0273-2289/91/3101—0043$02.00

Hydrolysis of Nucleic Acids in Single-Cell Protein Concentrates Using Immobilized Benzonase

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Received February 2, 1991; Accepted March 20, 1991

ABSTRACT

Hydrolysis of nucleic acids for single-cell protein concentrates has been carried out in one step using immobilized benzonase on corn cob. The immobilization is carried out by tosylation of primary alcohols of cellulose of corn cob. The immobilized benzonase is more stable vs pH changes than native benzonase, but the same optimum values of [Mg(II)] and temperature are obtained. The DNase activity is greater than the RNase activity. The percentage of DNA is reduced to 3–6% and that of RNA to 50%. The protein loss is negligible (1%). The enzymatic activity per weight unit of enzyme is greater in the case of benzonase that in reported data for other nucleases insolubilized on corn cob by the same procedure.

Index Entries: Benzonase; hydrolysis of nucleic acids; single-cell protein concentrates; immobilization of enzymes.

INTRODUCTION

Nucleases can be used to purify, from nucleic acids, proteins obtained by genetic engineering, and to obtain single-cell protein concentrates (SCP), free of nucleic acids, to be used in human diets. SCP can be obtained

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from bacteria, yeasts, fungi, and so on, and exhibits good nutritional properties. The protein level per dry biomass in SCP (50–70%) is higher than in foods, such as milk (25–30%), eggs (35%), and the like (*1,2*). Nevertheless, SCP have high levels of nucleic acids, specially RNA, (15–25% by dry biomass). This fact restricts the use of SCP in human diets because the ingestion of amounts of nucleic acids greater than 2 g daily increases the serum and urinary uric acid levels (*3*), giving secondary uricosuria. Therefore, the nucleic acids percentage must be reduced to $<2\%$.

Several methodologies have been used to carry out this diminution, e.g., acid and basic hydrolysis (*4,5*), and more recently, native exogenous nucleases (*6*). Recently, we have reported in this journal (*7*) that immobilized ribonuclease A and endonuclease *S. aureus* derivatives can be sequentially used to remove nucleic acids from SCP. The obtained reduction in nucleic acids amounts was very significant. Nevertheless, the process has some experimental difficulties owing to several facts; e.g., the most active enzyme—endonuclease *S. aureus*—that can hydrolyze RNA and DNA is very expensive and needs 10 m*M* [Ca(II)] to be activated, but this cation deactivates RNase A, an enzyme that only hydrolyzes a maximum of 80% of RNA. Thus, RNase A and endonuclease *S. aureus* cannot be coimmobilized to work in the same place. Therefore, these enzymes must be used sequentially. First, immobilized RNase A must be added to hydrolyze RNA in the SCP. Next, the enzymatic derivative must be removed and Ca(II) and immobilized endonuclease *S. aureus* added to the reactor to complete the hydrolysis of RNA and to hydrolyze DNA. Owing to the large amounts of RNA present in SCP, large amounts of endonuclease might be used if only this enzyme were used to hydrolyze all nucleic acids in SCP. This possibility is not useful from a practical point of view, because of the high cost of endonuclease *S. aureus*.

To avoid these experimental problems we have immobilized benzonase, a new enzyme obtained by genetic engineering that hydrolyzes DNA and RNA (*8*). The native enzyme has the optimum pH 8.0, and needs 2 m*M* [Mg(II)] to be activated. Benzonase has been immobilized by tosylation method on corn cob particles (*9*).

MATERIALS AND METHODS

Benzonase was from Merck. Yeast RNA sodium salt type IV and salmon testes DNA sodium salt type III were from Sigma.

Corn cob particles (EU-GRITIS 0.84–0.54 mm particle size, surface area 0.083 m^2/g) were kindly provided by P. Monsan (Bioeurope, Toulouse, France). Two different yeast cells were used as a source of SCP. *Saccharomyces cerevisiae* ECC) from the spent residue in a fermenting reactor, from

El Aguila, S.A. (Cordoba, Spain). Lyophilized yeast powder extract *S. cerevisiae* type I (Catalog. No YSC-1) was from Sigma.

S. cerevisiae Samples

A lyophilized sample of YSC-1 (10 g) was suspended in 100 mL of sterile water and the mixture left at room temperature for 6 h and centrifuged at above 6000*g* for 5 min.

The ECC residue (50 mL) from beer-fermenting reactor was centrifuged at 6000*g* for 5 min. The liquid was removed, the cells were resuspended in 10 mL of sterilized water, and the mixture was recentrifuged for 5 min.

