YEAST VOL. 5: 51–53 (1989)

Extraction and Rapid Inactivation of Proteins from *Saccharomyces cerevisiae* by Trichloroacetic acid Precipitation

ANTHONY P. H. WRIGHT*, MICHAEL BRUNS† AND BRIAN S. HARTLEY

Centre for Biotechnology, Imperial College of Science and Technology, London SW7 2AZ, U.K.

Received 20 May 1988

Methods currently used for the extraction of proteins from yeast involve relatively long time periods between sampling cells from a culture and analysis of their proteins by polyacrylamide gel electrophoresis–sodium dodecylsulphate. Often it is desirable to inactivate cellular metabolism rapidly after sampling and here we show that trichloroacetic acid precipitation techniques, often used for rapid extraction and inactivation of proteins from higher eukaryotes, can be adapted for use with organisms which have cell walls.

KEY WORDS — Yeast; protein; extract; trichloroacetic acid.

INTRODUCTION

The cell wall of yeast has always presented a barrier to the extraction of proteins and other macromolecules from the cell. To date this problem has been overcome either by physical disruption, with glass beads for example, or by treatment with cell wall degrading enzymes. Both are designed to maintain as many enzyme activities as possible but have the disadvantage that relatively long treatment times are needed after sampling the cells before the extract is amenable to analysis. During this time period many normal and doubtless abnormal cellular processes remain active leading to potential degradation or modification of proteins during preparation of the extract. For many experiments involving analysis of proteins by PAGE-SDS, Western blots or autoradiography, active extracts are not required and often it is important to inactivate cellular metabolism as quickly as possible after sampling. *Escherichia coli* samples can be prepared rapidly for electrophoresis by boiling cells in gel loading buffer (Mole and Lane, 1987) and a similar technique has also been reported for yeast (Hitzeman *et al.*, 1983) but we show here that yields are very low and may not represent all proteins equally. Samples from cells without cell walls or viruses can be prepared rapidly by precipitation with trichloroacetic acid (TCA) (Bruns *et al.*, 1986) but such techniques have not been reported for yeast. In this short report we show that TCA precipitation can be adapted to cause sufficient cell wall degradation to allow extraction of proteins from yeast.

Present addresses: *Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, F69 S-141 86 Huddinge, Sweden. †Heinrich-Pette-Institut für Experimentale Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, F.R.G.

0749–503X/89/010051–03 $05.00

MATERIALS AND METHODS

Strains and media

The yeast strain used was MC16, *MATa leu2 ade2 lys2 his4 SUF2* (Beggs, 1978). Media were according to Sherman *et al.* (1981).

Preparation of TCA-precipitated extracts

A known number of cells were sampled direct from growing cultures and added to an equal volume of ice-cold 20% TCA (samples of up to 0·7 ml can conveniently be treated in 1·5 ml microfuge tubes), mixed and incubated on ice for 60 min. The cells were then harvested by centrifugation at 12 000 × *g* in a microfuge at 4°C for 10 min. The supernatant was discarded and the pellet resuspended in 1 ml of ethanol at −20°C prior to incubation on ice for 30 min. The cells were then pelleted as before and the ethanol precipitation was repeated. After harvesting for the final time the pellets were dried briefly under vacuum to remove most

of the residual ethanol before resuspending in gel loading buffer (100 mM-Tris/HCl, pH 7·0, 10% glycerol, 4% SDS, 5% beta-mercaptoethanol, 0·02% bromophenol blue) at a concentration of 10^7 cells worth of extract per 20 µl. This was incubated in a boiling water bath for 5 min after which any residual cell debris was removed by centrifugation for 2 min in a microfuge, 20 µl of extract per gel slot was electrophoresed by standard PAGE-SDS techniques (Laemmli, 1970). This loading is sufficient for subsequent staining with Coomassie blue, immunoblotting or autoradiography.

