THE "SOLUBLE" AND "INSOLUBLE" POLYPHOSPHATES OF YEAST

by

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When yeast cells (*Saccharomyces cerevisiae*) are extracted with cold trichloroacetic acid, a polyphosphate fraction labelled "soluble" is separated from a second polyphosphate labelled "insoluble" which is retained in the residue; the latter fraction can be separated from the residue by further treatment[1]. These two polyphosphate fractions in yeast are reported to have different metabolic activities[1,2]. In a study of phosphorus metabolism in yeast, additional evidence has been obtained which substantiates the existence of these two fractions with different metabolic activities[3]. The purpose of this Communication is to explain why there is a separation into two phosphate fractions.

Since proteins below their isoelectric points are polyelectrolytes having polycations, and the polyphosphates exhibit polyanions[4], interaction between the two to form precipitates will become accentuated as the size of either the polycation or polyanion is increased. Thus, a given protein can be expected to preferentially precipitate long-chain from short-chain polyphosphates, as is demonstrated by Table I.

TABLE I

INTERACTION WITH ALBUMEN IN TRICHLOROACETIC ACID AS A FUNCTION OF POLYPHOSPHATE CHAIN LENGTH

Type of phosphate	*Number-average chain length*	*% Phosphorus precipitated**
Na_2O–P_2O_5 glass	16	25
Graham's salt	85	52
Graham's salt	105	58
Graham's salt	130	52
Graham's salt	230	84
Kurrol's salt	1600	100
Mixture of short-chain glass and Kurrol's salt	46% of total P as 16 chain length, 54% of total P as 1600 chain length	52

* Test system: 1.5 ml of 1% egg albumen solution added to 0.2 ml of 1% phosphate solution, washed down with 0.5 ml water to give clear solution. Precipitate formed on addition of 1 ml of 10% trichloro-acetic acid. Mixtures centrifuged and supernatants hydrolyzed in 1 *N* HCl at 100° C and estimated for orthophosphate colorimetrically[7]. Blanks run with substitution of egg albumen by water showed no precipitate.

The data in Table I show that the amount of polyphosphate coprecipitated with a moderately sized, water-soluble protein (egg albumen[5]) by trichloroacetic acid increases with increasing number average chain length of the polyphosphate. Using the known[6] size distribution of polyphosphate molecule-ions in the Na_2O–P_2O_5 glasses, the size of the phosphate below which no precipitation occurs with egg albumen under the conditions of the experiment can be calculated. This calculation indicates that a polyphosphate chain length of approximately 100 phosphorus atoms (equivalent to a molecular weight of 10,000 based on the $(NaPO_3)_x$) is the size below which no precipitation occurs. This figure holds for the four samples of Graham's salt and the one of Kurrol's salt tested but does not apply to the Na_2O–P_2O_5 glass having an average chain length of 16. In the case of

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this latter glass, a much smaller number, ca. 25 phosphorus atoms per chain, is calculated for the dividing line between precipitation and no precipitation.

The data given in the last line of Table I show that a polyphosphate with a chain length of 16 appears to be quantitatively separated from a polyphosphate having a chain length of 1600 by precipitation with albumen in the presence of trichloroacetic acid. The fact that a mixture which is approximately 50–50 in the long- and short-chain phosphates can be separated into two equal-sized fractions is in accord with the conclusion that the phosphate glass having the shortest average chain length should give only a trace of precipitate instead of the ca. 25% observed. Presumably, a large part of this 25% is attributed to coprecipitation or some mechanism other than the well-known "metaphosphate-protein reaction"[8].

The data presented suggest that the differential separation into two polyphosphate fractions, which occurs when yeast cells are partitioned with trichloroacetic acid, may be due to the difference in protein complexing properties between polyphosphates which differ in chain length. The observations do not appear to be an artifact, as has been suggested by SCHMIDT[9]. Naturally, the nature of the protein-polyphosphate interaction is dependent upon many variables, and a more comprehensive investigation of this phenomenon is under way. In addition, an attempt is being made to determine the chain length (molecular weight) of the polyphosphate fractions in yeast, using physical-chemical methods. The exact role of molecular size in determining the different metabolic activities of the polyphosphates in yeast is not clear at the present time.

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ENZYMIC DECOMPOSITION OF THE ACTIVE INTERMEDIATE IN CITRULLINE SYNTHESIS

by

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In 1949 one of the authors (S.G.) made the observation that skeletal and cardiac muscle homogenates when added to mitochondrial preparations of rat liver inhibited the reaction ornithine $\longrightarrow$ citrulline. Recent studies on the reaction ornithine $\longrightarrow$ citrulline[1,2,3] have shown this reaction to be composed of two main enzymic steps, reactions 1 and 2.

1. $CG^{**} + CO_2 + NH_3 + ATP \xrightarrow[Mg^{++}]{\text{Enzyme I}}$ Compound X + ADP

2. Compound X + Ornithine $\xrightarrow{\text{Enzyme II}}$ Citrulline + CG + $PO_4^{\equiv}$

* This work was done during the tenure by the senior author of an Established Investigatorship of the American Heart Association.

** The following abbreviations are used throughout this paper: CG, carbamyl-L-glutamate; Compound X, the active intermediate for citrulline synthesis[2] which contains one mole each of CG, CO_2, NH_3, and $PO_4^{\equiv}$. ATP and ADP, adenosine tri- and di-phosphates respectively; Enzymes I and II, enzyme systems as previously described[2]. All analytical methods and procedures have been described in preceding papers[1,2,3].