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Received for review April 26, 1983. Revised manuscript received March 5, 1984. Accepted March 13, 1984.

Dissociation of Yeast Nucleoprotein Complexes by Chemical Phosphorylation

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Chemical phosphorylation of yeast nucleoproteins using $POCl_3$ caused dissociation of nucleoprotein complexes. Subsequent precipitation of the phosphorylated proteins at pH 4.2 resulted in a protein preparation with low levels of nucleic acid contamination. However, there was an appreciable decrease in the recovery of proteins after phosphorylation. The advantages of the phosphorylation method over that of other chemical modification methods for decreasing the nucleic acid content of yeast proteins are also discussed.

Proteins from microbial sources, especially yeast, could significantly supplement the ever increasing demand for the world supply of food protein (Tannenbaum and Wang, 1975). However, the exploitation of these proteins for direct human nutrition is limited because of the high nucleic acid content and poor functionality of these proteins (Kihlberg, 1972; Sinskey and Tannenbaum, 1975; Vananuvat and Kinsella, 1975; Lipinsky and Litchfield, 1974). There are several methods currently available to reduce the nucleic acid content of single cell proteins (Newell et al., 1975a,b; Robbins et al., 1975; Shetty and Kinsella, 1979). These methods involve chemical and enzymatic treatment of homogenized yeast cells. Although these treatments effectively reduce the nucleic acid content, they have several detrimental effects on the nutritional and functional qualities of the isolated protein. For example, alkali treatment causes formation of potentially toxic compounds such as lysinoalanine (Shetty and Kinsella, 1980a,b) and enzymatic hydrolysis of nucleic acids results in concomitant degradation of proteins by endogenous proteases (Lindbloom, 1977). Chemical modification of yeast proteins with acid anhydrides such as succinic anc citraconic anhydrides effectively reduced the nucleic acid content of yeast proteins (Shetty and Kinsella, 1979, 1980a,b). However, the nutritional safety and acceptability of acylated proteins are yet to be determined. In our continuing effort to develop a simple and safe alternative method for the isolation of yeast proteins with low levels of nucleic acids, we studied chemical phosphorylation of yeast nucleoproteins using phosphorus oxychloride ($POCl_3$).

EXPERIMENTAL SECTION

Brewer's yeast (*Saccharomyces carlbergensis*) was obtained from Genesee Brewing Co. (New York). The yeast cells were washed 3 times with cold distilled water and disrupted by using a Dyno-Mill (Type KDL, Willy A. Bachofen, Manufacturing Engineers, Basel, Switzerland) at 5 °C. The pH of the homogenate was adjusted to 9.0, and the mixture was stirred for 30 min at 5 °C. The solution was then centrifuged at 15000*g* for 30 min at 5 °C to remove the cell wall and other insoluble materials.

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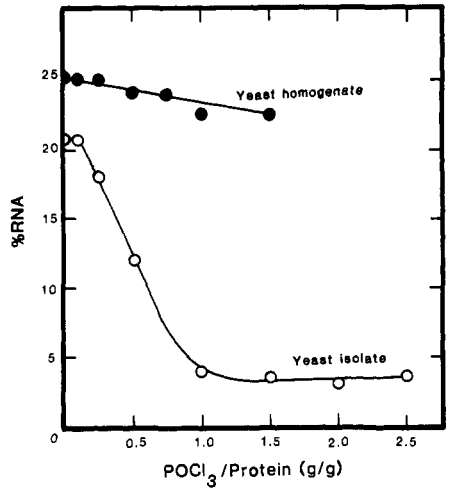

Figure 1. Effect of phosphorylation on the nucleic acid content of yeast proteins.

The nucleoproteins from the clear supernatant were recovered by isoelectric precipitation at pH 4.2, followed by centrifugation at 15000*g* for 30 min. The nucleoprotein precipitate was then dissolved in water at pH 9.0 and freeze-dried.

The protein and nucleic acids were estimated by the biuret and orcinol (Herbert et al., 1971) methods, respectively.

