THE PREPARATION OF RIBONUCLEOPROTEIN FROM YEAST*

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The present work was undertaken to study the distribution of yeast nucleoprotein in cell fractions after centrifugal separation, and to define conditions of extraction and purification in order to obtain the nucleoprotein in a relatively undegraded state. Bakers' yeast (Saccharomyces cerevisiae) is the classical source of ribonucleic acid (RNA), but relatively little work has been reported on the nucleoproteins of this organism. Khouvine and de Robichon-Szulmajster (1) have isolated nucleoproteins from yeast by extraction with 0.2 per cent sodium bicarbonate. The cells had been dried with acetone and ether at -10° and ground in a cold ball mill. Three nucleoprotein fractions were obtained by precipitation at 4.96, 4.32, and 2.25 pH. These authors have reported on the composition of the protein (1) and nucleic acid (2) components and have described the effects of some variations of yeast strain and treatment (2, 3). The presence in yeast of submicroscopic particles rich in pentose nucleic acid has been reported by several workers (4-7).

This paper reports the composition of several centrifugal fractions of ground yeast and of a nucleoprotein material isolated by pH adjustment.

EXPERIMENTAL

Yeast—Commercial bakers' pressed yeast was generously donated by the Consumers Yeast Company, Oakland, California. It had been grown on an aerated molasses wort supplemented with ammonium and phosphate ions. The yeast was stored at 4° and was used within a few days of harvesting.

Total Nitrogen—Total nitrogen was determined by a Kjeldahl method.

Total Phosphate—Total phosphate was determined by a modification of the method of Griswold, Humoller, and McIntyre (8), with p-methylamino-phenol sulfate as the reducing agent (9). This reagent, commonly used as

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a photographic developer, has better stability in storage than the aminonaphthol sulfonate used by Griswold et al. (8). The concentrations of the other reagents were changed and the heating time was lengthened.

Ribonucleic Acid—An assay method based on ultraviolet spectrophotometry was chosen, since yeast contains metaphosphate and non-nucleic acid pentose which may interfere with RNA determinations based on phosphorus determinations (10) or pentose estimations (11).

The method was developed from that of Scott and Fraccastoro as reported in abstract form (12) for tissue sections. The material, containing 50 to 500 γ of RNA, was extracted twice with 0.2 N perchloric acid to remove acid-soluble nucleotides. It was then washed once with ethanol and twice with ethanol-ethyl ether (3:1, volume by volume) to remove lipide materials. The residue was treated with 1 N sodium hydroxide for 1 hour at room temperature. The solution was acidified with perchloric acid (to a final concentration of 0.2 N hydrogen ion) to precipitate protein and deoxypentose nucleic acid (DNA). The precipitate was washed twice with 0.2 N perchloric acid.

The combined extract and washings were neutralized with sodium hydroxide and brought to 5 or 10 ml. volume with phosphate buffer (pH 7). The optical density of the solution was determined at 260 mμ and corrected for the density of a blank which lacked the tissue sample. The extraction and washing procedure used 1 ml. volumes in 10 X 75 mm. culture tubes at room temperature. The precipitates were separated by centrifugation.

As a standard, commercial yeast nucleic acid, which had been deproteinized according to Sevag, Lackman, and Smolens (13), dialyzed, and lyophilized, was used. It was subjected to the hydrolytic conditions of the method. Extraction of yeast for varying periods of time indicated that 1 hour was optimal.

The assay procedure was applied to a sample of thymus DNA provided by Dr. N. S. Simmons. The results, presented in Table I, indicate that the acid extraction and precipitation steps of the procedure should be accomplished at 2–4°. Perchloric acid and, to a lesser extent, hydrochloric acid and trichloroacetic acid slowly degrade DNA to acid-soluble fragments. The acids also render the DNA relatively susceptible to alkaline hydrolysis. After pretreatment with 0.2 N perchloric acid at room temperature for about 40 minutes (the time required for the two acid extractions of the regular assay procedure), 20 to 40 per cent of the DNA was degraded to acid-soluble fragments by 1 N sodium hydroxide at room temperature for 1 hour. The data indicate that the alkaline hydrolysis of DNA in this procedure is minimal if the acid pretreatment is carried out at low temperature. The results were checked with rat liver deoxypentose nucleoprotein (15) and calf thymus nucleohistone (16), contributed by Dr.
The degradation of DNA on alternate exposure to acid and alkali may lead to derivatives related to apurinic acid, with the subsequent rapid alkaline hydrolysis to yield acid-soluble nucleotide.

