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Yeast Functional Analysis Report

Rapid and reliable protein extraction from yeast

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Abstract

The methods currently used for protein extraction from yeast are either laborious or insufficiently reliable. Here I report a method for protein extraction for electrophoretic analysis that is both easy and reliable. In this method, yeast cells are subjected to mild alkali treatment and then boiled in a standard electrophoresis loading buffer. The method was tested for different strains of *Saccharomyces cerevisiae* and for yeast *Hansenula polymorpha* DL-1. It yields virtually complete extraction independently of the strain, growth conditions and protein molecular weight and allows working with small amounts of yeast cells grown on agar plates. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: yeast; protein extraction; SDS–PAGE

Introduction

The preparation of extracts of yeast proteins is a frequent procedure in yeast research. However, the methods for preparation of such extracts are either tedious or not sufficiently reliable. Lysis with glass beads (Conzelmann *et al*., 1988) is reliable, but fairly laborious, especially when dealing with large number of samples. Extraction by boiling in optimized SDS–PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) sample buffer (Horvath and Riezman, 1994) is quick, but works poorly with cells grown on minimal media, stationary cells, and gives a reduced yield of high molecular weight proteins. Extraction with NaOH and β-mercaptoethanol (Riezman *et al*., 1983) has to some extent the disadvantages of both previous methods. Here, I present a method that is both quick and reliable. This method involves brief alkali treatment of yeast cells followed by a standard 3 min boiling in the SDS–PAGE sample buffer.

Materials and methods

The yeast strains used in this study were *S. cerevisiae* 5V-H19 (*MAT*α *ura3 leu2 ade2-1 SUQ5;* Ter-Avanesyan *et al*., 1994) and *H. polymorpha* DL-1 (ATCC 26012). Yeast cells were grown on liquid or solid YPD (1% Yeast Extract, 2% peptone, 2% glucose) or minimal medium (0.7% Yeast Nitrogen Base, 2% glucose and appropriate amino acids) to 1–2 OD_{600} or to stationary phase (5–9 OD_{600}).

For the novel extraction protocol, designated here as post-alkaline extraction, about 2.5 OD_{600} (which constitutes about 2.3 mg of wet weight) of yeast cells were harvested by centrifugation from liquid culture or scraped off the agar plate using a bacteriological loop. These cells were resuspended in 100 µl distilled water, added 100 µl 0.2 M NaOH, incubated for 5 min at room temperature, pelleted, resuspended in 50 µl SDS sample buffer, boiled for 3 min and pelleted again. About 6 µl supernatant was typically loaded per lane of mini-gel (Bio-Rad Mini-Protean cell). The sample buffer (0.06 M Tris–HCl, pH 6.8, 5% glycerol, 2% SDS, 4% β-mercaptoethanol, 0.0025% bromophenol blue) was slightly modified from standard (Laemmli, 1970). It should be noted that the extraction efficiency did not noticeably depend on the SDS and β-mercaptoethanol concentration of the sample buffer in the ranges 1–3% and 2–5%, respectively.

Glass beads lysis (Conzelmann *et al*., 1988) and rapid protein extraction in optimized SDS

sample buffer (Horvath and Riezman, 1994) were performed as described. SDS–PAGE was performed according to Laemmli (1970).

Results and discussion

Development of the method was started as an attempt to improve the protocol of Riezman *et al.* (1983), in which the extraction of proteins is achieved by 10 min incubation in 0.2 M NaOH and 0.5% β-mercaptoethanol, followed by precipitation of the extracted proteins with trichloracetic acid (TCA), dissolution in SDS–PAGE sample buffer, and boiling. The acid precipitation and subsequent dissolution of proteins were the most tedious steps of this procedure, so I tried to replace the precipitation by titration of alkali with equivalent amounts of HCl, together with reduction of initial alkali concentration. The reduction of alkali to 0.1 M did not reduce the yield of protein and, surprisingly, the presence of β-mercaptoethanol in the alkaline solution proved to be unnecessary. However, one important difference was found. In the original method, proteins were released from cells by the combined action of alkali and β-mercaptoethanol. In contrast, the alkali (0.1 M NaOH) alone did not release any proteins even after 30 min incubation (Figure 1), but greatly enhanced the protein extraction during subsequent boiling in SDS–PAGE sample buffer. These observations allowed removing the alkali from treated cells by centrifugation, thus avoiding any use of acid. In this way, the new extraction method was formulated, as described in Materials and methods.