The yeast cells were broken in bidistilled water using a homogenizer (Braun Model MSK) with 0.25–0.30 mm glass beads (fron Braun Melsungen). Desintegration time was 2×1 min with 15 s intervals. The cell wall rupture was greater than 85%. The mixture was centrifuged and the supernatant was regarged as the extract of SCP.

Immobilization of Benzonase

The activation of corn cob was carried out according to the tosylation method previously described by the authors (*10*). The immobilization of benzonase was carried out for 3 h with slow stirring in the optimum buffer of the enzyme (pH 8.0) at 4°C.

Hydrolysis of Pure DNA and RNA

Hydrolysis of pure DNA and RNA was carried out in a methacrylate cylindrical batch reactor (34 mm inner diameter×78 mm high) independently. Three mL of DNA solution (0.5 mg/mL) or 1.5 mL of RNA solution (0.5 mg/mL) were added in 6 mL of buffer, pH 8.0, in different reactors. The mixtures were incubated at 37°C with 1 mL enzyme solution in the presence of a 2 m*M* [Mg(II)] and either substrate.

Samples of 25 μL were removed at different times and DNA and RNA hydrolysis were monitored by the increase in the absorbance at 260 nm using a UV-Vis spectrophotometer (Shimadzu model 2100).

Protein Determination

The amount of protein in the SCP samples was determined by the Lowry method (*11*), taking into account the indications of Peterson (*12*) regarding interfering compounds.

Nucleic Acid Determination

The Schmidt-Thannhauser procedure (*13*) was followed to separate DNA and RNA. DNA amount was determined by the diphenylamine

method (*14*), and RNA amount by difference between total amount of nucleic acids and DNA.

Hydrolysis of Nucleic Acids of SCP

The hydrolysis of nucleic acids of SCP was carried out in the methacrylate batch reactor, previously described. One mL of each extract (ECC or YSC-1) was added to the reactors independently with 19 mL of buffer, pH 8.0, 2 m*M* of Mg(II), and 1 g of insolubilized derivative of benzonase. The reaction was carried out at 37°C with stirring.

Samples of 1 mL were removed at different times and the percentage of RNA and DNA hydrolysis was tested by the difference between initial and final values of nucleic acids.

RESULTS

The immobilization of benzonase was carried out on a support whose activation degree was 3.9 μmol tosyl groups/g of support. The percentage of insolubilized enzyme was 14.25% and the remaining activity was 100%. These results were better than those obtained by other authors who tried to immobilize the enzyme on Sephacryl S-1000R by reductive amination (*15*).

Comparative Behavior of Native and Insolubilized Benzonase

If we compare the enzymatic activity of native and insolubilized enzyme, we can observe that the insolubilized enzyme is less sensitive to pH variation than native enzyme (Fig. 1a), but the optimum value of [Mg(II)] and temperature are similar in both cases (Fig. 1b and c). Therefore, we can say that the immobilization by tosylation method of benzonase on corn cob stabilizes the enzyme.

Hydrolysis of Nucleic Acids on SCP

The initial amount of protein, DNA, and RNA in SCP are shown in Table 1. We can observe that RNA is the main nucleic acid, as expected according to the literature (*2,16*). The YSC-1 sample shows higher values of RNA. This could be related to the preparation of this extract from lyophilized yeast rather than to the genetic characteristics of the cells. The values shown in Table 1 were chosen as 100% of DNA, RNA, and protein.

The hydrolysis of nucleic acids vs time is shown in Fig. 2. We can observe that the percentage of DNA hydrolyzed is greater than that of RNA. This has been observed in the hydrolysis of pure nucleic acids (*15,17*) and could be related to the intrinsic enzymatic activity of benzonase.

