

^{32}P and ^{51}Cr Labelled Sodium Polyphosphates: Preparation and Stability

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SUMMARY

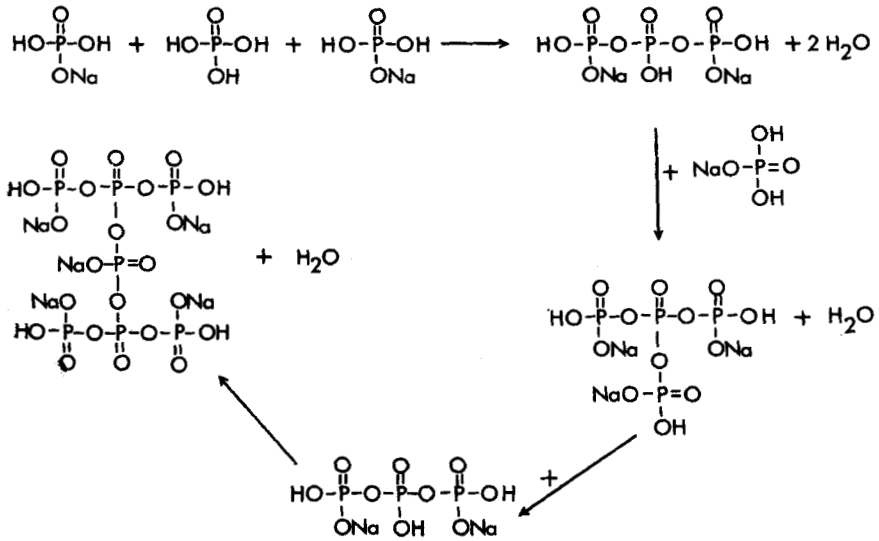
The preparation and stability of ^{32}P and ^{51}Cr labelled sodium polyphosphates have been studied. The influence of the chromium concentration on the solubility of the chromium-sodium polyphosphate indicates that its effects are more critical in the preparation of the cross-linked variety. Both polyphosphates showed a very good stability to the storage.

Polyphosphates are compounds with properties of interest for the biological sciences. Nucleoside polyphosphates (di- or triphosphates) are precursors of the nucleic acids. They also are carriers of the free energy of pyrophosphate bonds used in many synthetic and degradative biological reactions. From the medical point of view, ^{32}P -labelled-sodium polymetaphosphates have been used for the radiotherapy of bone malignancies ⁽¹⁾. In our laboratory, work has been carried out on the study of the possibilities of condensed phosphates, not only as radioactive compounds for the local irradiation of tumors, but also for the scintigraphic localization (scanning) of malignant lesions ⁽²⁾.

Despite the fact that the discovery of condensed phosphates is credited to work done in 1833 ^(3, 4) the chemistry of these compounds has been elucidated more recently ⁽⁵⁻¹³⁾. By dehydration of NaH_2PO_4 at high temperature, a water molecule is eliminated while a condensed diphosphate is formed (Fig. 1). This reaction could continue until an infinitely long chained sodium polyphosphate is formed. However, it is possible to regulate the length of the chain by chemical and/or physical means. On the other hand, if NaH_2PO_4 is dehydrated in presence of H_3PO_4 , crosslinked condensed phosphates are formed.

Ter-valent metal ions are precipitated by sodium polyphosphate but in most instances they are dissolved in excess of the reagent with the formation of stable complexes ⁽¹⁴⁾. Our approach to a gamma emitter labelling of

CROSS-LINKED:



LINEAL:

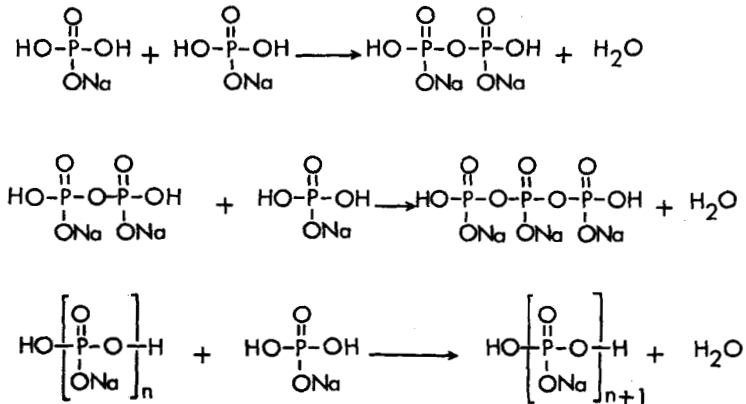


FIG. 1. Formation reactions of sodium polyphosphates.

condensed phosphates was developed on this basis, using chromic ion (Cr^{3+}) with the ^{51}Cr incorporated.

