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The Catalytic Hydrolysis of Oligophosphates by Ethylenediamine-Copper(II) and Poly-L-lysine-Copper(II) Complexes

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The hydrolysis of some oligophosphates (tetra-, penta- and hexaphosphates) has been studied at 45°C in the absence and in the presence of a metal complex, and the relation between the nature of the complex catalyst and the composition of the degradation products has been investigated on oligophosphates as substrates, especially on pentaphosphate. In the presence of the Cu(II)-en (1 : 1) complex, the intermediate chain of pentaphosphate is attacked to produce trimeta- and pyrophosphates as the main products; the hydrolytic reaction is apparently of a first-order kinetics which depends on the concentration of the catalyst. The Cu(II) - Poly-Lys complex shows a strong catalytic activity and attacks pentaphosphate exclusively, thus producing orthophosphate as the main product. The optimum conditions for the catalytic effect on the hydrolysis of pentaphosphate are as follows: 1) the ratio of the NH_2 repeat unit of Poly-Lys to a mole of pentaphosphate ($[-NH_2]_i/[P_5]_i$) is about 1.6 at pH 8, the concentration of Cu(II) being constant; 2) the molar ratio of Cu(II) to the NH_2 repeat unit of Poly-Lys ($[Cu]_i/[-NH_2]_i$) is 1.0: 3) the activity reaches a maximum at about pH 10. Attempts have been made to explain these results on the basis of the structure of the Cu(II) - Poly-Lys complex.

The catalytic action of various metal chelates on the hydrolysis of Sarine and DFP (diisopropyl phosphofluoridate) has been studied by Martell and his co-workers.[1-3] The catalytic hydrolysis of polyphosphates by metal ions and chelates has also been studied by several workers.[4-6] In view of the fact that metal ions play a significant role in the catalytic action of the natural enzyme, metal complexes are some of the most interesting catalysts. Previous investigations of the metal complex catalysts have almost exclusively been kinetic studies; little attention has been paid to such peculiarities in the catalytic action as the action of the natural enzyme.[7,8] The present work was undertaken to investigate the relation between the nature of the metal complex catalyst and the kind of degradation products of the substrate as described in a previous report.[9] Polyphosphates with the 4—6 degree of polymerization, i. e., oligophosphates, were used as substrates for the purpose described above.

The ethylenediamine-copper(II) (Cu(II)-en) and poly-L-lysine - copper(II) (Cu(II) - Poly-Lys) complexes were used as catalysts. Though both of the ligands are similar in N-coordination to copper, Poly-Lys is a polypeptide and may be expected to have some catalytic effect on the ability to form higher-order structures (e.g. the α-helix configuration).

Experimental

Reagents.—Oligophosphates were prepared by the same method as has been reported previously.[10] Trimetaphosphate was prepared by the pyrolysis of sodium dihydrogenphosphate containing ^{32}P.[11] Ethylenediamine and cupric nitrate, $Cu(NO_3)_2 \cdot 3H_2O$, were both of the G. R. grade and were used without further purification. The concentrations of cupric ion and ethylenediamine were standardized by means of chelatometric[12] and potentiometric titrations respectively. Poly-L-lysine was of the pure hydrobromide form with a molecular weight of 85000.

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[1] R. C. Courtney, R. L. Gustafson, S. J. Westerback, Hyytiainen, S. C. Charberek, Jr., and A. E. Martell, *J. Am. Chem. Soc.*, **79**, 3030 (1957).

[2] R. L. Gustafson and A. E. Martell, ibid., **84**, 2309 (1962).

[3] R. L. Gustafson, G. S. Charberek, Jr., and A. E. Martell, ibid., **85**, 598 (1963).

[4] R. Hofstetter and A. E. Martell, ibid., **81**, 4461 (1959).

[5] J. M. Rainey, *J. Inorg. & Nucl. Chem.*, **26**, 1415 (1964).

[6] W. Wieker and E. Thilo, *Z. anorg. Chem.*, **306**, 48 (1960).

[7] W. L. Koltun, R. N. Dexter, R. E. Clark and F. R. N. Gurd, *J. Am. Chem. Soc.*, **80**, 4188 (1958).

[8] J. P. Collman and D. A. Buckingham, ibid., **85**, 3039 (1963).

[9] Y. Moriguchi and M. Miura, This Bulletin, **38**, 678 (1965).

[10] M. Miura and Y. Moriguchi, ibid., **37**, 1522 (1964).

[11] Van Wazer, "Phosphorus and Its Compounds," Vol. I, Interscience Publisher, New York (1958), p. 618.

[12] K. Ueno, "Chelatometric Titration (Kireto Tekitei Hō)," Nankodo, Tokyo (1964), p. 148; V. Suk, M. Malat and A. Jenickova, *Collection Czechslov. Chem. Commun.*, **20**, 158 (1955).

Procedure.—The pH of the sample solution, which was made up of an oligophosphate and a catalyst, was adjusted by adding nitric acid or tetramethylammonium hydroixde. The ionic strength of the solution was maintained at 0.1 by adding tetramethylammonium nitrate. The pH of the solution was readjusted within ±0.5 pH unit at regular intervals. The solution (2.5−3.0 ml.) was kept at 45°C in a small test tube coated by silicone and was shaken vigorously at intervals. A part of the solution (0.05—0.1 ml.) was taken out at regular intervals, and the degradation products were identified and quantitatively determined by ion-exchange chromatography. Each degradation product was separated in response to the degree of polymerization by ion-exchange chromatography using ^{32}P as a tracer.[9,13] Its quantity, which was recorded on a chart by a linear count-rate meter, was determined by measuring the area of the separated peak on the chart by means of a planimeter. The H-form of Dowex 1 X4 (100—200 mesh) was used as the ion-exchange resin. The eluent used in the ion-exchange chromatography was an aqueous solution of sodium chloride, the concentration of which was continuously changed from 0.22 M to 0.6 M during elution. The pH of the eluent was adjusted to 9.2 by an ammonia-ammonium chloride buffer.