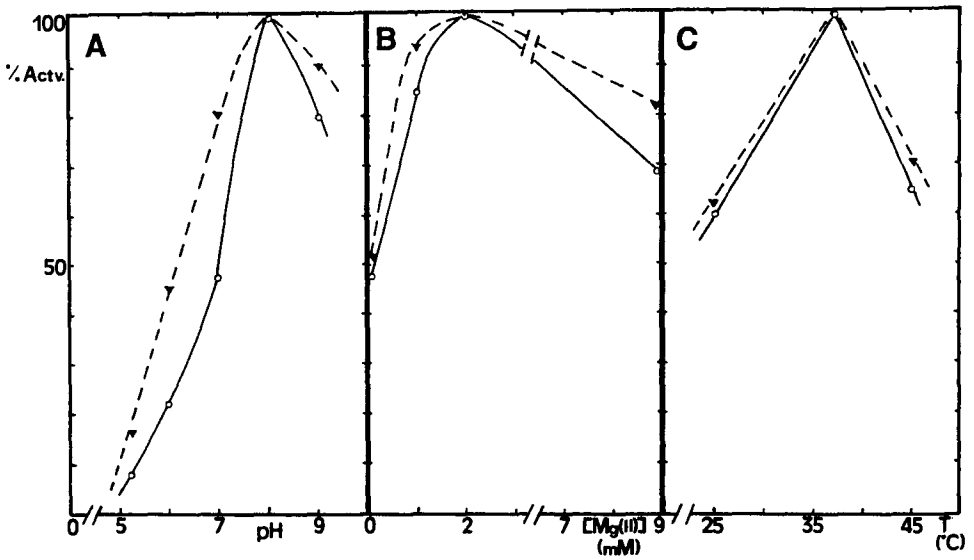

Fig. 1. Comparative behavior of native ○ and insolubilized ▲ benzonase on corn cob vs (a) pH, T=37°C; [Mg(II)]=2 m*M*; (b) [Mg(II)], T=37°C; pH 8.0; and (c) T, pH 8.0, [Mg(II)]=2 m*M*.

Table 1
Initial Amounts of Nucleic Acids and Proteins in the *Saccharomyces cerevisiae* Extracts

Yeast	RNA, μg/mL	DNA, μg/mL	Protein, mg/mL
YSC-1	3060	31.7	9.785
ECC	291	39.3	5.045

If we compare the enzymatic activity of immobilized enzyme in the absence of biomass (Fig. 3a) and in the presence of biomass (Fig. 3b), we can observe that enzymatic activity is lower in the second case than in the first. This might be related to the presence of a concentration of Mg(II) greater than 2 m*M* in the biomass that deactivates the enzyme, as it has been described earlier (Fig. 1b).

The excess of Mg(II) produces a conformational change that alters the active conformation of the enzyme. This fact has been detected by UV (*17*).

From Table 2 we can say that the hydrolysis of nucleic acids using immobilized benzonase reduce the percentage of nucleic acids to interesting values (<50%) without diminishing the percentage of protein (1%). Therefore, this treatment is useful for carrying out the hydrolysis of nucleic acids in SCP in mild conditions without alteration of proteins.

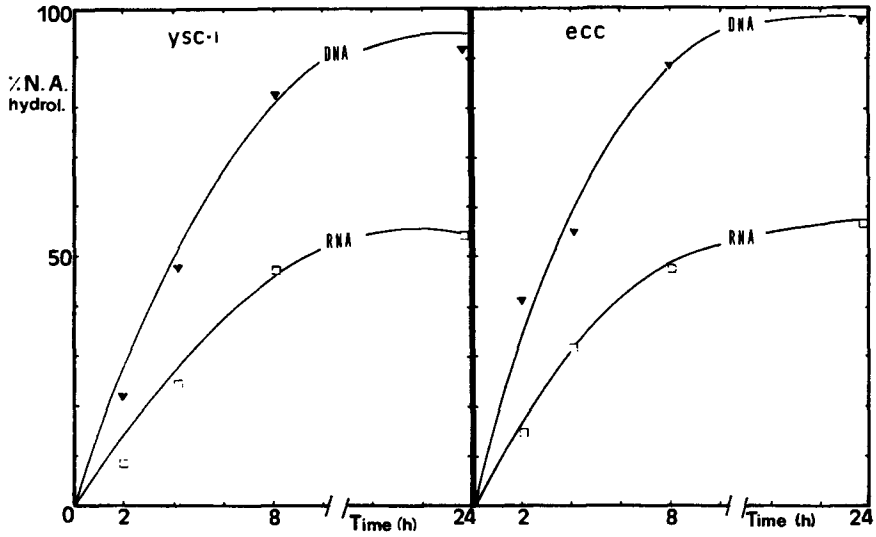

Fig. 2. Hydrolysis of nucleic acids of SCP. T=37°C; pH 8.0; [Enz]=0.56 μg/mL; [N.A.] in Table 1; V=20 mL.

