Enzymic Hydrolysis of Polyphosphate in the Gastrointestinal Tract

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Cyclic ring as well as linear polyphosphates including sodium trimeta-, sodium tetrameta-, sodium tripoly-, and sodium hexametaphosphate (Graham's salt) were found to be enzymically hydrolyzed in in vitro rat and porcine small intestine. Trimeta, tetrameta, tripoly, and hexameta were reduced by 24, 5, 80, and 10%, respectively, in in vitro rat intestine in 1 h, and by 10, 4, 3, and 15%, respectively, in in vitro porcine intestine in 1 h. The rate of hydrolysis in the in vitro intestine indicated an enzymic mechanism. The lack of polyphosphatase activity in heat treated intestines, with normal hydrolysis in intestines treated with antibiotic, confirms previous reports of phosphatase activity on the intestinal lumen.

Phosphates are incorporated into foods in broad areas of food processing. These inorganic salts have greatly differing effects on the functional properties of the food products in which they are used. This wide diversity of functionality is due, in part, to the broad range of structures possible in phosphate chemistry (Ellinger, 1972).

Through the condensation of orthophosphate, a multitude of polymers are possible. The commercially important sodium phosphate salts are described below (Table I). Hexametaphosphate (hexameta) as discussed here is the commercial product which is actually a Grahams's salt with an average chain length of about 12 (Van Wazer, 1958). It is not a ring phosphate. Two ring phosphates were studied; sodium trimetaphosphate (trimeta) is currently approved for use as a starch modifier and sodium tetrametaphosphate (tetrameta) is not presently used as a food additive. These ring phosphates also occur at trace levels in other polyphosphates as seen later in the analysis of the salts used.

Sodium orthophosphate (ortho) and polyphosphates currently have GRAS status, but some workers have questioned the safety of ring phosphates (Lehman, 1954) based on indications in the literature that rings may not hydrolyze on ingestion like other polyphosphates. It was the purpose of this work to compare hydrolysis of ring and linear polyphosphates in the gastrointestinal tract as this may relate to safety. It was a specific objective to show whether ring phosphates are hydrolyzed in the intestine.

Much contradiction exists in the literature concerning the metabolism of the condensed phosphates. Feeding studies with rats have shown similar chronic toxic effects for ortho, meta, and long chain phosphates (Hodge, 1964). Rothstein et al. (1953), who investigated the hydrolysis and absorption of glucose 6-phosphate, noted that hydrolysis of sodium tripolyphosphate (tripoly) and hexameta in rat intestine occurred and assumed the catalysis was due to enzymes bound to the intestinal lumen. Lang et al. (1955), however, reported that there was no evidence for phosphate enzymes in the gut which could act on polyphosphates. These workers also reported that 44–59% of potassium metapolyphosphate and 37–66% of hexameta fed to rats were found in the feces and, therefore, not hydrolyzed. Schreier and Noller (1955) repeated this with radioactive polyphosphates and found half the radioactivity to be absorbed and the remainder excreted in the feces as polyphosphate. These workers also reported that polyphosphates were enzymically hydrolyzed in homogenized intestinal mucosa of calves and humans, but that in rats, the intestinal bacteria were responsible for hydrolysis. In recent work, pyrophosphatase enzyme activity was observed on the brush border membranes of calf intestine (Chappelet-Tordo et al., 1974).

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Table I. Phosphates of Commercial Significance

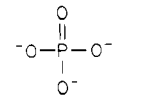
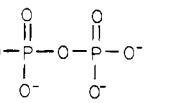
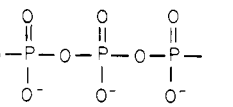
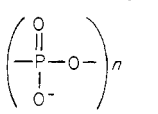
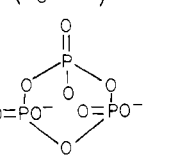
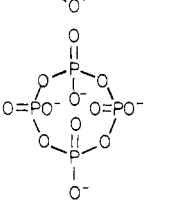
Name	Formula	Structural backbone
Trisodium orthophosphate	Na_3PO_4	
Tetrasodium pyrophosphate	$Na_4P_2O_7$	
Sodium tripolyphosphate	$Na_5P_3O_{10}$	
Sodium hexametaphosphate	$(NaPO_3)_n$	
Sodium trimetaphosphate	$Na_3P_3O_9$	
Sodium tetrametaphosphate	$Na_4P_4O_{11}$	