As a precipitate was formed in the oligophosphate-Poly-Lys system, it was separated by means of a centrifuge. A part of the supernatant solution was used for ion-exchange chromatography, and another part was used for the estimation of the ratio of the ^{32}P in the supernatant solution to that in the precipitate.

Results and Discussion

The experimental conditions and the results of hydrolytic degradation are summarized in Table I. A part of these results are quoted from Otani's data[13] for reference. As may be seen in Table I, the degradation ratio increases with the increase in the the chain length of oligophosphate and with the decrease in the pH of the sample solution. Cupric ion or its complexes show a catalytic action on the hydrolysis; this action is especially remarkable in the presence of the Cu(II)-Poly-Lys complex.

The Degradation in the Absence of Metal Ions and Complexes.—The hydrolytic degradations of tetra- and hexaphosphates at pH 8 and those of pentaphosphate at pH 4 and 8 are shown in Fig. 1 as a function of the reaction time. The ordinate of the figure, "Number of phosphorus atoms (%)," is expressed by the percentages of the total ^{32}P contained in the substrate and the degradation products. Each value of the degradation products is shown by the vertical distance between neighboring curves. These results, which are represented in Fig. 2a, can be expressed by the following equation:

$$k_1 t = 2.303 \log (100/P_n) \qquad (1)$$

where P_n is the percentage of oligophosphate with the n degree of polymerization at the reaction time, t, and k_1, the first-order rate constant.

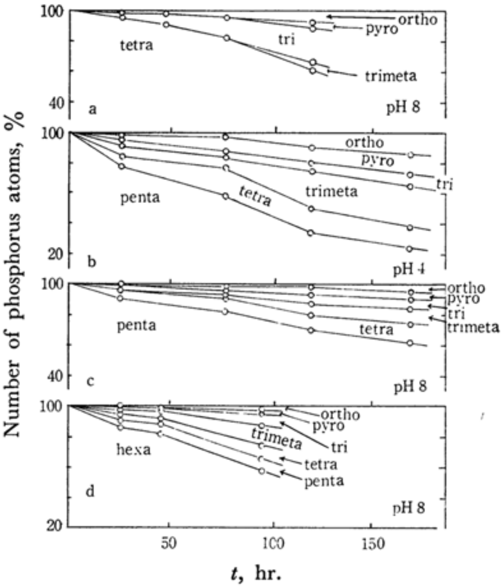

Fig. 1. The relation between the composition of degradation products and the reaction time in the absence of metal ion and complex. Initial concentration of oligophosphates, $[P_n]_i$; a: $[P_4]_i = 6.85 \times 10^{-3}$ M, b, c: $[P_5]_i = 4.07 \times 10^{-3}$ M, d: $[P_6]_i = 1.07 \times 10^{-2}$ M.

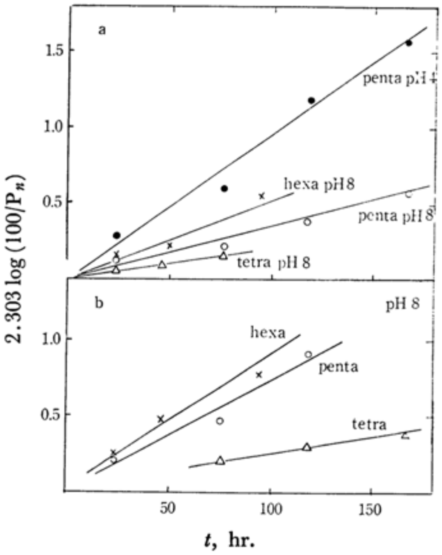

Fig. 2. The validity of the first-order kinetics (Eq. 1) in the hydrolytic gradation of oligophosphates.
a: no catalyst (cf. Fig. 1),
b: in the presence of Cu(II) (cf. Fig. 5)

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13) S. Otani, M. Miura and T. Doi, *J. Chem. Soc. Japan, Ind. Chem. Sect. (Kogyo Kagaku Zasshi)*, **66**, 593 (1963).

It has been found from several studies[13,14] that the hydrolysis of oligophosphates is of the first-order at low concentrations of substrate. This may also be confirmed as in Fig. 2a.

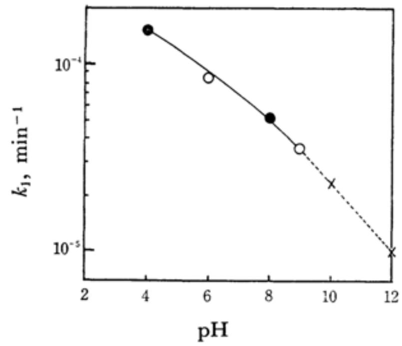

Fig. 3. The relation between the first-order rate constant in the hydrolysis of pentaphosphate and the pH in the absence of Cu(II) and complex.

●: Values obtained by the author (cf. Figs. 1b and 1c).
○: Values obtained by Otani et al.[13]
×: Extrapolated values.

The pH dependence of the first-order rate constant in the hydrolysis of pentaphosphate is shown in Fig. 3. Several stuides have been done on the pH-effect on the hydrolysis of polyphosphates,[6,13,15,16]; it can be concluded from these results that the hydrolysis rate increases with the decrease in pH because the hydrogen ion behaves as a catalyst, and that the terminal chain of polyphosphates is mainly attacked in the higher pH region, whereas the intermediate chain is also attacked in the lower pH region. These conclusions have been confirmed in the present study as may be seen in Figs. 1b, 1c and 3 and Table I; that is, the hydrolysis rate is greater at pH 4 than at pH 8, and the type of degradation is the terminal (a) and intermediate (b) chain attacks at pH 4, while it is the terminal chain attack (a) at pH 8.

$$\text{pentaphosphate} \rightarrow \text{tetra-} + \text{ortho-} \qquad \text{(a)}$$

$$\text{pentaphosphate} \rightarrow \text{trimeta-} + \text{pyro-} \qquad \text{(b)}$$