RESULTS AND DISCUSSION

Figure 1 shows the PAGE-SDS profiles of TCA-precipitated proteins (lanes 1–3) compared to a glass bead preparation (lane 4) and to a preparation in which cells were incubated at 100°C in loading buffer for 5 min prior to loading (lane 5). Each lane contains 10^7 cells worth of extract. The strain was MC16 grown to a density of $2{\cdot}3 \times 10^7$ cells/ml in YPD. The cell numbers in each of the samples for TCA precipitation were adjusted so that each initial 0·5 ml contained 10^7 (lane 1), 5×10^7 (lane 2) and 10^8 (lane 3) cells. They were then treated identically, as described in Materials and Methods, until finally resuspending in loading buffer, when the volumes were adjusted to make the original cell concentrations the same. The three preparations demonstrate that the efficiency of extraction is independent of cell density and that it is equivalent to that obtained with glass beads. Comparison of these extracts with the extract produced by boiling the cells in loading buffer (lane 5) demonstrates the importance of the TCA and ethanol precipitations. The treatment time in TCA can be varied according to the condition of the cells. For exponentially growing cells on YPD, 20–30 min may be sufficient, whereas stationary phase cells require longer (data not shown).

We have used this method in experiments to determine the rate of incorporation of ^{32}P and of ^{14}C-amino acids into yeast proteins. Only small numbers of cells are required so that culture volumes and therefore the amount of radioactive isotope can be kept small. We have also successfully used these extracts for immunoblotting techniques although we have found that electroblotting times should be increased to about double those normally used since proteins which have been TCA precipitated appear to transfer more slowly.

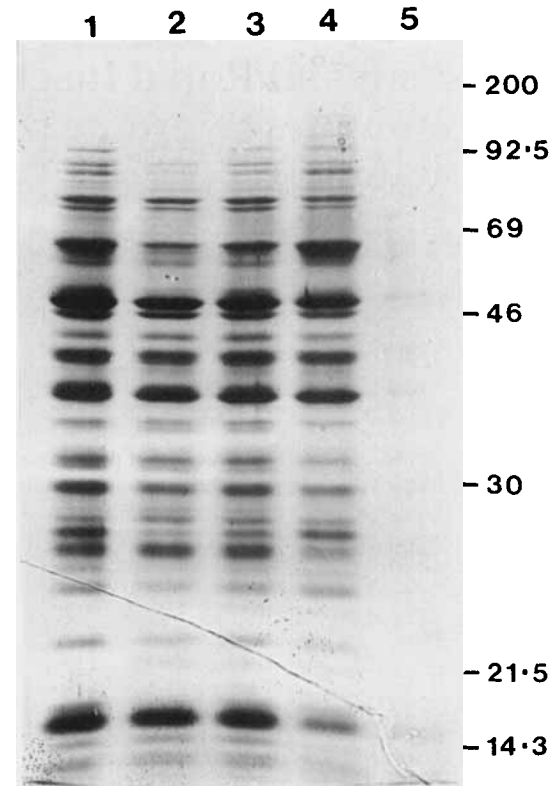

Figure 1. PAGE-SDS of TCA-precipitated protein extracts from yeast cells stained with Coomassie blue. Lanes 1–3 show the profiles of proteins TCA precipitated from cell suspensions at different cell densities (see text). Lane 4 contains an equivalent amount of an extract prepared by glass bead extraction in which cell breakage was greater than 95%. Lane 5 shows an extract produced from the same number of cells by heating at 100°C for 5 min in gel loading buffer. The migration of molecular weight standards (Amersham, no. RPN.756) is shown (kD).

ACKNOWLEDGEMENTS

We gratefully acknowledge support from the Science and Engineering Research Council (A.W.) and the Commission of the European Community (M.B.)

REFERENCES

Beggs, J. (1978). Transformation of yeast by a replicating plasmid. *Nature (London)* **275,** 104–108.

Bruns, M., Zeller, W., Rohdewohld, H. and Lehmann-Grube, F. (1986). Lymphocytic choriomeningitis virus. IX. Properties of the nucleocapsid. *Virology* **151,** 77–85.

Hitzeman, R. A., Chen, C. Y., Hagie, F. E., Patzer, E. J., Liu, C.-C., Estell, D. A., Miller, J. V., Yaffe, A., Kleid, D. G., Levinson, A. D., Oppermann, H. (1983).

Expression of hepatitis B surface antigen in yeast. *Nucleic Acids Res.* **11,** 2745–2763.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227,** 680–685.

Mole, S. E. and Lane, D. P. (1987). Production of monoclonal antibodies against fusion proteins produced in *E. coli*. In Glover, D. M. (Ed.). *DNA Cloning III: A Practical Approach*. IRL Press, Oxford/Washington, pp. 113–139.

Sherman, F., Hicks, J. and Fink, G. (1981). *Yeast Molecular Genetics Course Manual*. Cold Spring Harbor Publications, Cold Spring Harbor, New York, U.S.A.