When crystalline bovine serum albumin was carried through the procedure to give an indication of the extent of protein hydrolysis, negligible amounts appeared in the final solution. A test was made for possible adsorption of ribonucleotide material on the precipitated protein after alkaline hydrolysis and acidification. Repetition of the alkali and acid treatments yielded very little additional ultraviolet-absorbing material, indicating negligible adsorption of nucleate on this precipitate.

### Table I

**Degradation of Calf Thymus Desoxypentose Nucleic Acid** by Alkaline Hydrolysis after Acid Pretreatment

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Temperature of acid pptn.</th>
<th>Immediately acid-soluble</th>
<th>Apparent RNA*</th>
<th>Apparent DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 N HClO₄</td>
<td>20–25</td>
<td>20–25</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>0.2 N HCl</td>
<td>20–25</td>
<td>20–25</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>5% TCA</td>
<td>20–25</td>
<td>20–25</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>0.25 N HClO₄</td>
<td>2–4</td>
<td>2–4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>20–25</td>
<td></td>
<td>4</td>
<td>96</td>
</tr>
</tbody>
</table>

*The sample of calf thymus desoxypentose nucleic acid, provided by Dr. N. S. Simmons, was prepared as described by Simmons et al. (14).

† "Apparent RNA" is the designation for the fraction of desoxypentose nucleic acid rendered acid-soluble after the indicated acid pretreatment and the standard hydrolysis in 1 N NaOH for 1 hour at room temperature.

Any method of nucleic acid analysis which involves the separation of "nucleic acid" from acid-soluble nucleotides or other small nucleotides is empirical in the absence of more precise definition of the boundary between the two fractions. The boundary is set by means of fractionation, in this case the insolvability of nucleic acid or nucleoprotein in 0.2 N perchloric acid. The lower size limit set by this means is not known. It is also unknown whether configuration or composition is a factor in this separation.

**Yeast Grinding**—The yeast was ground with 2½ times its weight of levigated alumina in a porcelain mortar at 2–4°C (19). Cell counts of the ground yeast, after staining with Lugol's iodine solution, indicated that 65 to 75 per cent of the cells was broken. In addition to cells which appear to be entirely normal, others show degrees of breakage from deformed cells to empty cell wall ghosts.
**Centrifugal Fractionation**—Centrifugal conditions are expressed by the performance index \((P_i)\) proposed by Pickels (20), or by the product of centrifugation time (minutes) and this index \((t \times P_i)\). The performance index provides a more precise comparison of centrifugal conditions for various rotors or centrifuges than is possible with either relative centrifugal force or rotational speed (r.p.m.). It is defined by the equation

\[
P_i = \frac{(\text{r.p.m.})^2}{\log_2 R_2 - \log_2 R_1}
\]

in which \(R_2\) is the maximal radial distance and \(R_1\) is the minimal distance, the latter being a function of liquid level in the centrifuge tube. Table II lists the machines, the speed, centrifugal force, and time corresponding to the \(t \times P_i\) values used.