The dependence of the rate constants at pH 4 and 8 on the degree of the polymerization of oligophosphates is shown in Fig. 4. A part of the data are quoted from Otani's paper.[13] A similar study has been done at 60°C by Wieker.[17] The relation between the rate constant and the degree of polymerization can be expressed by Eq. 2, when the polyphosphate chain is attacked at random and the attack depends only on the statistical factor with relation to the degree of polymerization:

$$k_{\mathrm{P}_n} = (n-1)k_{\mathrm{P}_2} \qquad n > 2 \rightarrow n \gtrsim 2 \qquad (2)$$

where k_{P_n} is the rate constant of polyphosphate with the degree of polymerization, n, and k_{P_2}, the rate constant of pyrophosphate. Though the rate constant in Fig. 4 increases linearly with the degree of polymerization, neither of the two straight lines agrees with Eq. 2. This may indicate that the hydrolytic degradation of oligophosphates depends on the electrostatic effect caused by the charge on the polyphosphate chain.

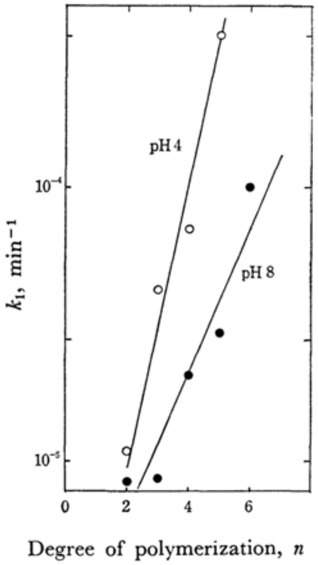

Fig. 4. The relation between the first-order rate constant and the degree of polymerization of oligophosphates.

The Formation of Trimetaphosphate.— As may be seen in Figs. 1a, 1c and 1d, the formation of trimetaphosphate increases with the degree of polymerization when the pH is kept constant. The formation of trimetaphosphate from tetraphosphate has recently been reported by Otani et al. of our laboratory.[13] In view of the fact that crystalline Kurrol's salt, which is a kind of polyphosphate, has a spiral structure with four tetrahedra, $-\mathrm{PO_3}-$, per pitch,[18] trimetaphosphate may be formed from the polyphosphate chain by such a mechanism as that the polyphosphate chain is constricted to form some spiral structure like Kurrol's salt, with trimetaphosphate then being formed from such a part, as is shown in Fig. 17.[19]

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14) J. P. Crowther and A. E. R. Westman, *Can. J. Chem.*, **32**, 42 (1954).

15) E. Thilo and W. Wieker, *Z. anorg. u. allgem. Chem.*, **291**, 164 (1957).

16) J. P. Crowther and A. E. R. Westman, *Can. J. Chem.*, **34**, 969 (1956).

17) W. Wieker, *Z. Elektrochem.*, **64**, 1047 (1960).

18) K. H. Jost and E. Thilo, *Cristallographia*, **6**, 828 (1961).

19) E. Thilo, "Advances in Inorganic and Radio Chemistry," Vol. IV, Academic Press Inc., New York (1962).

It may, therefore, be concluded that the probability of spiral formation to give trimetaphosphate increases with the increase in the degree of polymerization, and that even the tetraphosphate with four tetrahedra will probably form trimetaphosphate.

The Degradation in the Presence of Copper(II).—The results of hydrolytic degradation are shown in Fig. 5, where the molar ratio of cupric ions to oligophosphate is 1.0. The pH of the solution was kept at 4 or 8 for pentaphosphate and at 8 for tetra- and hexaphosphates. It has been reported[4–6] that the transition metals and a large quantity of alkali metals have a catalytic effect on the hydrolysis of polyphosphates. A comparison of Figs. 5a, 5c and 5d with Figs. 1a, 1c and 1d will indicate that the cupric ion acts as a weak catalyst that it seems to accelerate the formation of trimetaphosphate by the intermediate chain attack. The acceleration of the formation of ortho- and trimetaphosphates in the presence of alkali metals and alkaline earth metals has also been reported in the hydrolysis of polyphosphates with a high degree of polymerization by Wieker et al.[6] The results in Fig. 2b indicate that the hydrolytic reaction is of the first-order in the presence of cupric ions in not too high a concentration.

The Degradation in the Presence of the Cu(II)-en Complex.—The relation between the composition of degradation products and the reaction time for pentaphosphate at pH 4 and 8 are shown in Figs. 6b and 6c, and those for tetra- and hexaphosphates at pH 8, in Figs. 6a and 6d. The molar ratio of cupric ions and that of ethylenediamine to oligophosphate were 1.0 in each experiment. As may be seen in these figures, the Cu(II)-en complex has a considerable catalytic effect on the hydrolysis of oligophosphates at pH 8; the main products are pyro- and trimetaphosphates for the pentaphosphate substrate and tri- and trimetaphosphates for the hexaphosphate substrate. These results suggest that the Cu(II)-en complex catalyst attacks exclusively the intermediate chain of oligophosphates, thus forming trimetaphosphate.