Phosphorylation. Chemical phosphorylation of yeast nucleoprotein isolate (~1% aqueous solution) using phosphorus oxychloride ($POCl_3$) was performed as described elsewhere (Woo et al., 1982). The extent of phosphorylation was varied by changing the ratio of $POCl_3$ to protein in the reaction mixture. After phosphorylation, protein was precipitated by decreasing the pH to 4.2 and then recovered by centrifugation at 20000*g* for 30 min. In order to remove the residual $POCl_3$ in the protein precipitate, the precipitate was resuspended, washed, and centrifuged twice with 10 volumes of water at pH 4.2 and then dissolved in water at pH 8.5. The protein and RNA contents of the dissolved sample were determined.

In the case of experiments with yeast homogenate, phosphorylation was carried out before removing the cell wall materials. After phosphorylation, the samples were centrifuged at 15000*g* for 30 min to remove the cell wall materials. The supernatant, which contained the soluble proteins and nucleic acids, was adjusted to pH 4.2 to precipitate proteins. The protein precipitate was washed twice with 10 volumes of water at pH 4.2 and then dissolved in water at pH 8.5. The protein and nucleic acid contents of the sample were determined.

RESULTS AND DISCUSSION

Yeast nucleoproteins were phosphorylated by using $POCl_3$. The extent of phosphorylation of yeast nucleoproteins was varied by changing the ratio of $POCl_3$ to protein. Attempts to estimate the extent of phosphorylation using the methods described previously (Woo et al., 1982) were unsuccessful because of high phosphate content in the control. This may be due to either interference of nucleic acids with the method for phosphate determination or high levels of bound inorganic phosphates in the sample. Because of these problems, the extent of phosphorylation is expressed here as the ratio of $POCl_3$ to protein used for phosphorylation.

Proteins precipitated at pH 4.2 from the phosphorylated solution contained lower amounts of nucleic acids as the contaminant. The nucleic acid content of the isolated proteins decreased with the extent of phosphorylation

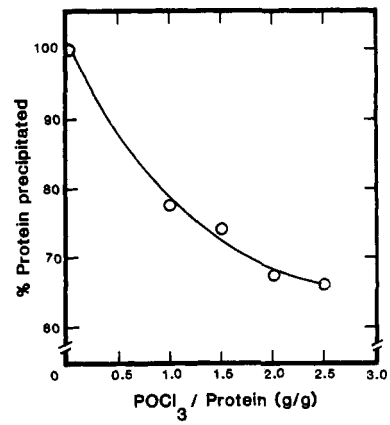

Figure 2. Effect of phosphorylation on the amount of protein precipitable at pH 4.2.

(Figure 1). Maximum reduction of nucleic acids occurred at a minimum $POCl_3$ to protein ratio of about 1.0 (g/g); under these conditions about 80% of RNA originally present in the nucleoprotein was removed. At higher ratios of $POCl_3$ to protein, there was no further decrease in the nucleic acid content of the isolated protein.

In the case of phosphorylation of yeast nucleoproteins in the presence of cell wall materials (i.e., yeast homogenate), there was no decrease in the nucleic acid content of the isolated protein at all ratios of $POCl_3$ to protein studied (Figure 1). Although the reason for this is not clear, it may be speculated that the cell wall materials may contain an excess number of $POCl_3$-reacting groups, which may decrease the availability of $POCl_3$ for protein phosphorylation.

Phosphorylation affected the recovery of yeast proteins. The amount of proteins precipitable at pH 4.2 decreased with the extent of phosphorylation (Figure 2). At a $POCl_3$ to protein ratio of 1.0, only about 80% of the total proteins precipitated at pH 4.2. Since the pK of substituted phosphate groups in proteins is about 2.0, it may be expected that phosphorylation may considerably decrease the isoelectric point of yeast proteins. Furthermore, if the lysyl residues are involved in phosphorylation (Woo et al., 1982), the elimination of the positive charges and concomitant introduction of phosphate groups may decrease the isoelectric point of these phosphoproteins. Under such circumstances, the recovery of proteins by precipitation at pH 4.2 will decrease. Attempts to recover the protein at lower pH resulted in increased nucleic acid contamination of the isolated protein, possibly because of coprecipitation of nucleic acids at lower pH.

The results presented here indicate that phosphorylation of yeast proteins induced dissociation of nucleoprotein complexes. The basic mechanism involved is phosphorylation of the protein increases the net negative charges on the protein; this in turn introduces a strong electrostatic repulsion within the nucleoprotein complexes and thus dissociates the protein and nucleic acids. Since the dissociated nucleic acids have an isoelectric pH around 1.5–2.0, the nucleic acids remain soluble during precipitation of protein at pH 4.2. This facilitates recovery of yeast proteins with low levels of nucleic acids.