To optimize the method, the influence of the duration of alkali treatment was tested (Figure 1). For more rigorous testing, stationary phase cells were used, which should have more robust cellular envelopes. For the maximum extraction, 5 min alkaline incubation was sufficient, while after 2 min incubation the extraction was almost complete for all proteins except those exceeding 100 kDa. For exponentially growing cells, 2 min incubation was sufficient for complete extraction (not shown). Carrying the alkaline incubation on ice was also tested, but this only slowed the process: the complete extraction required about 10 min incubation. It is worth noting that under some growth conditions the 5V-H19 strain produces low molecular weight red pigment, related to the block

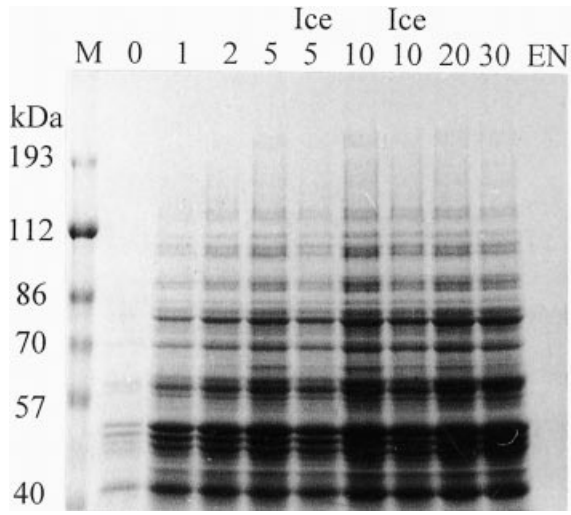

Figure 1. Optimization of the alkaline pretreatment. *S. cerevisiae* 5V-H19 cells were grown in liquid YPD to stationary phase, harvested, suspended in 0.1 M NaOH and incubated at room temperature or on ice, where indicated. After the time in minutes indicated at the top, aliquots were taken and used for protein extraction, as described in Materials and methods. 0, no NaOH incubation; EN, proteins extracted to NaOH after 30 min incubation. All samples represent equal amount of cells. M, molecular weight marker (Sigma cat. SDS-7B)

of the adenine synthesis pathway. This pigment was extracted to NaOH with about 30% efficiency in 5 min, while no protein extraction to NaOH occurred even after 30 min incubation.

The extraction efficiency of this method was compared to that of glass beads lysis (Conzelmann *et al.*, 1988) and boiling in optimized sample buffer (Horvath and Riezman, 1994; Figure 2). The post-alkaline method showed the same or slightly better extraction than the glass beads method and was clearly superior to the optimized boiling method. This experiment was performed using stationary phase cells, while for exponentially growing cells the difference with the optimized boiling method was less significant (not shown). The NaOH/β-mercaptoethanol method of Riezman *et al.* (1983) was not compared, but it was observed earlier that this method was inefficient towards cells grown on minimal media (Horvath and Riezman, 1994). This appears strange, since in this method the alkali-treated cells are present during boiling in SDS–PAGE sample buffer. A possible explanation is that the TCA precipitation somehow fixes