As may be seen in Figs. 5b and 6b at pH 4, the hydrolysis rate (cf. Table I) and the composition of degradation products when both ethylenediamine and cupric ions are present are similar to those when cupric ions alone are present. These results indicate that in both cases the same catalyst species acts in the hydrolysis of pentaphosphate. The species seems to be hydrated cupric ions

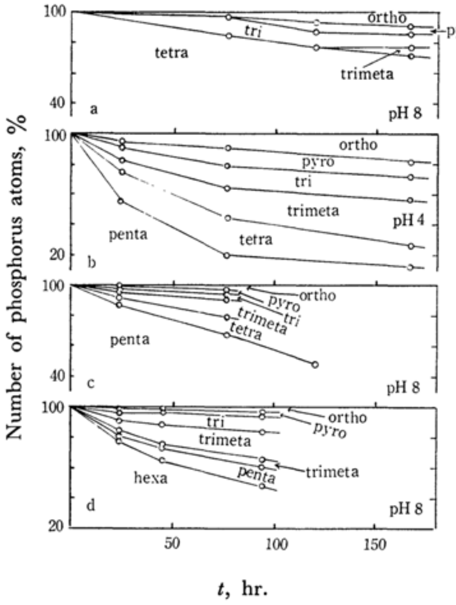


Fig. 5. The relation between the composition of degradation products and reaction time in the presence of Cu(II). Initial concentrations of oligophosphates,

$[P_n]_i$, and Cu(II), $[Cu]_i$; a: $[P_4]_i=6.60\times10^{-3}$ M, $[Cu]_i=6.60\times10^{-3}$ M, b, c: $[P_5]_i=4.40\times10^{-3}$ M, $[Cu]_i=4.40\times10^{-3}$ M, d: $[P_6]_i=1.03\times10^{-2}$ M, $[Cu]_i=1.03\times10^{-2}$ M.

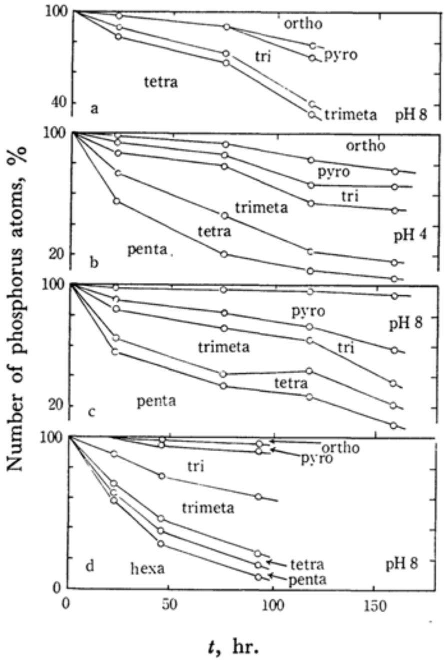
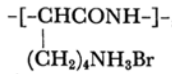


Fig. 6. The relation between the composition of degradation products and the reaction time in the coexistence of Cu(II) and ethylenediamine. Initial concentrations of oligophosphates, $[P_n]_i$, Cu(II), $[Cu]_i$ and ethylenediamine, $[en]_i$; a: $[P_4]_i=6.20\times10^{-3}$ M, $[Cu]_i=6.20\times10^{-3}$ M, $[en]_i=6.20\times10^{-3}$ M, b, c: $[P_5]_i=4.07\times10^{-3}$ M, $[Cu]_i=4.07\times10^{-3}$ M, d: $[P_6]_i=1.06\times10^{-2}$ M, $[Cu]_i=1.06\times10^{-2}$ M, $[en]_i=1.06\times10^{-2}$ M.

TABLE I. HYDROLYSIS OF OLIGOPHOSPHATES AND TRIMETAPHOSPHATE AT 45°C, IONIC STRENGTH 0.1 ($(CH_3)_4NNO_3$)

Oligophosphate P_n	Degree of polymerization	Condition of degradation					$-NH_2/P$*2	Degradation ratio (%)*3	Catalytic activity ratio*4	$k_1 \times 10^5$ (min^{-1})*5
		pH	Metal M	Ligand*1 L	Molar ratio					
					M/P_n	L/P_n				
Pyrophosphate P_2	2	4	—	—	0	0	0	0		1.3±0.2
		8	—	—	0	0	0	0		0.3
		8	Cu(II)	en	0.4	0.4	2/5	6.0		3.7±1.0
		8	Cu(II)	Poly-Lys	0.4	2.0×10^{-3}	2/5	2.1		
Tri- P_3	3	4	—	—	0	0	0	8.3		6.6†
		8	—	—	0	0	0	0.5	1.0	0.4
		8	Cu(II)	en	0.6	0.6	2/5	2.0	4.0	4.5±1.2
		8	Cu(II)	Poly-Lys	0.6	4.2×10^{-3}	2/5	7.1		
Tetra- P_4	4	8	—	—	0	0	0	4.4	1.0	3.8±0.4
		8	Cu(II)	—	1.0	0	0	$(16.5)_{70}$		4.0±0.4
		8	Cu(II)	en	1.0	1.0	2/4	15.5	3.5	13±2.0
Penta- P_5	5	4	—	—	0	0	0	23.6	1.0	15±0.1
		4	Cu(II)	—	1.0	0	0	43.7	1.9	39±7.0
		4	Cu(II)	en	1.0	1.0	2/5	44.5	1.9	43±2.0
		4	Cu(II)	Poly-Lys	0.71	3.6×10^{-3}	2/7	46.3	2.0	
		8	—	—	0	0	0	10.5	1.0	5.2±0.1
		8	Cu(II)	—	1.0	0	0	13.3	1.3	11±1.0
		8	Cu(II)	en	1.0	1.0	2/5	43.8	4.2	23±3.0
		8	Cu(II)	Poly-Lys	0.71	3.6×10^{-3}	2/7	60.3	5.8	
		8	Cu(II)	Poly-Lys	1.0	5.0×10^{-3}	2/5	77.9	7.4	
		10	Cu(II)	—	1.0	0	0	14.4		19±7.0
Hexa- P_6	6	8	—	—	0	0	0	15.5	1.0	10±4.0
		8	Cu(II)	—	1.0	0	0	24.1	1.6	17±2.0
		8	Cu(II)	en	1.0	1.0	2/6	42.0	2.7	42±2.0
Trimeta- P_3	3	8	—	—	0	0	0	$(1.6)_{70}$		
		8	Cu(II)	Poly-Lys	0.43	3.0×10^{-3}	2/7	21.4		
		8	Cu(II)	Poly-Lys	0.6	4.2×10^{-3}	2/5	32.6		

[*1] en: ethylenediamine $NH_2CH_2CH_2NH_2$; Poly-Lys: poly-Lysine



$-[-\underset{\substack{| \\ (CH_2)_4NH_3Br}}{C}HCONH-]-_n$ M. W. 85000

[*2] Number of repeat unit of Poly-Lys (side chain amino group $-NH_2$) or that of amino group of en per phosphorus atom of oligophosphate.

