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DISCLAIMER

ULTRAFILTRATION OF TRICHODERMA VIRIDE CELLULASE*

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INTRODUCTION

Concentration of enzymes and other biological macromolecules by membrane ultrafiltration has recently been studied by various workers. The fungal enzyme cellulase obtained from Trichoderma viride is a particularly interesting system due to the presence of a wide distribution of molecular weight compounds.[1)] Since the molecular sieve membranes present specific molecular weight cut-off points, a separation, according to molecular size, is relatively easy and less time consuming compared to other traditional biochemical methods. It is the object of this article to present results of ultrafiltration of T. viride cellulase by a molecular sieve membrane of cut-off point 30,000 (Amicon PM 30) over a wide concentration range and to correlate total protein recovery with individual enzyme activities.

MATERIALS AND METHODS

The strain of Trichoderma viride used was QM 6a obtained from U. S. Army Laboratories at Natick, Massachusetts. Enzyme activities were estimated using absorbent cotton for C_1 activity, CMC for C_x activity and Whatman

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Filter Paper #1 for filter paper activity as suggested by Mandels and Weber.[2] To account for dilution effect in analyzing column fractions, the time for hydrolysis was increased from 30 minutes to 160 minutes for C_x estimation. Protein was measured by the modified biuret method as suggested by Koch and Putnam.[3]

Concentration experiments were carried out in an Amicon model 200 ultrafiltration cell using PM 30 membrane at room temperature. Operating pressure was maintained at 20 psig. Column chromatographic experiments were run with Sephadex G-75 gels in 100 × 2.5 cm Sephadex laboratory columns. The eluting phosphate buffer (10 m.M. total phosphate, 100 m.M. NaCl) had a pH of 6.5. Column effluents were collected by Gilson automatic fraction collector. The entire chromatographic unit was enclosed in a constant temperature cabinet maintained at 4°C.

RESULTS AND DISCUSSIONS

Table 1 shows the results of concentration experiments in the range of concentration factor 2.0 to 10.00. The soluble protein distributes between the two liquid phases and a rough equilibrium value is reached after a concentration factor of 6.67. In this range C_1-cotton activity and filter paper activity are confined in the concentrate, none escaping in the filtrate. C_x activity, on the other hand, starts appearing in the filtrate after a concentration factor of 2.0 and its distribution follows the general pattern of soluble protein. The total protein recovery and the total C_x recovery remains fairly constant. Filter paper activity recovery decreases sharply initially in the lower concentration ranges but reaches more or less a constant value shortly. C_1-cotton activity recovery, however, decreases gradually at higher concentration factors over the entire experimental range.

Figure 1 and Fig. 2 depict results of chromatograph runs with concentrates (concentration factors 3.28 and 8.33, respectively) from the ultrafiltration experiments. The protein peak after fraction number 60 in Fig. 1 is not present in Fig. 2 due to its passage through the membrane to the filtrate.* This, according to Table 1, corresponds to an increase of total protein percentage in filtrate from 37 to 48%. As shown in Fig. 2, C_x activity is distributed over the various fractions where as C_1-cotton activity is only found between fractions number 24 and 37. These fractions were pooled together and an estimation of C_1-cotton activity of this pool could only account for about 5% of the total activity present in the concentrate. This, together with results shown in Table 1, suggest that C_1 activity is strongly dependent upon the simultaneous presence of C_x activity. Theoretical estimation of percent total protein in the filtrate derived from a stirred tank model on the high pressure side and assuming a perfect separation across the membrane according to molecular size closely matched the experimental observations for an initial amount of 49% of under 30,000 molecular weight fraction.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the assistance of Jerry Levine of Biodynamics Group, Lawrence Berkeley Laboratory in setting up the chromatograph experiments.

*Also, the peak between fractions 45 and 53 becomes shorter from Fig. 1 to Fig. 2.

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2) Mandels, M., Weber, J., Advan. Chem. Ser., 95, 391 (1969).

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Table 1. Culture filtrates twice centrifuged at 10,000 rpm 4°C for 10 minutes. 200 cc of supernatant (average protein content 382 µg/ml, 0.80 filter paper activity) used in the ultrafiltration cell for all concentration experiments. No reducing sugar present.

