BINDING AND RELEASE FROM CELL WALLS: A UNIQUE APPROACH TO THE PURIFICATION OF AUTOLYSINS

W. Claiborne Brown

Department of Biology, University of California, San Diego
La Jolla, California 92037

Received April 10, 1972

SUMMARY

Autolysin is purified eleven-fold from 5 M salt extracts of intact cells by adsorption and elution from cell walls.

Several recent developments offer fascinating possibilities for studying the structure and function of autolysins. One is the finding that autolysins can be extracted from cell walls by strong salt solutions (1,2). Another is the finding in this laboratory that autolysins can be extracted from intact cells of several bacteria (submitted for publication). Since a major objective of this laboratory is to study the properties of purified autolysins, the latter development is considered particularly significant because of the potential for processing large quantities of enzyme. While extraction of intact cells is certainly a more efficient method of isolating autolysins, problems are encountered in purification because the extract contains material other than autolysin which absorbs strongly at 260 and 280 nm.

The finding first reported by Shockman *et al*. (3) that SDS-treated cell walls bind autolysin provided a possible solution to this problem. This report describes a preliminary purification procedure which incorporates the basic features of these isolated findings. The results show that the autolysin in whole cell extracts can be adsorbed and eluted from cell walls resulting in a modest increase in specific activity.

Cell walls were prepared and treated with boiling 2% sodium dodecyl

sulfate (SDS) as described previously (4). Bacillus subtilis SR22 was grown at 37 C with rotary shaking in trypticase soy broth (TSB;BBL) to late exponential phase (80-100 Klett units). Cells were harvested and stored at -70 C until used. Enzyme assays were performed using a Beckman DBG-T spectrophotometer. The decrease in absorbance at 600 nm was recorded from the 0-1A scale with the recorder set for 10 mv sensitivity. The reaction mixture contained the following in a total volume of 2.0 ml: 500 μM $MgCl_2$; 2.0 mg of cell walls and 50 mM tris (hydroxymethyl) aminomethane (tris), pH 8.0, and 0.2 ml of cell extract. One unit of enzyme causes a decrease in absorbance of 0.001/minute at 600 nm. Protein was estimated from the 260/280 ratio using a monograph prepared by California Biochemical Corporation.

The procedure for purifying autolysin from whole cell extracts is illustrated in Figure 1. Enzyme activity and absorbance at 260 and 280 nm were measured at each state of purification. Figure 2 shows that the substrate cell walls do not lyse when suspended in buffer. However,

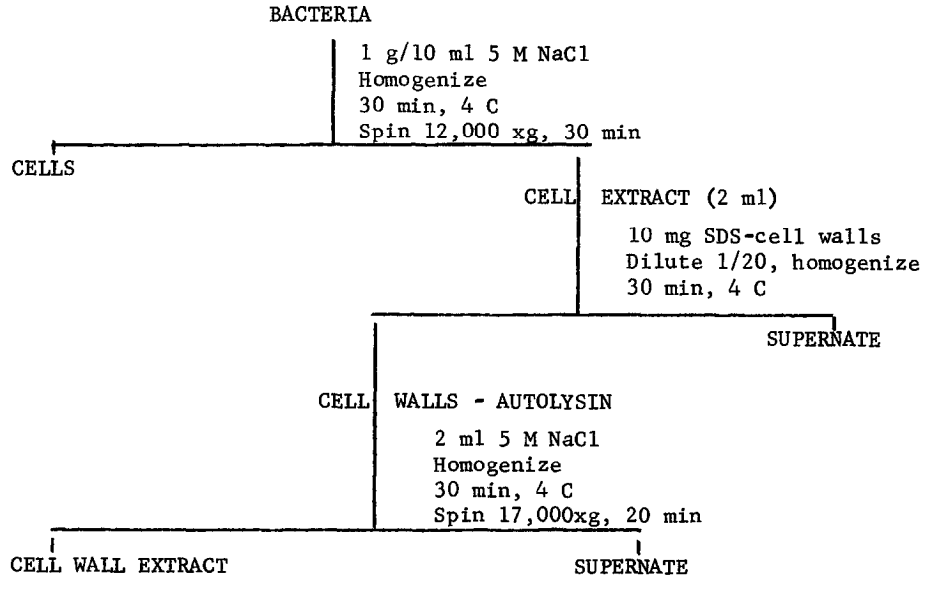

Figure 1. Flow sheet for purification of autolysin from whole cell extracts.