[*3] Percentage of substrate degradated at the reaction time 22 hr. except $(\ )_{70}$ which is the one at 70 hr.

[*4] Ratio of the degradation ratio in the presence of catalyst to that in the absence of catalyst at the same pH.

[*5] First-order rate constants (hydrolysis rate) estimated from Eq. 1. (†: quoted from Otani's data[13]).

(aquo-complex), since little Cu(II)-en 1 : 1 chelate exists at pH 4. On the other hand, the form of the Cu(II)-en complex, which predominantly has a catalytic effect at pH 8, seems to be monohydroxo, Cu en(OH)(H_2O), or dihydroxo, Cu en-$(OH)_2$, chelate.[2)]

The degradation ratio at the reaction time of 70 hr. and the apparent rate constant are plotted against the concentration of Cu(II)-en in Fig. 7. The initial concentration of pentaphosphate and the molar ratio of copper(II) to ethylendiamine were kept constant. The degradation ratio and the apparent first-order rate constant, k_{obs}, estimated by using Eq. 5, increase linearly with the concentration of catalyst. The simplest kinetics which is applicable to the results seems to be represented by Eqs. 3—6:

$$\text{pentaphosphate} \xrightarrow{{}^1k_1} \text{degradation products} \quad (3)$$

$$\text{pentaphosphate} + \text{catalyst} \xrightarrow{{}^2k_1} \text{degradation products} + \text{catalyst} \quad (4)$$

$$k_{obs}t = 2.303 \log (100/P_5) \quad (5)$$

$$k_{obs} = {}^1k_1 + {}^2k_1C \quad (6)$$

where k_{obs} is the apparent first-order rate constant (observed value) and C, the concentration of the metal complex catalyst. The value extrapolated to the zero concentration of the catalyst, ${}^1k_1$, does not agree with the value observed in the absence of the Cu(II)-en complex, k_{obs}. This may show that the catalytic effect of hydrogen ions and that of the Cu(II)-en complex on the hydrolysis of pentaphosphate are not always independent, but correlate with each other.

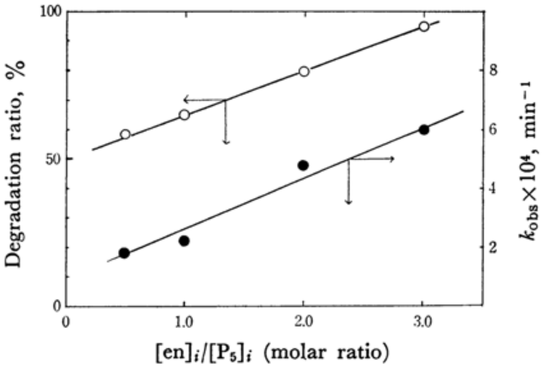


Fig. 7. The dependence of degradation ratio (%) at the reaction time 70 hr. and the apparent first-order rate constant (Eq. 5) on the Cu(II)-en catalyst concentration at pH 8.
Initial concentration of pentaphosphate, $[P_5]_i$: $[P_5]_i$: 4.07×10^{-3} M, molar ratio of Cu(II) to ethylenediamine: 1.0.

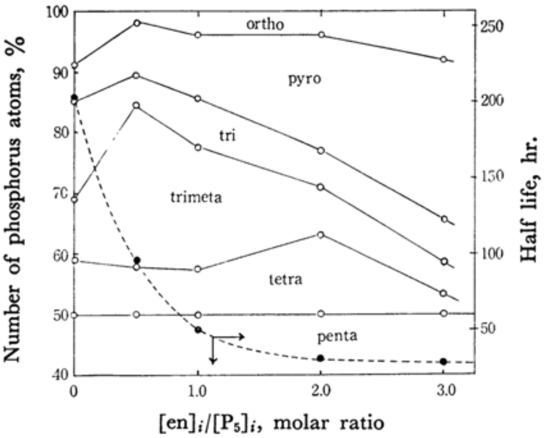


Fig. 8. The dependence of the composition of degradation products at half left on the Cu(II)-en catalyst concentration (life ordinate scale) and the dependence of the half life in the hydrolytic degradation of pentaphosphate on the Cu(II)-en catalyst concentration (right ordinate scale) at pH 8 (cf. Fig. 7).

The dependence of the composition of degradation products at half-life on the catalyst concentration is shown in Fig. 8. The molar ratio of copper(II) to ethylenediamine in each experiment was kept constant at 1.0. The formation of pyrophosphate increases with the concentration of Cu(II)-en, as may be seen in Fig. 8. From the fact that the pyrophosphate and the triphosphate are hardly degraded in the presence of the Cu(II)-en complex (cf. Table I), it seems reasonable to conclude that the reaction shown in Eq. 7 becomes predominant with an increase in the concentration of the Cu(II)-en complex.

$$\text{pentaphosphate} \longrightarrow 2\ \text{pyro-} + \text{ortho-} \quad (7)$$

The Degradation in the Presence of the Cu(II)-Poly-Lys Complex.—The catalytic effect of the Cu(II)-Poly-Lys complex on the hydrolysis of oligophosphates was studied by using pentaphosphate; the results are shown in Figs. 9—16. Figures 9—11 show the dependence of the ^{32}P

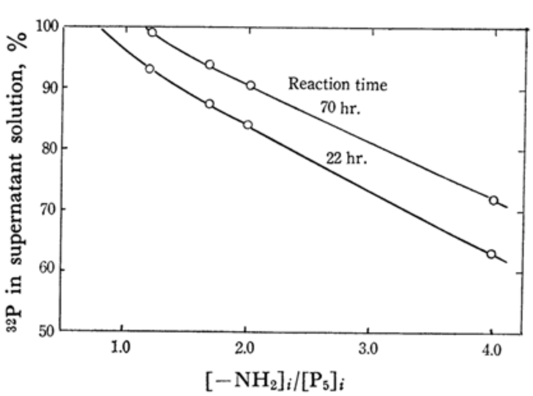


Fig. 9. The dependence of ^{32}P percentage in the supernatant solution of pentaphosphate-Poly-Lys-Cu(II) system on the Poly-Lys concentration (repeat unit molar concentration, $[-NH_2]$) at pH 8.