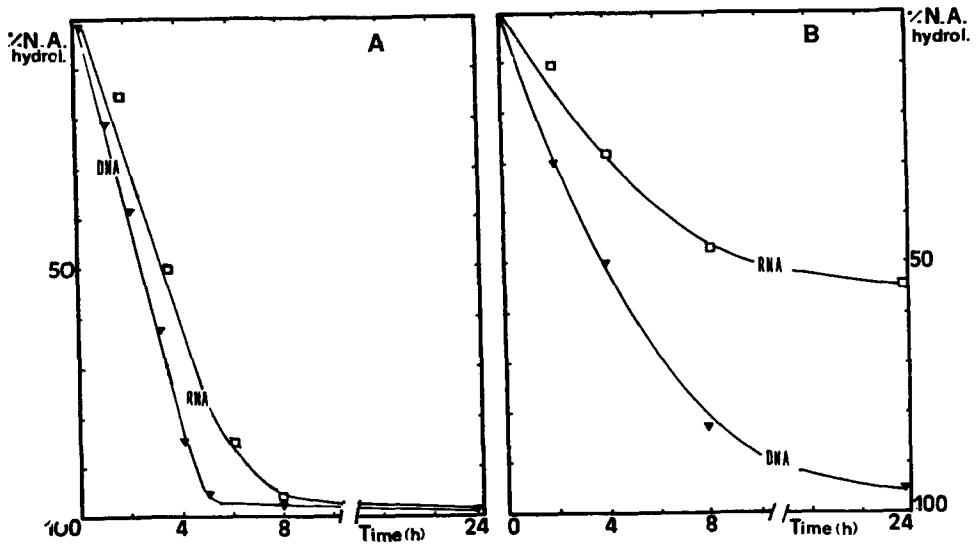

Fig. 3. a. Hydrolysis of pure nucleic acids. T=37°C; pH 8.0; [DNA]=130 μg/mL; [RNA]=65 μg/mL; [Enz]=0.5 μg/mL; [Mg(II)]=2 m*M*. b. Hydrolysis of nucleic acids in SCP from YSC-1. T=37°C pH 8.0; [DNA]=1.5 μg/mL; [RNA]=153 μg/mL; [Enz]=0.56 μg/mL.

Table 2
Percent of Hydrolyzed RNA and DNA
in SCP by Insolubilized Benzonase on Corn Cob

Yeast	Time, h	% Hydrolyzed RNA	% Hydrolyzed DNA	% Protein loss
YSC-1	8	47	81	—
YSC-1	24	53	94	1.1
ECC	8	49	88	—
ECC	24	56	97	1.1

T=37°C; pH=8.0; [Enz]=0.56 μg/mL; V_o=20 mL.

Finally, because Mg(II) does not inhibit RNase A, which presents an optimal pH 8.0, like benzonase, this enzyme could be coimmobilized with benzonase to provide an efficient biocatalyst to hydrolyze the nucleic acids in SCP. These results will be reported in the near future.

DISCUSSION

The immobilization procedure does not change the optimum amount of the cofactor 2 m*M* Mg(II) as in the case of endonuclease *S. aureus* (*7*), but the thermostability is not increased.

The main effect of the immobilization method is the stabilization vs pH (Fig. 1a). This fact has not been observed in the case of RNase A and endonuclease *S. aureus* (*7*).

The enzymatic activity of immobilized benzonase is greater vs DNA than vs RNA as in the case of native enzyme (*15,18*). This is the opposite activity to that observed with immobilized and native RNase A, which only hydrolyzed RNA (*7,9*). Because both enzymes are active at the same pH (8.0) and the Mg(II) does not strongly affect RNase A, both enzymes could be coimmobilized on corn cob particles by our tosylation method to provide an effective biocatalyst to diminish the total amount of nucleic acids of SCP.

Finally, we can observe in Table 2 that the protein loss produced by this procedure is negligible (1%). This percentage is lower than that reported by the authors using, sequentially, immobilized RNase A and endonuclease *S. aureus* (6%) (*7*), and lower than the data reported in the literature, e.g., native exogenous RNase A with SCP from *C. albicans*, 16% (*18*), or alkaline or acid hydrolysis (*19–21*).

On the other hand, the hydrolysis of nucleic acids is carried out to the same extent in both cases. This behavior is different from that observed in the case of endonuclease *S. aureus* (*7*), which presents different behaviors vs the nature of the hydrolyzed sample.

YSC-1 89% RNA hydrolyzed at 8 h.
ECC 69% RNA hydrolyzed at 8 h.

These values were obtained with 22.8 μg of enzyme (7) and the results obtained in the case of benzonase were obtained with 11.5 μg. Therefore, the enzymatic activity per microgram of enzyme is greater in the case of benzonase than in the case of endonuclease *S. aureus*. Therefore, the procedure described improves the previous procedure reported by the authors, because (i) only one hydrolysis step is carried out; (ii) the protein loss is lower with this new enzyme; and (iii) the enzymatic activity per microgram enzyme vs RNA is better in the case of benzonase than in the case of endonuclease *S. aureus*.

ACKNOWLEDGMENTS

This work has been supported by the Spanish C.I.C.Y.T. (Grant BIO 88-0241).

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