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THE FORMATION OF CONDENSED PHOSPHATE IN AQUEOUS SOLUTION

BY A. BECK AND L. E. ORGEL

THE SALK INSTITUTE FOR BIOLOGICAL STUDIES, LA JOLLA, CALIFORNIA

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In view of the central role of adenosine triphosphate and related compounds in metabolism, the abiological synthesis of condensed phosphates is relevant to the problem of the origins of life.¹⁻³ Here we describe experiments on the condensation of phosphate to pyrophosphate and of linear triphosphate to trimetaphosphate in aqueous solution. We have used three condensing agents—potassium cyanate,

dicyandiamide (cyanoguanidine), and the water-soluble diimide, 1-cyclohexyl-3-(2-morpholinyl-(4)-ethyl)-carbodiimide metho-*p*-toluenesulfonate (CDI).

Experimental.—Solutions were prepared and left to stand at room temperature. The experiments were analyzed 1–3 days after preparation. In a number of cases the analysis was repeated after a few weeks; in no case were the yields of products changed significantly. Analyses were carried out by the paper chromatographic method of Kolloff.⁴ Since orthophosphate reacts with cyanate to give carbamylphosphate, which can be mistaken for pyrophosphate on the chromatogram, it was necessary to destroy cyanate and carbamylphosphate. To achieve this result we acidified the solutions to pH 1,⁵ and after an hour neutralized and heated the sample for 10 min in boiling water.⁶ As a complexing agent for calcium, ethylenediaminetetraacetic acid (0.05 *M*) was added prior to chromatography. The solvents 90-7 and 92D-5⁴ were used for the detection of pyrophosphate and trimetaphosphate, respectively. Solutions of known concentration were run simultaneously with the samples, and the yields were evaluated by comparing visually the intensities of the spots.

The lower limit of detection was generally 0.1–0.5 per cent conversion, depending on the nature and concentration of the solution. (It is not advisable to increase the concentration by evaporating or freeze-drying the solution prior to chromatography, for condensation can take place under such conditions.) The tripolyphosphate solutions were filtered prior to analysis and it is possible that a small part of the trimetaphosphate was lost through coprecipitation and adsorption.⁷

Results.—Carbodiimide-orthophosphate: No condensation to pyrophosphate was detected in solutions 0.01–0.5 *M* with respect to orthophosphate and 0.1 *M* in CDI. We carried out a variety of experiments with solutions in the pH range 1–7 and would have detected pyrophosphate at concentrations greater than 0.00005 *M*.

Carbodiimide-tripolyphosphate: When a solution 0.1 *M* in CDI and 0.01 *M* in $\text{Na}_5\text{P}_3\text{O}_{10}$ was left to stand overnight, more than 50 per cent of the tripolyphosphate was converted to trimetaphosphate. (See *Note added in proof.*) Lowering the pH to ≈ 5 (by cation exchange) did not affect the yield, though most of the uncondensed tripolyphosphate then decomposed.

Dicyandiamide-orthophosphate: We could not confirm that up to 1.5 per cent yields of pyrophosphate are formed from 0.001 *M*–0.01 *M* solutions of phosphoric acid and dicyandiamide.¹ We could detect no pyrophosphate when 0.01 *M* solutions of phosphoric acid and dicyandiamide were used; when both reagents were 0.1 *M* (pH ≈ 1), the pyrophosphate spot was sometimes just visible and sometimes not visible at all. The limit of detection was 0.00005 *M* pyrophosphate (0.1% conversion).

Dicyandiamide-tripolyphosphate: No trimetaphosphate was found in unbuffered solutions containing 0.1 *M* sodium tripolyphosphate and 0.1 *M* dicyandiamide. Five-tenths per cent conversion could have been detected.

Cyanate-orthophosphate: No condensation to pyrophosphate was detected in homogeneous solutions of phosphate and cyanate with or without calcium salts. However, a system with initial concentrations of 0.2 *M* H_3PO_4 , 0.2–0.4 *M* KCNO , and 0.0005 CaCl_2 , from which calcium phosphate precipitated, yielded 1–2 per cent pyrophosphate, thus confirming the results of Miller and Parris.² If precipitation of calcium phosphate was prevented by addition of a suitable electrolyte (1 *M*

ammonium acetate), no pyrophosphate was formed. On the other hand, when ten times more calcium was added, and a much stronger precipitate obtained, the yield of pyrophosphate increased roughly in proportion to the amount of calcium. Once again, when precipitation was prevented (by making the solution 0.5 *M* in acetic acid (pH \sim 5), no pyrophosphate was detected. The pH in all these cases was between 5 and 6.5.