TABLE II. HYDROLYTIC DEGRADATION OF PENTAPHOSPHATE IN THE PRESENCE OR IN THE ABSENCE OF PRECIPITATE AT 45°C, pH 8

No.	^{32}P (%) In the supernatant solution Ortho-	Pyro-	Tri-	Tetra-	Penta-	Trimeta-	In the precipitate As penta	Total
1	13.9	10.0	6.0	20.6	30.1	9.6	9.6	100
2	3.0	7.5	6.1	9.1	39.2	7.5	27.6	100

No. 1 Degradation was carried out in the presence of precipitate at 45°C for 22 hr.

No. 2 After degradation for 5 hr. at 45°C in the presence of precipitate, the supernatant solution separated from the precipitate was kept at 45°C for 17 hr.

$[P_5]_i$, $[-NH_2]_i/[P_5]_i$ and $[Cu]_i/[-NH_2]_i$ were 1.63×10^{-3} M, 1.43 and 0.5, respectively, in both cases.

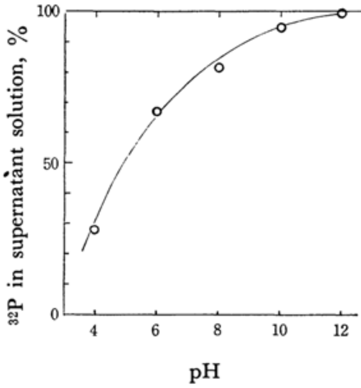


Fig. 10.

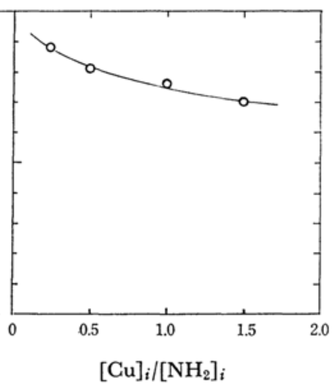


Fig. 11.

Fig. 10. The pH dependence of ^{32}P percentage in the supernatant solution of pentaphosphate - Poly-Lys - Cu(II) system.
$[Cu]_i/[-NH_2]_i$ and $[-NH_2]_i/[P_5]_i$: kept constant, 0.5 and 1.43, respectively.

Fig. 11. The dependence of ^{32}P percentage in the supernatant solution of pentaphosphate - Poly-Lys - Cu(II) system on the number of Cu(II) per repeat unit of Poly-Lys at pH 8.
$[-NH_2]_i/[P_5]_i$: kept constant, 1.43.

percentage in the supernatant solution on the concentrations of Poly-Lys, hydrogen ions and cupric ions respectively. These experiments were carried out at 45°C for 22 hr.; all the ^{32}P in the precipitate was assumed to be of the pentaphosphate form in estimating the degradation ratio.** Figure 12 shows the relation between the composition of the degradation products and the reaction time. The molar ratios of cupric ions to pentaphosphate are 0.71 and 1.0 in Figs. 12a and 12b respectively. The repeat unit of Poly-Lys per pentaphosphate is 1.43 in Fig. 12a and 2.0 in Fig. 12. The pH dependence of the hydrolysis of pentaphosphate in the presence of the Cu(II) - Poly-Lys complex is shown in Fig. 13, where all the experimental conditions except the pH value are the same as those in the case of Fig. 12a. Figure 14 shows the dependence of the degradation ratio on the concentration of the Cu(II)-Poly-Lys complex, the ratio of cupric ions to the repeat unit of Poly-Lys being kept constant at 1 : 2. Figure 15 shows the relation between the concentration of cupric ions and the degradation ratio, where the ratio of the repeat unit of Poly-Lys to pentaphosphate is kept constant at 1.43. Figure 16, obtained. from the experiments in Fig. 14, shows the relation between the composition of the degradation products and the concentration of the Cu(II)-Poly-Lys complex. All of the initial concentrations of pentaphosphate in Figs. 9—15 are kept constant at 1.60×10^{-3} M.

Although the catalytic action of various amino-acids and synthetic polypeptides has been studied in the hydrolysis of DFP[3,20] and *p*-nitrophenylacetate[21,22] as substrates, the peculiar action of

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** It could not be determined in this case whether or not all ^{32}P in the precipitate remained in the pentaphosphate form; therefore, in practice, it seems probable that the degradation ratio of pentaphosphate is somewhat greater than the value obtained in the present paper.

20) T. Wagner-Jauregg, B. E. Hackley, Jr., T. A. Lies, O. O. Owens and R. Proper, *J. Am. Chem. Soc.*, **77**, 922 (1955).

21) S. Saito, This Bulletin, **38**, 4 (1965).

22) J. Noguchi and T. Saito, "Polyamino Acids, Polypeptides and Proteins," Ed. by M. A. Stahmann, Univ. of Wisconsin Press, Madison (1962), p. 313.