Concentration Factor	Total Protein Recovery	Distribution of Total Protein		Total C_x Recovery	Distribution of Total C_x Activity		Recovery of Filter Paper Activity	Recovery of C_1-Cotton Activity
		Concentrate	Filtrate		Concentrate	Filtrate		
	%	%	%	%	%	%	%	%
2.00	98.88	76.32	22.56	62.28	62.28	Trace	100.00	62.50
3.28	95.61	58.93	36.68	57.37	43.18	14.19	82.39	48.35
5.00	95.83	53.83	42.00	60.61	27.63	32.98	67.53	42.07
6.67	96.17	48.10	48.07	64.45	20.95	43.50	58.50	43.09
8.33	96.00	47.80	48.00	61.30	15.08	46.22	60.50	37.10
10.00	95.81	47.31	48.50	62.87	15.62	47.25	62.67	31.36

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FIGURE CAPTIONS

Fig. 1. Column 85 × 2.5 cm G-75 Sephadex, fraction size 5 ml, eluting buffer (10 m.M. total phosphate, 100 m.M. NaCl) pH 6.5, flow rate 5-8 ml/hr, temp. of operation 4°C.

Sample Characteristics: Concentrated 3.28:1 by Amicon PM 30 membrane from solca floc grown T. viride QM 6a culture filtrate, sample size 3 ml.

Fig. 2. Column 85 × 2.5 cm G-75 Sephadex, fraction size 5ml, eluting buffer pH 6.5 (10 m.M. in phosphate, 100 m.M. in NaCl), flow rate 5-8 ml/hr, temp. of operation 4°C.

Sample Characteristics: Concentrated 8.33:1 by Amicon PM 30 membrane from solca floc grown T. viride QM 6a culture filtrate, sample size 5 ml.

