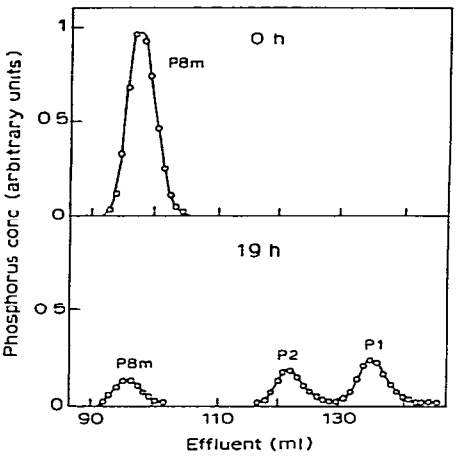
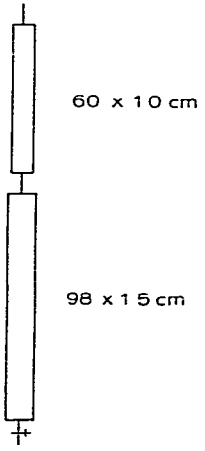


Fig. 3. Anion-exchange chromatograms of the hydrolysis products of octametaphosphate.

Fig. 4. Gel chromatograms of hydrolysis products previously fractionated by anion-exchange chromatography.

Exactly 1 ml of 0.1 *M* sodium hydroxide solution was required for neutralization of 1 ml of test solution after hydrolysis. Thus the hydrogen ion concentration in the test solutions can be considered to be constant during the period of hydrolysis. This was confirmed by measuring the pH of the test solutions after completion of hydrolysis.

From the results described above, it can be concluded that the hydrolysis of P_{8m} is evidently first order in the octametaphosphate concentration. In addition, from the investigation of the hydrolysis of other condensed phosphates, it can be expected



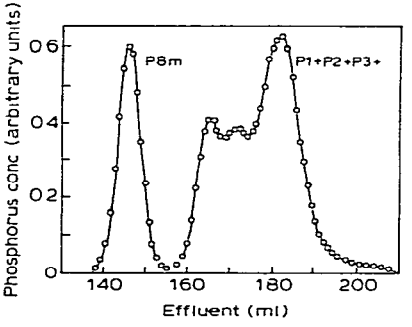


Fig. 5. Combined anion-exchange and gel chromatographic columns.

Fig. 6. Chromatogram of a mixture of octameta- and linear phosphates on the combined columns.

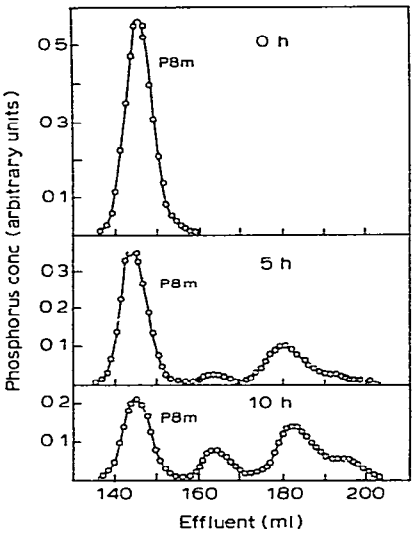


Fig. 7. Chromatograms of the hydrolysis products of octametaphosphate on the combined columns.

to be also first order in the hydrogen ion concentration. The reaction rate can then be written as

$$- d[P_{8m}]/dt = k[H^+] [P_{8m}] \quad (2)$$

where $[P_{8m}]$ is the concentration of P_{8m} at time t. If $[H^+]$ is constant, this equation can be integrated to:

$$[P]_t = [P]_0 \cdot \exp(- k[H^+] t) \quad (3)$$

The rate constants and half-lives at various temperatures are listed in Table I.

A plot of the logarithms of the rate constants *versus* the reciprocal of the absolute temperature was linear (Fig. 9). From the slope the Arrhenius activation

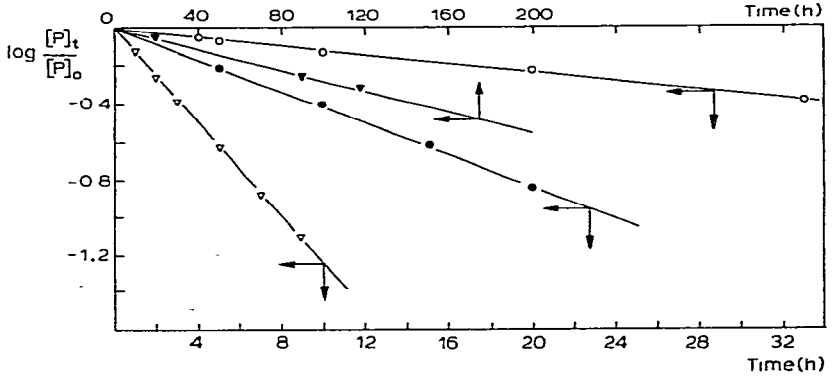


Fig. 8. Plots of $\log [P]_t/[P]_o$ *versus* time for the octametaphosphate hydrolysis at 20°C (▼), 30°C (○), 40°C (●) and 50°C (▽).