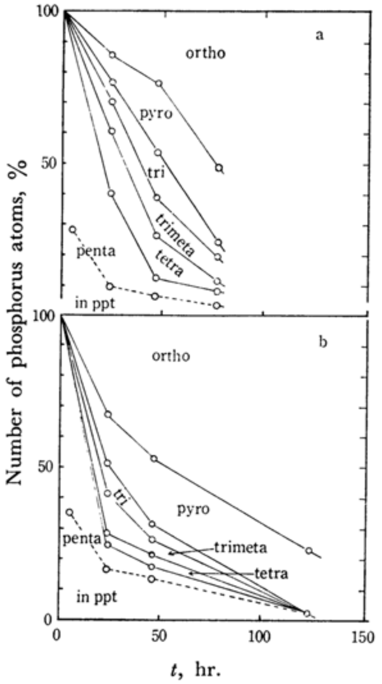

Fig. 12. The relation between the composition of degradation products and the reaction time in the coexistence of Cu(II) and Poly-Lys at pH 8.
Broken line: ^{32}P as pentaphosphate form in the precipitate (%).

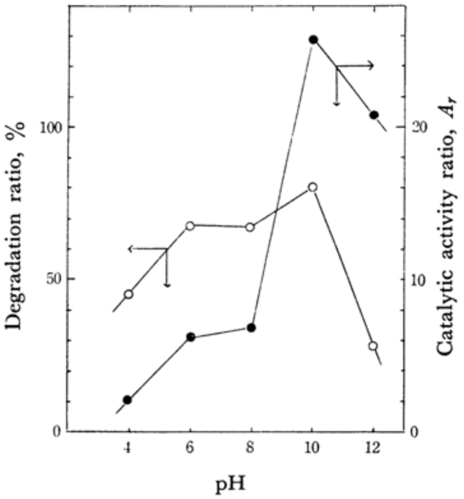

Fig. 13. Effect of pH on the hydrolysis of pentaphosphate in the coexistence of Cu(II) and Poly-Lys. Reaction time: 22 hr.
Left ordinate scale: degradation ratio (%)
Right ordinate scale: catalytic activity ratio, A_r, defined by

$$A_r = D_r / D_{r_0}$$

D_r: Degradation ratio (%) in the coexistence of Cu(II) and Poly-Lys.
D_{r_0}: Degradation ratio (%) in the absence of Cu(II) and Poly-Lys (cf. Fig. 3).

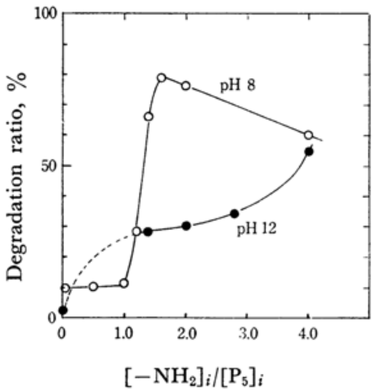

Fig. 14. The dependence of degradation ratio of pentaphosphate on the concentration of Cu(II)-Poly-Lys. Reaction time: 22 hr.

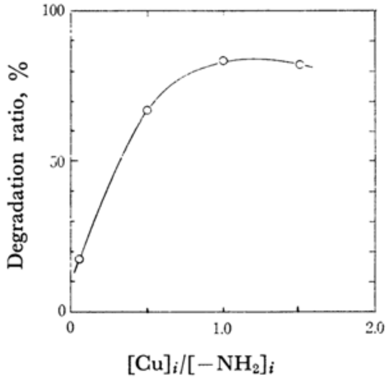

Fig. 15. The relation between the concentration of Cu(II) and the degradation ratio of pentaphosphate in the coexistence of Cu(II) and Poly-Lys at pH 8. Reaction time: 22 hr.

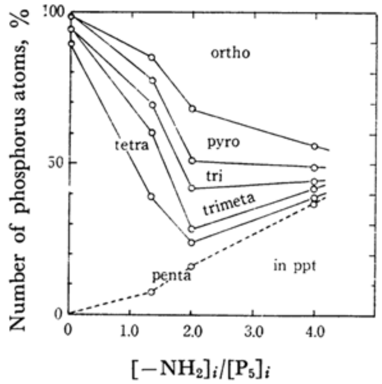

Fig. 16. The relation between the composition of degradation products and the number of Poly-Lys repeat unit per pentaphosphate in the coexistence of Cu(II) and Poly-Lys at pH 8 (cf. Fig. 14). Reaction time: 22 hr.
Broken line: ^{32}P as pentaphosphate form in the precipitate (%).

the catalyst on the substrate has not been investigated, probably because the substrates are too simple. As may be seen in Table I and Figs. 6 and 12, the catalytic action of the Cu(II) - Poly-Lys complex is very remarkable and differs from that of the Cu(II)-en complex in the main product of the hydrolytic degradation. The main product in the former case is orthophosphate, while those in the latter are pyro- and trimetaphosphates. Though ethylenediamine and Poly-Lys are both N-coordination ligands to copper(II), the catalytic effects in the hydrolysis of pentaphosphate differ from each other in the main products. This is probably due to the specific structure of Poly-Lys and to the remarkable catalytic action to be decribed below.

Orthophosphate may be formed from pentaphossphate by the following reactions:

$$\begin{aligned}&\text{pentaphosphate} \longrightarrow \text{tetra-} + \text{ortho-}\\&\qquad\text{tetra-} \longrightarrow \text{tri-} + \text{ortho-}\\&\qquad\text{tri-} \longrightarrow \text{pyro-} + \text{ortho-}\end{aligned}\tag{8}$$

$$\begin{aligned}&\text{pentaphosphate} \longrightarrow \text{tri-} + \text{pyro-}\\&\qquad\text{tri-} \longrightarrow \text{pyro-} + \text{ortho-}\end{aligned}\tag{9a}$$

$$\begin{aligned}&\text{pentaphosphate} \longrightarrow \text{trimeta-} + \text{pyro-}\\&\qquad\text{trimeta-} \longrightarrow \text{pyro-} + \text{ortho-}\end{aligned}\tag{9b}$$

As may be seen in Figs. 12a, 12b and Table I, pyrophosphate does not degrade at all practically and the degradation ratios of tri- and trimetaphosphates are much less than that of pentaphosphate. From these facts in the presence of the Cu(II) - Poly-Lys complex, it seems reasonable to assume that the predominant reaction in bringing about the remarkable formation of orthophosphate in the initial stage of the reaction is not the intermediate chain attack represented by Eq. 9a or 9b, but the terminal chain attack, Eq. 8.