The present experimental work deals with both the preparation and stability of those condensed phosphates used in our biological experiences. Both, ^{32}P -labelled lineal and crosslinked sodium polyphosphates were used for local irradiation, while for studies on tumor localization ^{51}Cr -labelled lineal and crosslinked sodium polyphosphates were the choice.

PREPARATION OF SODIUM POLYPHOSPHATES.

The condensed phosphates used in this experimental work were prepared as follows :

- (a) *Lineal.* 100 mg of NaH_2PO_4 were heated in a platinum crucible at $950\text{--}1,000^\circ\text{C}$ for 7 hours. Then, after immediately cooling to room temperature the preparation was dissolved with continuous stirring in 5 ml of distilled water at 4°C (overnight).
- (b) *Crosslinked.* A mixture of 69 mg of NaH_2PO_4 plus 48 mg of 95 % H_3PO_4 was processed as described in (a).

To obtain the corresponding chromium complexes, 0.5 mg and 0.1 mg of Cr^{3+} (as CrCl_3) were added to (a) and (b) respectively, before heating.

Both radioactivities, ^{32}P -orthophosphate carrier-free and ^{51}Cr (as CrCl_3), were neutralized (the stock solutions were acid). In the first case this was done by addition of NaOH , and in the second case by evaporation, before being added to the reaction mixture. The presence of free acid in any case will be reflected in the condensed phosphate composition.

In all the cases the glassy melted phosphate dissolves very slowly and it is kept at 4°C in order to minimize hydrolysis. When the chromium complexes were prepared, they were filtered through a membrane filter (Millipore, pore size $0,25\ \mu$) after dissolution, with the purpose to retain any insoluble material.

EFFECTS OF THE RATIO P/Cr ON THE CHROMIUM-SODIUM POLYPHOSPHATE COMPLEXES.

The influence of increasing amounts of Cr on the physico-chemical characteristics of the complexes was studied by measuring the amount of insoluble material formed. After dissolution, the preparations were filtered through membrane filter ($0,25\ \mu$), and both filtrate and filter were counted in a well-type scintillometer. The percentage of soluble Cr as a function of the ratio P/Cr is plotted in Figure 2.

STABILITY TEST.

Lineal and crosslinked sodium polyphosphates (with both ^{32}P and ^{51}Cr labels) prepared as described above (20 mg/ml) were stored at 4° C. Samples were analyzed chromatographically 1 day, 7, 15 and 30 days after preparation. By ascending chromatography on paper using Ebel's (15) solvents system :

- (1) Basic solvent : isopropyl alcohol 40 ml + isobutyl alcohol 20 ml + H_2O 39 ml + ammonium hydroxide (22° Bé) 1 ml;
- (2) Acid solvent : isopropyl alcohol 75 ml + H_2O 25 ml + trichloroacetic acid 5 g + ammonium hydroxide (22° Bé) 0.2 ml.

It is possible to separate bidimensionally all the hydrolysis products. Figure 3 is a typical autoradiography of a chromatogram. In order to determine the percentage of each component, after developing the autoradiography, the chromatogram areas matching the spots were cut off and counted in a well-type counter (for ^{51}Cr) and after elution with 5% H_3PO_4 in a liquid scintillation counter (16). Table 1 includes those values for the different hydrolytic products.

Also, the possibility of analytical determination of the hydrolytic products by electrophoresis on Whatman 3MM paper using 0.02 M NaHCO_3 buffer has been assayed.

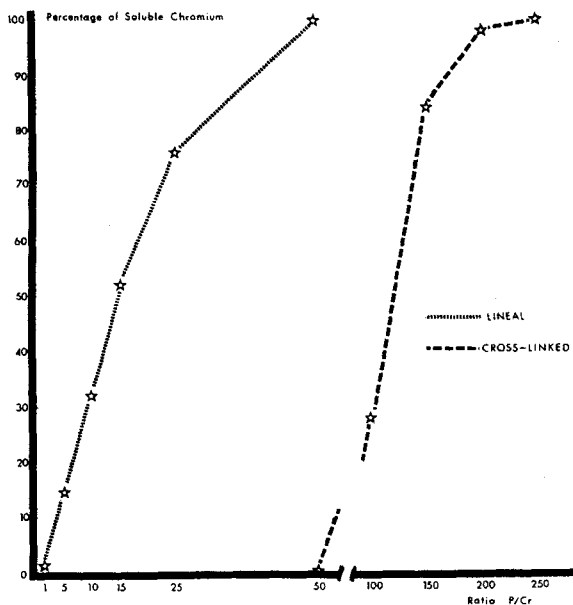


FIG. 2. Influence of chromium concentration on chromic-sodium polyphosphates' solubility.