Cyanate-tripolyphosphate: We carried out many experiments using 0.01–0.02 *M* tripolyphosphate and wide ranges of concentration of potassium cyanate (0.2–1 *M*), acetic acid (0–0.8 *M*), and calcium chloride (0–0.5 *M*). Whenever the solution remained homogeneous, we were unable to detect trimetaphosphate.

If the solution was made strongly acid (>0.5 *M* acetic acid), cyanuric acid and other cyanate polymers were precipitated. Then yields of up to 5 per cent trimetaphosphate were obtained. Trimetaphosphate in yields of up to 1 per cent could also be detected in solution at higher pH's in the presence of sufficient Ca^{2+} to form a precipitate.

Discussion.—The condensation of phosphate to pyrophosphate is expected to be second order in phosphate, and hence to be favored by high phosphate concentrations. On the other hand, the condensation of tripolyphosphate to trimetaphosphate, while also involving the formation of a pyrophosphate bond, should be first order in tripolyphosphate. While the first reaction is the more interesting from the point of view of prebiological energy production, it seemed likely that the second reaction could be made to yield a greater percentage conversion in the presence of convenient concentrations of condensing agents. Both reactions involve the condensation of two negatively charged phosphate groups and so should be susceptible to catalysis by positive ions.

Our experiments show that the yields of pyrophosphate from phosphate in homogeneous aqueous solutions are very small. While we cannot exclude the possibility that a better condensing system was present on the primitive earth, we have used favorable laboratory conditions with representatives of three widely discussed classes of condensing agents with very limited success. We conclude, tentatively, that inorganic pyrophosphate production, if it was important, is more likely to have occurred thermally, heterogeneously,² or photochemically.³

The condensation of tripolyphosphate to trimetaphosphate by the soluble carbodiimide (CDI) has no direct biological significance. However, insofar as CDI typifies a very large class of condensing agents, for example, various ketenes, nitriles, and acetylenes, it may suggest that the formation of cyclic triphosphate compounds is likely under any conditions in which phosphate condensation itself is favored. In this connection, the central role of cyclic trimetaphosphates in the synthesis of polynucleotides in nonaqueous solutions should be noted.⁸

Summary.—Potassium cyanate solutions would not bring about the condensation of orthophosphate to pyrophosphate or of tripolyphosphate to trimetaphosphate in homogeneous solutions; both condensations did occur, however, when sufficient calcium ion was added to form a phosphate precipitate. In the case of trimetaphosphate the condensation also took place in the presence of the precipitate formed from cyanate at low pH's.

Cyanamide dimer, if it caused the condensation of orthophosphate to pyrophos-

phate, never did so in yields greater than 0.1 per cent; no formation of trimetaphosphate from tripolyphosphate was observed.

With a water-soluble carbodiimide, 1-cyclohexyl-3-(2-morpholinyl-(4)-ethyl)-carbodiimide metho-p-toluenesulfonate, we were unable to show condensation of orthophosphate to pyrophosphate, whereas tripolyphosphate was converted to trimetaphosphate in >50 per cent yield.

Note added in proof: Using 1-ethyl-3-(3-dimethyl amino) propyl carbodiimide hydrochloride, provided by Dr. Sheehan, we were able to isolate the trimetaphosphate as the benzidine salt, and to identify it by its IR spectrum.

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TWO METHODS FOR ESTIMATING COMPRESSION AND SOUND VELOCITY AT VERY HIGH PRESSURES*

BY ORSON L. ANDERSON†

LAMONT GEOLOGICAL OBSERVATORY OF COLUMBIA UNIVERSITY, PALISADES, NEW YORK

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Precision sound velocity measurements made at relatively low pressure (less than 10 kilobars) can be used to estimate sound velocity and volume compression at very high pressures. If the parameters of a continuous analytic function are sufficiently accurately determined at low values of the independent variable, the function may be evaluated at high values of the independent variable. Consequently, only materials undergoing compression without phase change are treated.

What is new is the presentation of two functions suitable for representing volume dependence upon pressure (compression curves) and the evaluation of the parameters in these functions from acoustic data taken at low pressures but with high precision. The parameters are the bulk modulus and its higher derivatives. These new results also apply to older work on the equation of state: in particular, Birch's equation of state¹ relating pressure and density given by

$$P = \frac{3B_0}{2} \left\{ \left(\frac{\rho}{\rho_0} \right)^{7/3} - \left(\frac{\rho}{\rho_0} \right)^{5/3} \right\}, \quad (1)$$

where B_0 is the bulk modulus at ambient pressure.

The features and advantages of the various equations of state are not discussed here; rather, acoustic data is used to evaluate two particular compression equations. Isothermal expressions are needed, since this is the condition of the measurement of