TABLE I
RATE CONSTANTS AND HALF-LIVES OF OCTAMETAPHOSPHATE HYDROLYSIS AT VARIOUS TEMPERATURES

	20°C	*30°C*	*40°C*	*50°C*
k	$6.06 \cdot 10^{-42}$	0.265	0.963	2.86
	110	26	7.2	2.4

energy for scission of a P–O–P linkage was estimated as 25.2 kcal/mole. This value is equivalent to that obtained for hydrolysis of other condensed phosphates in acidic media[2].

In spite of the low reliability of previously published data, which were limited by the analytical methods, the kinetic parameters of tri- and tetrametaphosphate hydrolysis can be estimated by extrapolation of the data in Fig. 10 on p. 169 of ref. 2 and the data in ref. 10, respectively. The half-lives of tri- and tetrametaphosphate at 50°C were estimated as 0.87 h and 6.9 h, respectively. Future studies will involve the hydrolysis of tri- and tetrametaphosphate in acidic aqueous solutions.

Since the kinetics of hexametaphosphate hydrolysis at pH less than 4 were not studied, details of the hydrolysis kinetics of cyclic phosphates are unknown. However, so far as the data on tri-, tetra- and octametaphosphate can be compared, it can be assumed that tetrametaphosphate has the highest stability to acidic hydrolysis and that trimetaphosphate has the lowest, among the cyclic phosphates. The instability of trimetaphosphate is probably due to the strain in the six-membered ring. The observed decrease in stability of cyclic phosphates with increasing ring size above four is in accord with the increase in proton affinity with increasing degree of polymerization[11].

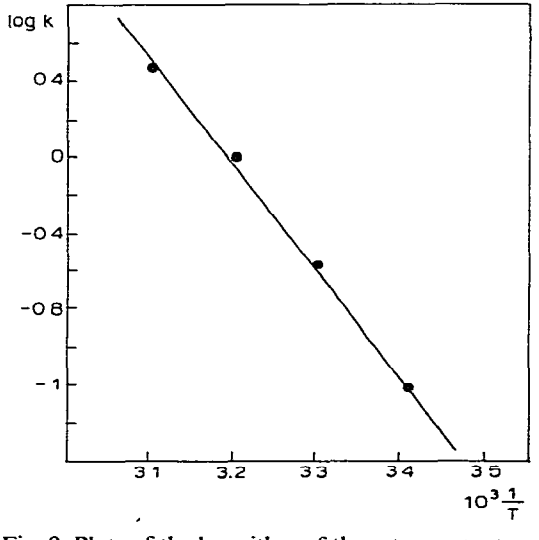


Fig. 9. Plots of the logarithm of the rate constant *versus* the reciprocal of absolute temperature.

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REFERENCES

1 J. R. Van Wazer, *Phosphorus and Its Compounds*, Vol. I, Interscience, New York, 1958.
2 R. K. Osterheld, in E. J. Griffith and M. Grayson (Editors), *Topics in Phosphorus Chemistry*, Wiley, New York, 1972.
3 R. M. Healy and M. L. Kilpatrick, *J. Amer. Chem. Soc.*, 77 (1955) 5258.
4 J. Crowther and A. E. R. Westman, *Can. J. Chem.*, 34 (1956) 969.
5 E. J. Griffith and R. L. Buxton, *Inorg. Chem.*, 4 (1965) 549.
6 U. Schülke, *Z. Anorg. Allg. Chem.*, 360 (1968) 231.
7 E. J. Griffith and R. L. Buxton, *J. Amer. Chem. Soc.*, 89 (1967) 2884.
8 G. Kura and S. Ohashi, *J. Chromatogr.*, 56 (1971) 111.
9 S. Ohashi, G. Kura, Y. Shimada and M. Hara, *J. Inorg. Nucl. Chem.*, 39 (1977) 1513.
10 E. Thilo and W. Wieker, *Z. Anorg. Allg. Chem.*, 29 (1957) 164.
11 G. Kura and S. Ohashi, *J. Inorg. Nucl. Chem.*, 38 (1976) 1151.