EXPERIMENTAL RESULTS AND DISCUSSION.

As shown in Figure 2, the amount of Cr reacting with the polyphosphate formed through the dehydration of NaH_2PO_4 has a marked influence on the solubility of the condensed phosphate. This influence is more striking in the preparation of the cross-linked variety. While for the lineal type a 100% of solubility is obtained with a ratio P/Cr of about 50, the crosslinked variety requires a ratio P/Cr of about 250. This behaviour can be related to the higher availability of -OH or -ONa groups in the lineal form (Fig. 1) which are bound to the Cr. The solubility of the chromic sodium polymetaphosphate seems

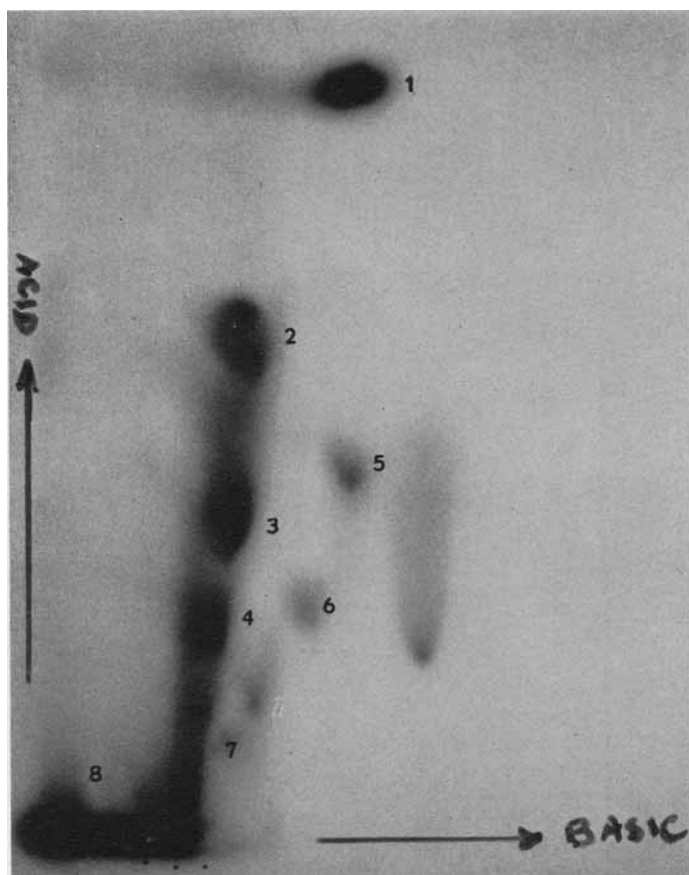


FIG. 3. Autoradiography of a chromatographic separation of sodium polyphosphate's hydrolytic products : 1) orthophosphate, 2) pyrophosphate, 3) tripolyphosphate, 4) tetrapolyphosphate, 5) trimetaphosphate. 6) tetrametaphosphate, 7) hexametaphosphate 8) Graham's salt.

to be highly influenced by the number of -OH and -ONa groups not bound to Cr. When the ratio P/Cr decreases, a considerable amount of insoluble material is formed and the physico-chemical characteristics of this insoluble fraction are like the chromium polymetaphosphate (Cr_{n/3}[H₂P_nO_{3n+1}]) described by Thilo *et al.*⁽¹⁷⁾, which also is isomorphic with the Fe^{III}[H₂P_nO_{3n+1}]

Concerning the stability, both sodium polyphosphates, lineal and cross-linked, show a very low degree of orthophosphate formation. The hydrolysis seems to be restricted to the short-chains or rings usually formed during the dehydration. The variation of high polyphosphate content is much less significant under the experimental conditions used. On the other hand, when the ⁵¹Cr-sodium polyphosphate stability was studied, most of the radioactivity was found in molecules having more than 4 phosphorus atoms. In this case the

TABLE 1. Chromatographic analysis of ³²P-sodium polymetaphosphates and ⁵¹Cr-sodium polymetaphosphates solutions stored at 4° C — (as percentage of the total radioactivity in the chromatogram).