Table II. Species Distribution of the Phosphate Salts Utilized in This Investigation

	Ortho	Pyro	Tri-meta	Tri-poly	Tetra-meta	Tetra-poly	⩾5
STP	0.6	2.7	0.4	96.3	0		
Trimeta	0.4	0.6	97.9	1.1	0		
Tetrameta	1.0	5.3	0.5	0.3	92.9		
Hexameta ($\bar{n}$ = 12)	0.6	1.3	1.9	1.6	1.5	1.9	92.2

Linear polyphosphates have been shown to undergo extensive hyrolysis when injected into rats and rabbits but ring phosphates did not significantly hydrolyze (Gosselin et al., 1952). Only a small portion of trimeta was converted into ortho. This was the only report of hydrolysis of the ring phosphates in vivo. The relative lack of evidence for enzymes for ring phosphate hydrolysis has been the major reason for concern over possible toxicity of ring phosphates.

Mitchell (1958) investigated enzymic polyphosphate hydrolysis in simulated digestive fluids. This worker reported that no enzymic hydrolysis took place in either simulated gastric or intestinal fluid and, therefore, no hydrolysis of polyphosphates occurred after ingestion. In order to clarify the reported literature, the purpose of this

Table III. Phosphate Distribution with Time of Incubation at 37 °C in Rat Small Intestine[a]

	Time, min	Ortho	Pyro	Trimeta	Tripoly	Tetrameta	Tetrapoly	$n > 4$
Tripoly	0	1.1	4.3	0.3	95.7			
	10	42.1	8.9	0.4	48.7			
	30	80.7	4.2	0.2	15.0			
	60	74.8	7.5	0.0	17.7			
Trimeta	0	0.5	0.6	96.9	2.0			
	10	4.0	0.3	91.9	3.8			
	30	13.9	0.3	82.5	3.2			
	60	25.8	0.4	72.5	1.3			
Tetrameta	0	1.7	7.0	0.2	0.0	91.1	0.0	
	10	10.8	0.0	0.0	0.0	89.1	0.1	
	30	10.2	0.0	0.0	0.0	89.6	0.0	
	60	13.9	0.0	0.0	0.0	86.1	0.0	
Hexameta	0	1.0	1.0	1.8	1.5	1.1	1.7	90.8
	10	10.1	1.0	5.8	1.6	0.0	2.0	79.6
	30	14.7	0.4	4.2	1.0	0.0	1.0	78.6
	60	15.5	0.4	3.6	0.9	0.0	0.9	78.9

[a] Average of two determinations per salt.

Table IV. Phosphate Distribution with Time of Incubation at 37 °C in Porcine Small Intestine[a]

	Time, min	Ortho	Pyro	Trimeta	Tripoly	Tetrameta	Tetrapoly	$n \geq 4$
Tripoly	0	1.1	4.3	0.3	95.7			
	10	8.0	10.0	0.5	81.5			
	30	11.5	12.7	0.6	75.2			
	60	18.1	16.4	0.6	65.0			
Trimeta	0	0.5	0.6	96.9	2.0			
	10	6.6	0.8	91.6	1.1			
	30	8.4	1.0	88.8	1.8			
	60	10.9	2.0	86.6	1.5			
Tetrameta	0	1.7	7.0	0.2	0.0	91.1	0.0	
	10	7.1	3.6	0.0	0.1	88.8	0.4	
	30	12.0	0.6	0.0	0.0	87.1	0.4	
	60	12.1	0.2	0.0	0.0	87.2	0.4	
Hexameta	0	1.0	1.0	1.8	1.5	1.1	1.7	90.8
	10	9.0	1.2	3.9	1.4	0.0	1.5	80.1
	30	7.5	1.5	8.8	1.5	0.0	1.8	78.9
	60	12.5	0.7	7.3	1.5	0.0	1.2	75.9

[a] Average of two determinations.

study was to investigate the nature of ring and chain polyphosphate hydrolysis in the gastrointestinal tract by investigating the specificity of phosphatase enzymes in the intestine.

EXPERIMENTAL SECTION

The sodium salts of tripoly, trimeta, tetrameta, and hexameta used throughout the investigation were produced by Monsanto Co. (St. Louis, Mo.). Distribution of phosphate species in these salts is shown in Table II.