The composition of the precipitate which is formed by the interaction between a part of pentaphosphate and the Cu(II) - Poly-Lys catalyst can not be determined in the present work. Although the precipitate may also contain the degradation products from the reaction (8), (9a) or (9b), it is probable that most of the oligophosphates in the precipitate are pentaphosphate because the ^{32}P contained in the precipitate decreases with an increase in the reaction time (cf. broken lines in Figs. 13a and 12b) and in the degradation products in the supernatant solution. From the facts that there is remarkable difference between the degradations in the presence and in the absence of the precipitate and that the degradation ratio decreases upon the separation of the precipitate (Table II) it is found in the initial stage of the reaction that a great portion of the Cu(II) - Poly-Lys catalyst exists in the precipitate with a part of pentaphosphate and that the catalyst is liberated with the degradation of pentaphosphate from the precipitate to a bulk solution. The formation of precipitates by the interaction between polyphosphate and Poly-Lys depends on the pH, the concentration of Poly-Lys and that of cupric ions (Figs. 9—11), and the interaction seems to be considerably electrostatic in character.[23]

As may be seen in Figs. 13—15, there are three optimum conditions for the catalytic effect of the Cu(II) - Poly-Lys complex: 1) the remarkable catalytic effect appears only when the molar ratio of Poly-Lys to pentaphosphate, $[-NH_2]_i/[P_5]_i$, reaches a value which is larger than about 1.2 and attains a maximum at about 1.6; 2) the catalytic activity increases with the molar ratio of copper(II) to the NH_2 repeat unit of Poly-Lys, $[Cu]_i/[-NH_2]_i$, and attains a maximum at 1.0, and 3) the catalytic activity reaches a maximum at about pH 10. These results lead us to the following conclusions: 1) when the concentration of Poly-Lys is much lower than that of pentaphosphate, hardly any catalytic activity appears because the cupric ions which play an important role in the catalytic effect are masked by the complexation with pentaphosphate; 2) although copper(II) complexes with various compositions and structures may exist in the pentaphosphate - Poly-Lys - Cu(II) system, some of these species have the most remarkable catalytic activity; that is, the Cu(II)-Poly-Lys complex with 2 : 1 composition ratio of the side chain amino group to copper(II) or the hydroxo-complex with a 1 : 1 composition ratio acts on pentaphosphate and forms a mixed chelate such as that shown in Fig. 18a or 18b, so that the pentaphosphate is liable to suffer decomposition by the Cu(II) - Poly-Lys chelate[4], and 3) in view of the fact that the configuration of Poly-Lys becomes α-helix in the pH region above 10.5,[24] the results shown in Fig. 13 may indicate that there is some relation between the α-helix configuration of Poly-Lys and its catalytic activity.

It may be assumed from the variation in the color of the precipitate of the pentaphosphate-Poly-Lys - Cu(II) system that the complex species at pH 8—10 is different from that at 12. The

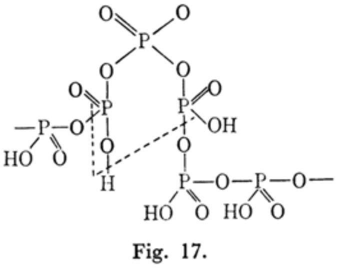

Fig. 17.

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23) G. Felsenfeld and S. Huang, *Biochim. et Biophysica Acta*, **34**, 234 (1959).

24) P. Doty, K. Imahori and E. Klemperer, *Pro. Nat. Acad. Sci.*, **44**, 424 (1958).

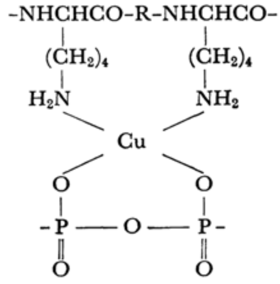


Fig. 18a.

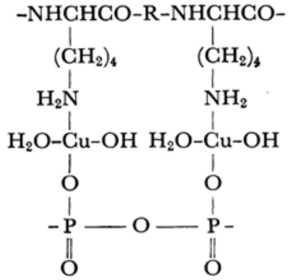


Fig. 18b.

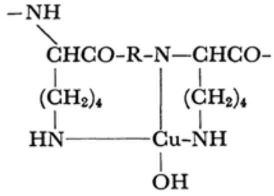
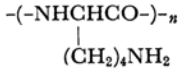


Fig. 18c.

R: 

$$-(\text{-NH}\underset{(\text{CH}_2)_4\text{NH}_2}{\underset{|}{\text{C}}}\text{HCO-})\text{-}_n$$

copper(II) atom in the light blue-green complex at pH 8—10 may be coordinated with one or two nitrogen atoms of the amino group of Poly-Lys (e. g. Fig. 18a or 18b), while that in the red-purple complex at pH 12 may be coordinated with the nitrogen atom of the peptide bond, –CONH–, in the main chain of Poly-Lys as well as the nitrogen atom of the side chain (e. g. Fig. 18c). As may be seen in Figs. 13 and 14, the former complex is catalytically more active than the latter because the active sites of copper(II) for pentaphosphate are occupied by nitrogen atoms of Poly-Lys. The color of the latter complex may be due to the biuret reaction from the interaction between cupric ions and polypeptides at high pH values. The chelate shown in Fig. 18a, 18b or 18c is an example of the conceivable structures; the structure with relation to the α-helix configuration can not be discussed in detail in this report for lack of data of the optical rotatory dispersion.

The oligophosphate - Poly-Lys - Cu(II) system is not suitable for the kinetic investigation because the precipitate is formed in this system. Kinetic studies of the oligophosphate - Poly-α, L-glutamate - magnesium(II) and -copper(II) systems are in progress.

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