	³² P-Sodium polymetaphosphates							
	1 day		7 days		15 days		30 days	
	L	C	L	C	L	C	L	C
Orthophosphate	—	—	0.4	0.5	0.4	0.6	1.2	5.6
Pyrophosphate	3.5	1.4	9.1	7.1	6.5	7.3	9.9	12.4
Triphosphate	3.9	2.9	6.8	5.1	3.2	4.6	5.9	4.2
Trimetaphosphate	7.4	7.0	9.7	11.8	12.3	8.6	14.4	18.5
Tetra- + hexametaphosphates	34.4	39.7	26.0	26.5	31.4	31.2	26.9	18.8
High polyphosphates (Graham's salt)	50.8	49.0	48.0	48.9	46.2	47.8	41.7	40.5
	⁵¹ Cr-Sodium polymetaphosphates							
Orthophosphate	—	—	—	—	—	—	—	—
Pyrophosphate	—	—	—	—	0.8	1.2	1.4	1.8
Triphosphate	8.0	6.6	4.1	3.2	3.4	3.0	1.9	2.6
Trimetaphosphate	—	0.6	1.1	0.7	1.3	1.0	1.0	1.3
Tetra- + hexametaphosphates	32.5	33.3	11.5	12.0	10.6	10.3	2.8	4.9
High polyphosphates (Graham's salt) ORIGIN	59.5	59.5	83.1	83.9	83.9	84.5	92.9	89.4

L = lineal.

C = Cross-linked.

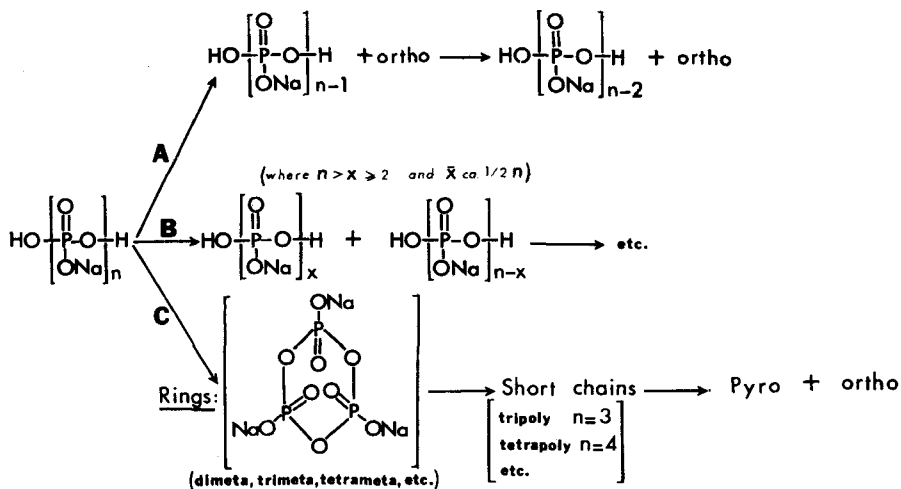
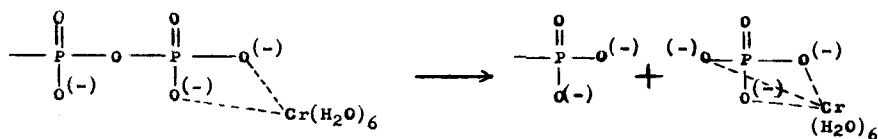


FIG. 4. Hydrolytic pathways of sodium polyphosphates.

hydrolytic pattern seems different (from the point of view of the ^{51}Cr radioactivity) from that of the sodium polymetaphosphate. It seems that in any case the final hydrolytic product is chromic phosphate. This assumption is substantiated by the fact that while the radioactivity decreases in all the other fractions, an increase is observed in the amount remaining in the origin (Table I). Under the conditions of the chromatographic study, the chromic phosphate does not migrate.

The hydrolysis of long chain polyphosphates, under our experimental conditions proceeds according to the paths A and C in Figure 4, i.e. splitting off of end groups and ring formation, while in acid solution all the three paths are involved^(18, 19). This rate of hydrolysis is very low for the sodium polyphosphates. On the contrary, the chromium sodium polyphosphates show a higher rate of hydrolysis reflected in the increase of radioactivity which does not migrate (chromic phosphate), and which is from 5 to 25 times higher after 30 days. This higher formation of orthophosphate is in agreement with other authors' findings^(20, 21, 22) that the hydrolysis is catalyzed by metal cations, and interpreted as following the formation of an activated complex by chelation of the hydrated metal ion :



The polyphosphates are not well separated by electrophoresis. The lineal form migrates 7.5 cm, while cross-linked and orthophosphate 7.0 and 9.5 cm respectively after a 45 minutes run using 0.02 M NaHCO_3 as buffer and a voltage gradient of 18 V/cm. Anyway, this method may have application for the determination of the Cr-sodium polyphosphate hydrolysis rate, since the hydrolysis product (Cr-PO_4) does not migrate while the original polyphosphate does, with a migration similar to that of sodium polyphosphate.

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