The phosphate species were separated and quantified by the paper chromatographic method of Karl-Krupa (1956) as described by Kolthoff et al. (1961) with the substitutions of acetone for 2-propanol in the acidic solvent and 7.6 g of ammonium carbonate for 10 ml of 15 N ammonia in the alkaline solvent. When analyzing the phosphate distribution of tripoly, trimeta, and tetrameta, development in just the acidic solvent gave sufficient resolution for quantification. However, two-dimensional development was used for analysis of the hexameta samples due to its greater complexity.

In Vitro Intestinal Hydrolysis. In vitro digestion was done by a modification of the method of Rothstein et al. (1953). Adult male Wistar derived rats of about 250 g were fasted for 2 days to clear the intestine and then sacrificed with CO_2. The small intestine was immediately excised. The small intestine was flushed with 20 ml of warm saline and tied off into three sections One milliliter of 3% phosphate salt solution was injected into each section. The phosphate solutions had been adjusted to pH 7.5 with 0.2 N NaOH or 0.5 N HCl prior to use (the pH of simulated intestinal fluid) (U.S. Pharmocopoeia, 1974). The sections were incubated in 37 °C saline for 10, 30, and 60 min. After the period of incubation, the section was cut above a funnel and the contents and a 3-ml saline wash were collected and frozen until analyzed. Two intestines were used per salt and the sections of each intestine used for each incubation period were randomized.

Table V. Phosphate Distribution of Sodium Tripolyphosphate after Incubation in Variously Treated Rat Intestine Segments[a]

Treatment recvd by small intestine segment	Incubation time, min	Ortho	Pyro	Trimeta	Tripoly
Initial STP	0	0.8	4.2	0.4	94.6
24 h in saline	30	26.4	12.6	0.2	60.5
24 h in saline then boiled 5 min	30	0.8	4.2	0.4	94.6
24 h in concentrated antibiotic solution	30	21.8	13.4	0.1	64.5

[a] Average of three determinations/treatment.

Porcine small intestines were excised from hogs during slaughter at a local slaughter house and were taken to the laboratory. Elapsed time between removal of the intestines

Table VI. Effect of 2 Weeks Prefeeding Rats a Ring Phosphate on the Phosphate Distribution after 30-min Incubation in the Small Intestine[a]

Phosphate prefed for 2 weeks	Av daily intake, g/day	Ortho	Pyro	Trimeta	Tripoly	Tetrameta	Tetrapoly
0.5% tetrameta	27.7	18.9	0.0		0.0	80.8	0.4
0.5% Na_2HPO_4	25.4	19.2	0.4		0.0	80.3	0.2
0.5% trimeta	26.9	13.9	0.4	85.0	0.7		
0.5% Na_2HPO_4	28.4	16.4	0.5	82.6	0.8		

[a] Average of two determinations.

and initiation of the hydrolysis study was 60–90 min. The first 2 m of each small intestine were used. These were flushed with 200 ml of warm saline and cut into 50-cm sections. The sections were tied, filled with 25 ml of 3% test solution, and incubated at 37 °C for 10, 30, and 60 min. The phosphate solution had been adjusted to pH 7.5 prior to use. One intestine was used per salt and the sections used for incubation periods were randomized. After the incubation period, the section was cut over a funnel and flushed with 50 ml of 37 °C saline. The total effluent was frozen until analyzed.

To determine the hydrolytic agent in rat intestine, three rat small intestines were obtained as previously described and treated prior to incubation. One section of each intestine was held in 37 °C saline for 24 h as a control, one section was held in saline for 24 h, then placed in boiling water for 5 min, and the third section was held for 24 h in antibiotic solution (1000 units/ml penicillin, 2.5 μg/ml Fungizone, and 1000 μg/ml streptomycin) (Grand Island Biological Co., Grand Island, Nebr.) to reduce microbial flora. Tripoly was injected into the intestine sections and incubated 30 min.

RESULTS AND DISCUSSION

Hydrolysis of polyphosphates occurred rapidly in in vitro rat intestine (Table III). In 60 min, both tripoly and trimeta are substantially hydrolyzed. This rate suggests that enzymic hydrolysis is likely to be involved, since tripoly and trimeta both have extremely slow rates of hydrolysis in water at this pH (Shen and Morgan, 1973). The rate of hydrolysis of linear polyphosphates with $n \geq 5$ and tetrameta rings in rat intestine was slower than tripoly, although no measurable hydrolysis was reported by Mitchell in 7 days at this pH in simulated intestinal fluid.