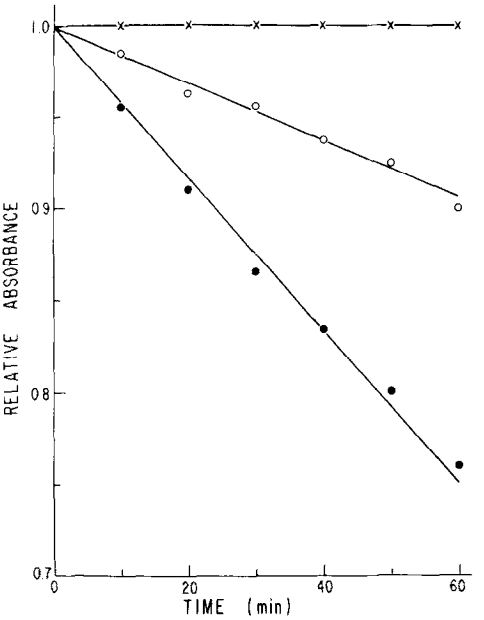

Figure 2. Autolysins of SDS-treated cell walls before and after binding of autolysin. The cell walls were treated as described in Fig. 1. x—x before binding; o—o after binding; •—• after removal of autolysin.

after mixing with whole cell extracts followed by dilution to 0.25 M salt, the cell walls bind autolysin as indicated by a dramatic change in slope. The autolysin is subsequently removed from the cell walls by three extractions with 5 M NaCl. Concomitantly, the rate of autolysis of the residual cell walls is decreased by a factor of 4.

The degree of purification achieved by this process is summarized in Table 1. By binding and release from cell walls the autolysin was

TABLE 1. Purification of autolysin from whole cell extracts

Step	Total Units	Total Protein (mg)	Specific Activity	Yield %
Cell Extract	451	17	26	100
Cell Wall Extract[a]	247	0.9	274	55

[a]The three salt extracts were combined and assayed for protein and enzyme.

purified approximately 11-fold with a 55% yield of activity. Many repetitions of this procedure yielded final purifications varying from 5- to 15-fold and recoveries of 35 to 65%. The procedure also resulted in some purification from UV absorbing material as indicated by the $A_{280/260}$ which changed from 0.63 to 0.78.

The procedure described represents another refinement in the purification of autolysins. While the bulk process presented in this report is suitable for processing small samples, the ultimate advantage of this technique will be realized if it can be adapted to column operations. Experiments are in progress to develop such a system.

ACKNOWLEDGEMENTS

This investigation was supported by National Science Foundation grant GB-30238X; a grant from the California Division, American Cancer Society 549; funds from a Ford Foundation grant 70-436 to Third College; and funds from Brown-Hazen fund of the Research Corporation for the purchase of the Beckman DBG-T spectrophotometer. I am indebted to Carrie Wilson and Betty Koll for their skillful technical assistance and to Bernice King, a Third College student, for her general laboratory assistance. I also thank F. E. Young for his helpful suggestions during this investigation.

REFERENCES

1. Pooley, H. M., Poores-Juan, J. M. and Shockman, G. D. Biochem. Biophys. Res. Comm. 38:1134-1140 (1970).
2. Fan, D. P. J. Bacteriol. 103:488-493 (1970).
3. Shockman, G. D., Thompson, J. S. and Conover, M. J. Biochemistry 6: 1054-1065 (1967).
4. Brown, W. C. and Young, F. E. Biochem. Biophys. Res. Comm. 38:564-568 (1970).