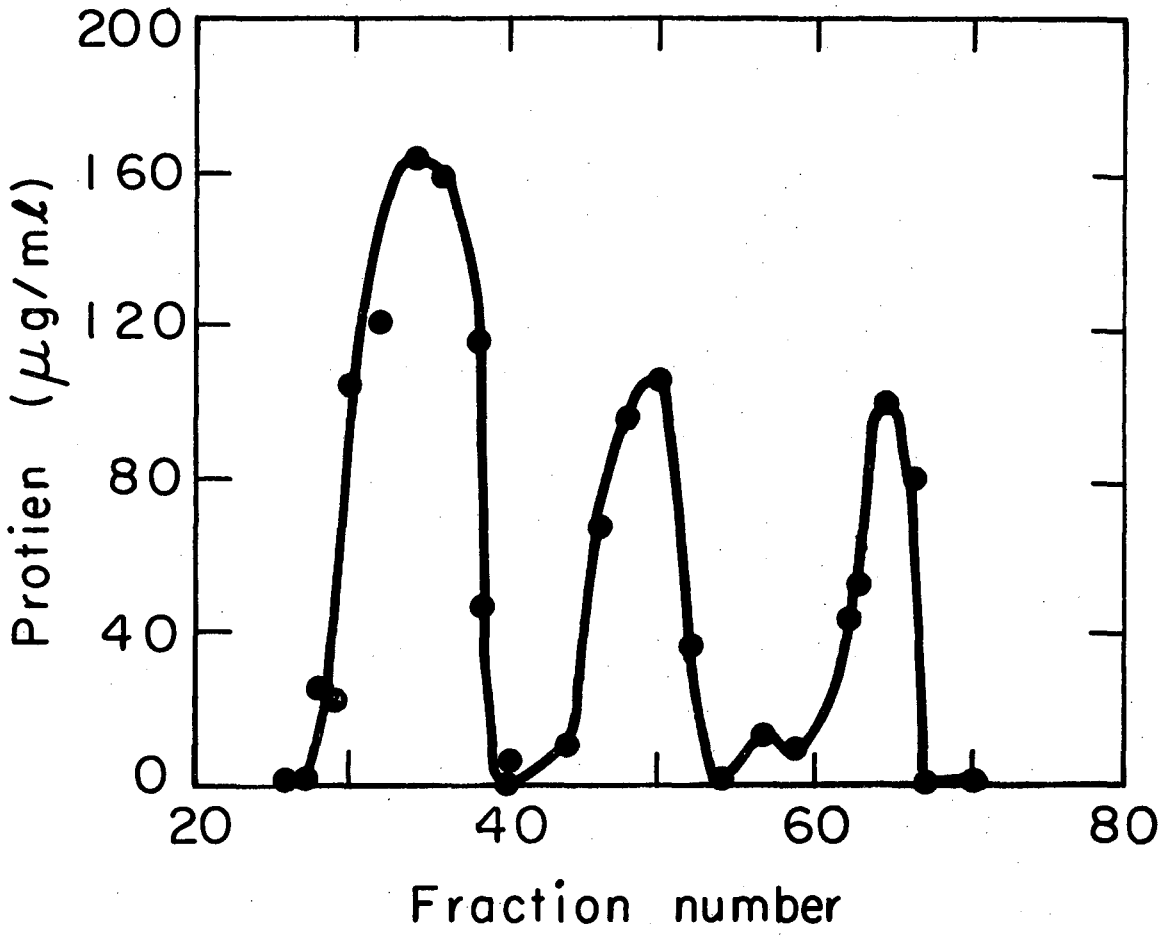

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Fig. 1

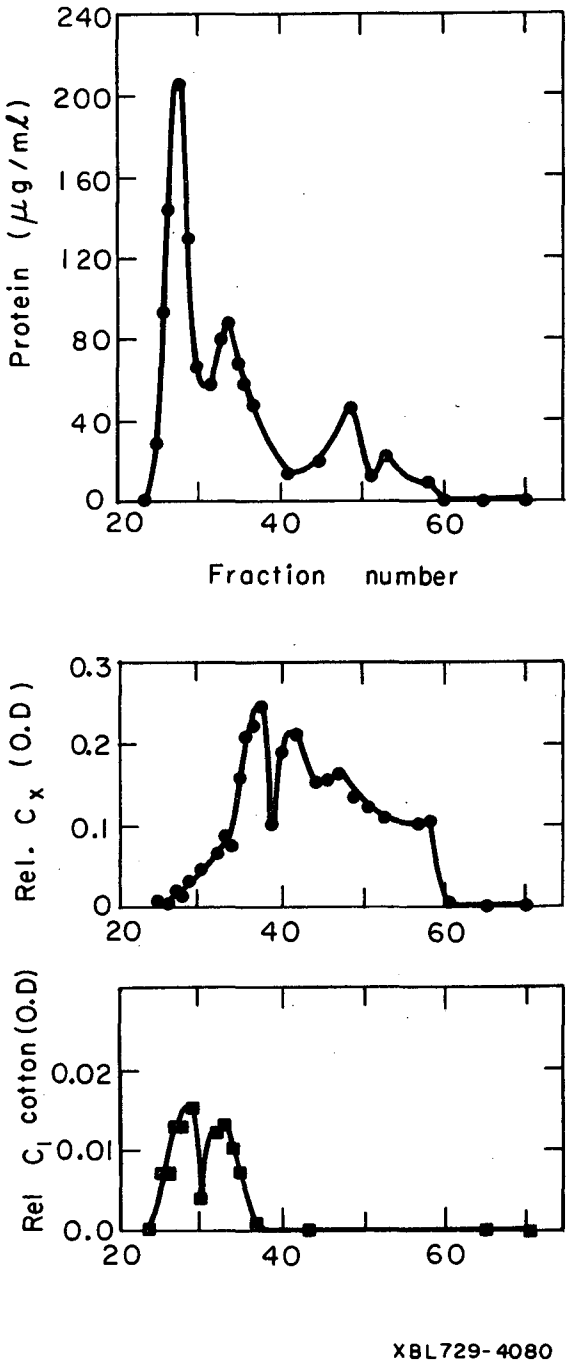
240
200
160
120
80
40
0
Protein (μg/mℓ)
20
40
60
Fraction number
0.3
0.2
0.1
0
Rel. C_x (O.D)
20
40
60
0.02
0.01
0.0
Rel C_1 cotton (O.D)
20
40
60
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Fig. 2

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