Chemical modification of yeast proteins with succinic and citraconic anhydrides has been shown to decrease the nucleic acid content in the isolated proteins (Shetty and Kinsella, 1979, 1980a,b). The basic mechanism involved in these methods is similar to that of phosphorylation as described above. The major disadvantage with the succinylation method is that the isopeptide bond of succinyl–lysine is not acid hydrolyzable; this impairs the

bioavailability of lysine. Although citraconylation is reversible under acidic conditions, and hence would not impair the bioavailability of lysine, the nutritional safety of citraconylated proteins is yet to be studied. In this respect phosphorylated proteins may have an advantage over either succinylated or citraconylated yeast proteins. Since most of the milk proteins are phosphoproteins, it may be expected that the phosphorylated yeast proteins will be nutritionally safe and may possess good functional properties.

Registry No. $POCl_3$, 10025-87-3.

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Received for review March 23, 1984. Accepted June 22, 1984.

Trimethylamine Oxide Prevents Insolubilization of Red Hake Muscle Proteins during Frozen Storage

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The enzymic breakdown of trimethylamine oxide (TMAO) to dimethylamine and formaldehyde has been suggested to cause changes in the physicochemical properties of frozen, stored, gadoid muscle via the interaction of the proteins with formaldehyde. In the experiments reported here we demonstrate that in the absence of significant concentrations of formaldehyde a decrease in the level of TMAO of and by itself leads to a change in solubility of the muscle proteins of red hake during frozen storage. Betaine, which does not participate in the reaction catalyzed by the enzymic system responsible for the degradation of TMAO, also can inhibit the solubility change of protein during frozen storage.

During frozen storage of fish, muscle proteins undergo denaturation due to a variety of causes (Shenouda, 1980). Among these are the denaturation that is caused by the increase in salt concentrations or change in pH following removal of water by ice formation. Interactions with lipids can denature proteins due to their surfactant effects, while lipid oxidation can change protein conformations by modifying side groups or by inducing cross-linking of proteins by a free radical process (Karel et al., 1975). Gadoid fish contain an enzymic system for the breakdown of trimethylamine oxide (TMAO) to dimethylamine and formaldehyde (Amano and Yamada, 1964; Yamada et al., 1969; Dingle et al., 1977; Crawford et al., 1979). It has been suggested that the formaldehyde produced cross-links the proteins in the muscle tissue, forming a three-dimensional network causing textural toughness (Amano and Yamada, 1965; Castell et al., 1973; Gill et al., 1979). There is, however, no direct evidence that cross-linking of proteins in fish muscle is caused by formaldehyde produced enzymically, nor is there any direct evidence that chemical changes in fish muscle proteins are brought about by reaction of the proteins with formaldehyde. Nevertheless, there have been reports that indicate that formaldehyde concentrations equivalent to those found in frozen stored fish muscle can cause polymerization of some proteins (Ohnishi and Rodger, 1979; Owusu-Ansah and Hultin, 1984). We also have evidence that much of the formaldehyde that is produced from the enzymic decomposition of TMAO reacts with the small molecular compounds in the muscle tissue (Banda and Hultin, 1983). Thus, it is probably premature to assign all of the changes in texture in frozen stored gadoid muscle to cross-linking of proteins by formaldehyde.

Recently, Yancey et al. (1982) discussed the evolution of various osmolyte systems that protect cells from water stress. Methylamines, including trimethylamine oxide, are examples of such osmolytes. Water stress in living organisms could be caused by high or fluctuating salinity, dehydration, or freezing. Species tolerant to frost restrict ice formation to the extracellular spaces of the tissue. This concentrates the salts in the cell that would denature the proteins without the protective osmolytes. Freezing fish muscle post-mortem concentrates the solutes of the cell, and we thought it possible that the naturally occurring protective osmolyte of fish muscle, trimethylamine oxide, might protect the muscle proteins from denaturation caused by the stress of solute concentration during freezing. The results reported in this paper show that trimethyl-

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0021-8561/84/1432-1032$01.50/0