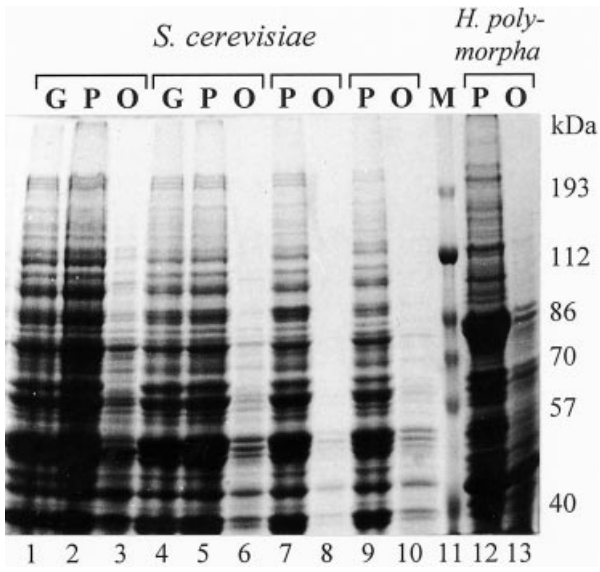

Figure 2. Comparison of different methods for protein extraction. *S. cerevisiae* 5V-H19 cells were grown to stationary phase in liquid YPD (lanes 1, 2, 3), in liquid minimal medium (lanes 4, 5, 6), on YPD agar plates (lanes 7,8), or on minimal agar plates (lanes 9, 10). *H. polymorpha* DL-1 cells were grown on YPD plate (lanes 12, 13). Proteins were extracted using either the glass beads method (G), post-alkaline extraction (P), or extraction in optimized SDS sample buffer (O). Equivalent amounts of cells were used for extraction in each comparison group. M, molecular weight marker (lane 11)

proteins within cells, interfering with their extraction during subsequent boiling in the sample buffer.

The post-alkaline method appears to yield virtually complete extraction of proteins, since no proteins could be extracted by any method from the cells, already extracted by this method (not shown). The method was tried successfully with several haploid *S. cerevisiae* strains of different origin (shown only for 5V-H19) and with methylotrophic yeast *H. polymorpha* (Figure 2). The samples obtained by this method were suitable for Western blotting. In our laboratory, such samples were routinely stained for the Sup45 (50 kDa) Sup35 (80 kDa) and Hsp104 (100 kDa) proteins, reproducibly giving high signal intensity.

It should be noted that yeast cells survive 5 min treatment with 0.1 M NaOH. To test this, the treated cells were plated on YPD plate, where most of them produced colonies. Therefore, such incubation could affect the levels of some proteins (or their modification states). For example, some increase in heat shock proteins may be expected. Normally, no significant effects of this kind would occur in 5 min. Even 30 min incubation did not change the general appearance of protein sample (Figure 1). I did not observe any significant alterations in the levels of heat shock Hsp104 protein and Sup35, judging by comparison with glass beads extraction. However, if such effects are of concern, the duration of NaOH incubation may be reduced. Two-min incubation is sufficient for exponential or early stationary cells, and further reduction may be achieved using 0.3 M NaOH instead of 0.1 M. It is also advisable to minimize the time between the alkaline incubation and boiling in the sample buffer.

The method allows to simplify not only the protein extraction, but also the growth of yeast samples. It allows using small amount of cells, grown on agar plates, thus obviating the need for liquid culture. Typically, a yeast streak of 2×10 mm in size is sufficient to prepare a protein sample for several loadings on mini gel. Up to several tens of yeast samples can be grown on a single Petri dish. A big or even medium-sized yeast colony can give enough material for Western blot analysis, which usually requires less protein than Coomassie Blue staining. Such an approach would not be possible with the glass bead method, which requires a relatively high minimum amount of cells for the efficient extraction, or with the optimized boiling procedure, which does not work efficiently with plate-grown cells.

The mechanism by which mild alkali enhances protein extraction is not clear. However, it should be noted that the yeast cell wall includes a group of proteins, which are not extracted by SDS and β-mercaptoethanol, but may be extracted under mild alkali conditions (30 mM NaOH at 4°C overnight; Mrsa *et al.*, 1997). These proteins belong to, or are related to, the Pir family (proteins with internal repeats). They are highly *O*-glycosylated and their *O*-linked saccharides are covalently linked to other cell wall components, most likely β1,3-glucans. Under alkaline conditions, *O*-chains tend to be cleaved off in a process called beta-elimination. Thus, the liberation of the *O*-glycosylated proteins by alkali may be one of the causes for the observed effect.

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