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The Preparation from Baker's Yeast of Yeast Nucleic Acid Containing a High-Polymer Fraction

G. JUNGNER and L.-G. ALLGÉN

Biochemical Department, Karolinska Institutet, Stockholm, Sweden

Dielectric studies of a number of ribose nucleotides (RNA) have led to attempts to produce more highly polymerized specimens with a view to establishing the dielectric characteristics of the RNA molecule.

In the preparation of RNA from yeast, difficulties are encountered both in the extraction and in the separation of the protein impurities. The methods applied are based, as a rule, on the use of alkalis, heat, or mechanical disintegration followed by extraction with salts; or on combinations of these processes. RNA is sensitive to such treatment, however, and rarely yields highly polymerized specimens. Earlier methods have usually involved treatment with alkali in order to split the very firmly bound nucleic acid-protein complex. If heat is used in addition, the specimens obtained are very pure as judged by their low protein content, but they show evidence of depolymerization. Treatment with salts, which may appear to be very mild, also has a disintegrating effect [1].

A number of relatively highly polymerized components are found in commercial specimens, despite the fact that their production is based on treatment with alkali under heat. We varied the concentration of alkali and the heat treatment and found that it was possible to produce specimens with a higher content of highly polymerized RNA than the commercial specimens. By varying the alkali concentrations (between 0.01 and 0.05 N NaOH) and using different boiling times, from just bringing to the boil to one hour or more boiling, fractions were obtained with a molecular weight of up to 15 000 to 20 000. Such fractions can be obtained in several ways. The nature of the specimens varies chiefly in respect of the protein content, since milder treatment results in considerably greater contamination with protein. This, how-

ever, can be reduced by subsequent treatment with chloroform and amyl alcohol according to the method introduced by Sevag, Lackman and Smolens [2].

The specimens obtained after heating are considerably more polydisperse. We found it most satisfactory to use relatively strong alkali at low temperature, although this method could be questioned. In principle it leads to the procedure indicated by Johnson and Harkins [3], which has also been used by Chantrenne [4]. In addition, it has been found suitable for the production of pentose polynucleotides of other origins [5]. By adopting a modified procedure we obtained a specimen containing a relatively well-defined high-polymer fraction of RNA with a molecular weight of 60 000—70 000. In view of this fraction's high molecular weight, considerably above that found earlier in specimens from yeast, it can be studied dielectrically. The high-polymer part of the specimen represents only some 10 per cent. Nevertheless its properties — which interest us mainly in respect of its structure from a dielectric point of view — are so outstanding that we have hitherto confined ourselves to characterizing this fraction.

It should be stated, however, that the attempts to separate and isolate the high-polymer molecules with the acid precipitation indicated by Levene [6] only resulted in the elimination of very low components.

Another procedure, which also gives better results than the methods used for commercial production, involves alkali treatment and extraction with picric acid (Levene and La Forge [7]). It gives a fairly well-defined fraction with a molecular weight of approximately 30 000.

There is no reason to describe our study of the different methods of production, since it was found that an essential point in the preparation of high-polymer specimens is to proceed rapidly. Generally it may be said that irrespective of the variations of method, the results depend essentially on the length of time to which the specimen is exposed to the possibility of decomposition. It has therefore been a matter of weighing the respective advantages of a specimen with a high degree of polymerization and a moderate contamination by proteins and of a purer specimen having a lower degree of polymerization. Even the mild method of Sevag *et al.*[2] takes up so much time that the specimen becomes depolymerized.

A very good analogy to the pronounced instability of RNA is furnished by the investigations made by Cohen and Stanley [8] on polynucleotides isolated from the tobacco mosaic virus. The limited stability which they reported agrees very well with our experience of the high-polymer fraction in yeast, although the latter appears to be even more labile. It is surprising that even in a relatively dry condition, with a 10—15 per cent moisture content, the high-polymer fraction of the specimen is destroyed in a few weeks at room tem-

perature. Such depolymerization also takes place at low temperatures and with specimens as free from moisture as possible. In fact, we found it impossible to work with any but freshly prepared specimens.

It is not possible to determine the extent to which the instability is due to an enzymatic action or to a spontaneous tendency to depolymerization. It appears probable that enzyme action is greater during extraction but that later the specimen is itself unstable, possibly after it has ceased to be bound to the proteins.

We therefore tried to find a simple and reproducible method. The following modification of Johnson and Harkins' method [3] was found serviceable. Growing baker's yeast, in portions of 100 g, with a moisture content of approximately 70 per cent, is washed by suspension in distilled water and centrifuged until a colourless supernatant is obtained. The yeast suspension is cooled to approximately 0° C and treated for two hours with NaOH in 5 per cent solution. The suspension is then neutralized with acetic acid to pH 6. Hyflo Super Cel, 5 per cent by weight, is suspended in the solution which is then suction-filtered. After acidifying with HCl (Congo), the filtrate is precipitated with 4 volumes of ethanol. The precipitate is centrifuged off and dissolved by addition of 0.1 *N* NaOH. The solution is again treated with Hyflo and filtered. The pH is adjusted to 6.0 and the solution precipitated with 4 volumes of ethanol, after which the preparation is dried with ethanol and ether. Freezing-drying is sometimes used. The entire procedure is carried out at a low temperature, around 0° C, and is completed in the course of 12 hrs.

The specimens obtained by this procedure are not entirely protein-free but appears from analysis and the biuret test to contain approximately 5 per cent of protein. In some experiments, the specimens were further treated according to the procedure of Sevag *et al.*[2] but it was found extremely difficult to remove all the proteins. Destruction of part of the high-polymer fraction generally occurs at the same time, and in any event the specimens are less homogeneous. On the other hand, it is profitable to submit the specimen to dialysis for a short time. Using cellophane it is found that a number of low-polymer nucleotides are removed even in a few hours.

The method suggested involves the use of strong alkali, and the question arises as to how far this affects the high-polymer fraction. A report on this point will be submitted in another paper . For the present, we would merely stress that the high-polymer fraction has a high polarity. It is readily digestible with crystalline ribonuclease prepared according to Kunitz and is entirely unaffected by desoxyribonuclease.

SUMMARY

A comparative study was made of different earlier methods for the production of yeast nucleic acid from baker's yeast.

Specimens with a small proportion of relatively highly polymerized fractions can be obtained in several ways. The most advantageous method involves short treatment at low temperature with relatively strong alkali.

A specimen with a relatively monodisperse, highly polymerized fraction having a dielectric molecular weight of approximately 65 000 is obtained by a modification of Johnson and Harkins' method.

The high-polymer fraction is very unstable even when in air-dry condition with a low moisture content. The preparation must be performed rapidly and the investigations made on freshly prepared specimens.

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