Chappelet-Tordo et al. (1974) reported that the intestinal phosphatase enzymes were nonspecific and cleaved several very different organic phosphates as well as inorganic pyrophosphate. There is some probability, then, that the same enzyme that cleaves the linear polyphosphates is active on the ring phosphates. The differences in rate of hydrolysis between species of phosphates could be due to anion fit in the active site.

Porcine intestine also gave significant hydrolysis of both ring and chain phosphates (Table IV). In general, the rate of hydrolysis for each compound was similar to that in rat intestine. The hydrolysis rate of tripoly deviated most from the rat hydrolysis rate, and was slower in porcine intestine. Retention time in porcine intestine would be significantly longer than in rat intestine so that there may be no greater likelihood of porcine excretion of unhydrolyzed polyphosphate.

To establish whether that hydrolysis was due to enzymes or to bacteria, sections of intestines from the rats were treated separately to eliminate either bacterial action or enzymic action. The relative rate of tripolyphosphate hydrolysis after holding the intestine sections in saline at 37 °C for 24 h and in high concentration antibiotic solution indicated that hydrolysis was not due to microbial action (Table V). However, the lack of hydrolysis in the heat-treated intestine sections confirmed the role of enzymes bound to the intestine as reported by Rothstein et al. (1953).

Conditioning rats by adding ring phosphates to the diet for 14 days prior to assay did not increase the amount of hydrolysis of that salt compared to rats fed a diet with increased ortho (Table VI). The lack of induction of specific metaphosphatase enzymes indicates that ring phosphates are probably cleaved by the enzymes normally present in the intestine for hydrolysis of other organic and inorganic phosphates.

Mitchell (1958) looked only at secreted digestive fluids as a source of polyphosphatase enzymes, but failed to consider the bound enzymes in the intestinal lumen reported by Rothstein et al. (1953). Polyphosphatase enzymes do exist in the intestine which are now shown to be active in the hydrolysis of both ring and chain phosphates. The rapid hydrolysis of ring and chain phosphates observed in both rat and porcine in vitro intestine supports the concept of metabolic similarity of ring and chain polyphosphates in determining safety for food use.

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LITERATURE CITED

Chappelet-Tordo, D., Fosset, M., Iwatsubo, M., Gashe, C., Luazdonski, M., *Biochemistry* **13**, 1788 (1974).
Ellinger, R. H., "Phosphates as Food Ingredients", Chemical Rubber Co. Press, Cleveland, Ohio, 1972.
Gosselin, R. E., Rothstein, A., Miller, G. J., Berke, H. L., *J. Pharmacol. Exp. Ther.* **106**, 180 (1952).
Hodge, H. C., *Food Cosmet. Toxicol.* **2**, 147 (1964).
Karl-Krupa, E., *Anal. Chem.* **28**, 1091 (1956).
Kolthoff, I. M., Elving P. J., Sandell, E. B., "Treatise on Analytical Chemistry", Part II, Vol. 5, Interscience, New York, N.Y., 1961, p 384.
Lang, K., Schachinger, L., Karges, O., Blumenberg, F. K., Rossmueller, G., Schmutte, I., *Biochem. Z.* **327**, 118 (1955).
Lehman, A. J., *Assoc. Food Drug Off. U.S. Q. Bull.* **18**, 98 (1954).
Mitchell, L. C., *J. Assoc. Off. Agric. Chem.* **41**, 185 (1958).
Rothstein, A., Meier, R. L., Scharff, T. G., *Am. J. Physiol.* **173**, 431 (1953).
Schreier, K., Noller, H. G., *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol.* **227**, 199 (1955).
Shen, C. Y., Morgan, F. W., in "Environmental Phosphorus Handbook", Wiley, New York, N.Y., 1973, p 24.
"U.S. Pharmacopoeia. XIX", United States Pharmacopeial Convention, Inc., Rockville, Md., 1975.
Van Wazer, J. R., "Phosphorus and Its Compounds", Vol. I, Interscience, New York, N.Y., 1958, p 776.

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