**Table II**

Summary of Centrifugal Conditions

<table>
<thead>
<tr>
<th>Machine</th>
<th>r.p.m.</th>
<th>Relative centrifugal force (average)</th>
<th>Time</th>
<th>(t \times P_i \times 10^{-4})</th>
</tr>
</thead>
<tbody>
<tr>
<td>International PR-1 rotor No. 284</td>
<td>2,000</td>
<td>620</td>
<td>20</td>
<td>1.2</td>
</tr>
<tr>
<td>Spinco L rotor No. 21</td>
<td>8,100</td>
<td>4,300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>15,000</td>
<td>22,600</td>
<td>30</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>21,000</td>
<td>44,000</td>
<td>20</td>
<td>127</td>
</tr>
<tr>
<td>Spinco L rotor No. 40</td>
<td>40,000</td>
<td>105,000</td>
<td>100</td>
<td>2430</td>
</tr>
<tr>
<td></td>
<td>40,000</td>
<td>105,000</td>
<td>120</td>
<td>2920</td>
</tr>
</tbody>
</table>

The ground yeast was suspended in distilled water (4 ml. per gm.) and stirred 30 to 60 minutes. The suspension was centrifuged at \(t \times P_i = 1.2 \times 10^8\) to sediment alumina, whole cells, and coarse débris. The supernatant fluid, called the yeast extract, was lyophilized. Portions of this extract were resuspended in distilled water for further centrifugation.

**Preparation of Nucleoprotein by pH Adjustment**—For comparison with the centrifugal fractions, a nucleoprotein material was extracted by stirring ground yeast with water for 2 hours, maintaining the pH at 7.0 with sodium hydroxide. The suspension was centrifuged at \(t \times P_i = 97 \times 10^8\). The pH of the supernatant fluid was adjusted to 4.0 with hydrochloric acid, stirred for 1 hour, and centrifuged as before. The pellet was suspended in water, the pH adjusted to 7.0, and the process repeated. The solution was dialyzed 17 hours against flowing glass-distilled water with stirring, and was then lyophilized. All operations were carried out at about 4°.
Results

Centrifugation of the ground yeast and alumina at $t \times P_i = 1.2 \times 10^8$ gives a sediment containing the alumina and whole cells and coarse débris. This fraction is referred to as the initial residue. Centrifugation at $t \times P_i = 20 \times 10^8$ gives a rather small, buff-colored, opaque pellet consisting of microscopically visible particles. $t \times P_i = 2920 \times 10^8$ yields a reddish brown translucent pellet which contains very few particles which are visible with the light microscope. The supernatant fluid is very faintly cloudy and has a whitish floating layer which mixes readily with the body of the liquid. It has not been possible to separate this floating layer cleanly. The distribution of components in the centrifugal fractions in a

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of Material in Centrifugal Fractions of Yeast</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Centrifugation conditions, $t \times P_i (\times 10^8)$</th>
<th>Per cent of component of original dry sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t \times P_i$</td>
<td>Dry weight</td>
</tr>
<tr>
<td>Initial residue*</td>
<td>1.2</td>
<td>58</td>
</tr>
<tr>
<td>Low speed pellet</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>High “ “</td>
<td>2920</td>
<td>10</td>
</tr>
<tr>
<td>Supernatant fluid</td>
<td>2920</td>
<td>24</td>
</tr>
</tbody>
</table>

* The values are determined by the difference between the ground yeast suspension and the first supernatant fluid, since it is difficult to sample the alumina-containing sediment.

A typical experiment is given in Table III. The composition of the fractions is given in Table IV.

The composition of the nucleoprotein material isolated by pH adjustment is shown in Table IV. The yield of nucleoprotein was 1.8 per cent of the yeast (dry basis) and it contained 6.1 per cent of the total yeast RNA.

Effects of Storage—The problem of storage is critical because it is impossible to do all the required analyses immediately while continuing the fractionation scheme. The variables introduced by different degrees of cell breakage, possible differences in stirring efficiency, etc., preclude the use of different fresh preparations for each analysis in a comparative series.

