Polyphosphates are widely known because their use in water treatment. Recently the attention was driven to some interesting biological properties of those compounds which are relevant to pathological processes as aortic calcification and malignant growths. In all the cases these properties appear to be related to their ability to chelate divalent cations. Since the interpretation of the reactions involved as well as the effectiveness of their cation sequestering action is related to the chemical structure, the usefulness of these compounds is dependent on their ability to withstand hydrolysis. With these facts in mind, the hydrolysis of linear and cross-linked sodium polyphosphates was radiometrically studied.

Materials and methods

The $^{32}$P-labeled linear and cross-linked sodium polyphosphates were prepared as described elsewhere. 10 mg of each variety of polyphosphate in 0.5 ml of distilled water were hydrolyzed by heating in a boiling water-bath (95—96 °C). Samples were withdrawn at different times and analyzed by ascending paper chromatography at 4 °C. Whatman 3MM paper and Ebel’s acid solvend were used. After an overnight run a fairly good separation of five well defined fractions was obtained: a) high polymers, b) tetrametaphosphate, c) trimetaphosphate, d) pyrophosphate, and e) orthophosphate. Fig. 1 shows the 3 hours hydrolysis products of both linear and cross-linked sodium polyphosphates. For a quantitative evaluation, after being scanned the chromatograms were cut in sections corresponding to the activity peaks. Then the radioactivity was eluted from the paper with 1% $\text{H}_3\text{PO}_4$ and counted in a liquid scintillation counter. The corresponding quenching corrections were made by addition of a $^{32}$P internal standard. The radioactivity values corresponding to the different fractions plotted as percentage of the total $^{32}$P in function of the hydrolysis time are shown in Fig. 2 and 3.

Reprints request to Dr. LEOPOLDO J. ANGHILERI, Klinikum Essen, Tumorforschung der Ruhr-Universität, D-4300 Essen, Hufelandstr. 55.

* Present address: Klinikum Essen, Tumorforschung der Ruhr-Universität, 43 Essen, Hufelandstrasse 55, West Germany.


3 L. J. ANGHILERI, J. Lab. Comp. VI, 166 [1970].

* Composition of the chromatographic solvents according to Ebel:

Basic: 40 ml isopropyl alcohol + 20 ml of isobutyl alcohol + 39 ml of distilled water + 1 ml of ammonium hydroxide (22° Be).

Acid: 75 ml of isopropyl alcohol + 25 ml of distilled water + 5 g of trichloracetic acid + 0.3 ml of ammonium hydroxide (22° Be).

An ascending bidimensional chromatography using first the basic and then the acid solvent of Ebel was performed on a sample of 1 hour hydrolysis. The radioactivity was located by autoradiography (Figs. 4 and 5) and the percentage of total $^{32}$P corresponding to each spot counted as described above (Table I).

Results

Fig. 2 shows that the hydrolysis to orthophosphate is a slow rate reaction, being lower for the cross-linked form than for the linear one. Pyrophosphate also is formed slowly, but contrarily to orthophosphate its formation is higher for the cross-linked variety (Fig. 3). After heating hours a peak in tetrameta-, tripoly-, and trimetaphosphate concentration is readied. Beyond this point their concentration as well as that of high polymers decreases while the orthophosphate formation increases.

The Ebel's acid solvent was chosen because the fairly good separation obtained with a low polyphosphate degradation by the solvent action. Since in radiochromatographic determinations very low amounts of material are used (approximately in the range 50–100 μg), the possibility of a polyphosphate-solvent interaction should be considered. This is the reason why despite the fact that the bidimensional chromatography was not the solvent of choice, the radioactivity was located by autoradiography (Figs. 4 and 5).

Table I. Chromatographic analysis of 1 hour hydrolysis products of sodium polyphosphates (Figs. 4 and 5). — As percentage of total $^{32}$P in each fraction.

<table>
<thead>
<tr>
<th>Polypolysphate</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-linked</td>
<td>8.4</td>
<td>9.5</td>
<td>11.2</td>
<td>10.3</td>
<td>6.5</td>
<td>4.8</td>
<td>2.0</td>
<td>1.3</td>
<td>1.6</td>
<td>43.1</td>
</tr>
<tr>
<td>Linear</td>
<td>6.2</td>
<td>14.4</td>
<td>2.0</td>
<td>0.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>77.0</td>
</tr>
</tbody>
</table>

J. P. Ebel, Bull. Soc. chim. biol. 20, 991 [1943].

Fig. 4. Bidimentional chromatography of linear sodium polyphosphate hydrolytic products (Notations in Table I).
phate to orthophosphate $^6$:

$$
\begin{align*}
\text{n phosphorus atoms} & \\
\text{polyphosphate} & \\
\rightarrow & (n-1)\text{-chain + orthophosphate} \\
\rightarrow & (n-2)\text{-chain + orthophosphate} \\
\rightarrow & \text{short chain (tripoly-)} \\
\rightarrow & \text{shorter chain (pyro-) + ortho}
\end{align*}
$$

In addition, despite the fact that the branching point in the cross-linked form is more rapidly hydrolyzed than the linear chains, a higher rate of ring formation can be expected. Therefore, since ring degradation is reflected in a high pyrophosphate formation it is reasonable to expect a higher pyrophosphate concentration during the cross-linked polyphosphate hydrolysis. As seen in Fig. 3 this assumption is corroborated by the experimental results. On the other hand, the bidimensional chromatographic findings of higher concentration of metaphosphates, or of polyphosphates which could be the result of ring degradation (Table I) still strengthens this hydrolysis interpretation.

It is interesting to point out that the linear form shows no hydrolytic formation of metaphosphates (Table I) and its high pyrophosphate concentration can be related to a degradation via triplyphosphate formation. Concerning this it should be mentioned that trimetaphosphate has been found present in polyphosphate solutions $^7$ and coincidentally triplyphosphate is the intermediate in its hydrolysis to orthophosphate.

Finally it can be stated that the linear sodium polyphosphate is hydrolyzed by boiling water (95 - 96 °C) through a degradation path which produces a higher proportion of orthophosphate than with the cross-linked form. On the other hand, this variety forms more pyrophosphate. This difference seems to be related to a metaphosphate formation missing or at least occurring to a much lesser extent during the linear polyphosphate hydrolysis. Coincidentally, the biological fate of these two forms of sodium polyphosphate seems to fit to a similar pattern of higher metaphosphates formation in the case of cross-linked polyphosphate. Furthermore, this characteristic hydrolytic behavior appears explaining the peculiar in vivo distribution of the cross-linked polyphosphate $^8$.


$^8$ L. J. ANGHILERI and E. S. MILLER, to be published.