Lyophilizing was chosen as the method least likely to affect the materials under study, although loss of solubility of small particle fractions upon freezing and drying was noted by Claude (21). Pirie (22) found that freezing whole tobacco leaf caused a lowered yield of nucleoprotein. Lyophihized yeast fractions were found to contain 2.6 to 4.6 per cent moisture,
as determined by drying at 110°, 30 mm. of Hg pressure, for 5 hours. This amount of moisture may be sufficient to permit significant enzyme action to occur. Such action, however, is very much slower than with the moist material. For example, in a fresh sample of the nucleoprotein prepared by pH adjustment, less than 5 per cent of the nucleotide material was soluble at pH 4. After storage of the lyophilized sample at 4° for 1 year, about 20 per cent of the nucleotide was soluble at pH 4. After storage in water solution at 4° for 1 week, the acid-soluble nucleotide was 66 per cent.

**TABLE IV**

**Composition of Fractions from Yeast**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Centrifugation conditions $t \times P_i \times 10^8$</th>
<th>Per cent of dry weight of fraction</th>
<th>Weight ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total N</td>
<td>Total P</td>
</tr>
<tr>
<td>Ground yeast suspension.</td>
<td>1.2</td>
<td>9.6</td>
<td>1.16</td>
</tr>
<tr>
<td>Extract (supernatant) .......</td>
<td></td>
<td>10.1</td>
<td>1.14</td>
</tr>
<tr>
<td>Low speed pellet .............</td>
<td>20</td>
<td>4.9</td>
<td>0.75</td>
</tr>
<tr>
<td>High “ ” “ ” .................</td>
<td>2920</td>
<td>11.2</td>
<td>2.40</td>
</tr>
<tr>
<td>Supernatant fluid ............</td>
<td>2920</td>
<td>11.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Nucleoprotein prepared by pptn. at pH 4, solution at pH 7 ...............</td>
<td></td>
<td>14.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Calculated on the basis of 9.5 per cent P in RNA.
† Calculated as before; the remaining P is the phosphate of acid-soluble nucleotide.

**DISCUSSION**

The method of grinding was one of the chief difficulties in this study. The grinding must be vigorous in order to break the tough cell walls that internal structures may also be disrupted, a problem which has been emphasized by others (7, 23). Also, the incomplete cell breakage makes it difficult to study the coarse débris fraction, especially since alumina is also present. More complete cell breakage could be obtained with improved grinding, but the problem of concomitant breakage of internal structure offers no simple solution.

It is apparent from the data in Tables III and IV that there is a concentration of RNA in the submicroscopic particle fraction. The unbroken cells and coarse débris, sedimentable at $t \times P_i = 1.2 \times 10^8$, included 58 per cent of the total RNA. Of this quantity, thirty-two parts would be considered held in unbroken cells on the basis of microscopic count, the remaining twenty-six parts being held with the débris.

It is possible that most of the RNA in the coarse débris is in submicro-
scopic particles held to larger structures by weak attachment. These attachments, once broken, would allow the ribonucleoprotein to appear in the small particle fraction, without noticeable addition to the large particle fraction. It is also possible that most of the RNA in the coarse débris fraction is an integral part of cell walls or other large structures.

The composition of the nucleoprotein material isolated by pH adjustment is similar to that of the submicroscopic particle fraction. Phosphate determinations of pellet and supernatant fractions during the preparation by pH adjustment indicated that both aggregation (failure to redissolve at pH 7) and disruption (failure to precipitate at pH 4) during manipulation contributed to the low yield.

Additional studies of the properties of the various fractions are being pursued.

SUMMARY

Bakers' yeast was ground with levigated alumina and extracted with water. The ground yeast was separated centrifugally into four fractions. The ribonucleic acid was concentrated in two fractions, the submicroscopic particles and the coarse débris. Intermediate size particles and the supernatant fluid were relatively poor in ribonucleic acid. A ribonucleoprotein material was isolated by precipitation at pH 4 and solution at pH 7. Its composition resembled that of the submicroscopic particle fraction.

In connection with the determination of the RNA content of centrifugal fractions and of the ribonucleoprotein, the "apparent RNA" arising from thymus DNA subjected to acid precipitation and alkaline hydrolysis treatments was determined. The "apparent RNA" is a function of the acid used and of the temperature during the necessary contact time.

BIBLIOGRAPHY

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