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Gel filtration chromatography for the quantitative purification and stabilization of cellulases of turbid solutions

The evaluation of cellulase activity of rumen liquor, silage extracts and some filtrates of fungal growth media (crude enzyme preparations) is complicated by the turbidity of the solutions. The turbidity interferes with established colorimetric enzyme assays. Purification of the assay medium subsequent to the incubation and prior to the colorimetric reducing sugar determination[1,2], although possible[3,4], was found to be too cumbersome for routine analysis. Instead we adapted existing qualitative gel filtration chromatographic techniques[5] to the quantitative purification of turbid crude enzyme preparations.

Experimental

Columns consisted of 4.5 × 15 cm precision bore glass tubing with sintered glass retaining plate. The tubing was collapsed below the sintered glass plate to give minimum hold-up of liquid. A 2 mm layer of silver sand was placed on the sinter and the columns were filled with suspensions of swollen Sephadex G-75 (bead form 65–100 mesh). The bed was allowed to settle at a buffer flow rate of 10 ml/h. The operating buffer flow rate was 20 ml/h and the columns were located in a room at 21° ± 1°. The load maximum was 3.5 mg of protein. A sodium phosphate buffer, pH 6.3, was used as eluting solvent (0.01 M in total phosphate, 0.1 M in NaCl and 0.005 M in NaN_3). The cellulase activity eluted in the 30–80 ml elution fraction. For routine use fractions were collected in graduate cylinders. At the completion of the cellulase elution, the columns were regenerated by increasing the flow rate to maximum possible. Regeneration was completed in 90–120 min.

Cellulase activity of the crude enzyme preparation was established by the method of FELSTENSTEIN[6] using ion-exchange columns[7] to purify the assay medium after incubation. The crude enzyme preparations were applied to the column. In some instances purified C_1 (activity toward insoluble cellulose) and C_x cellulase[8] (activity towards soluble cellulose) (*Trichoderma viride*) were added. The results are shown in Table I.

TABLE I

RECOVERY OF CELLULASE ACTIVITY FROM GEL FILTRATION COLUMNS

Source of crude enzyme preparation	*Amount of protein applied to column (mg)*	*Cellulase activity applied to column*		*Recovery of cellulase activity*	
		C_x *units*[b]	C_1 *units*[b]	C_x	C_1
Filtered rumen	3.1	42.3	5.0	97	98
liquor	1.1	17.6	1.3	96	93
Aqueous silage	3.4	21.1	16.2	103	102
extracts	2.0	13.0	11.8	104	100
Trichurus cylindricus	2.9	165.2	100.0	97	101
culture filtrate[a]	0.5	37.4	17.1	94	98

[a] A 6 day culture was used, wheat bran being the major growth substrate. The filtrate was dark brown and turbid.

[b] For definition of units, see ref. 8.

Results

The recovery of cellulase activity from the columns was complete. Added activity was also recovered quantitatively. The method is applicable to protein solutions having a high concentration of cellulase activity as well as to those having low cellulase activity. The phosphate ions did not interfere with the incubation nor with the color development. In general the level of activity in the eluted fraction was sufficiently high, eliminating the need for the concentration of the eluent. When concentration and removal of buffer salts was necessary, a Diaflo ultra filtration cell (Amicon Corporation) was used. The columns have been reused for a number of months and no degeneration has been observed.

The C_1 cellulase activity of filtered rumen liquor was reported to be low and variable[6]. In our preliminary studies[9] the instability of the C_1 enzymes was recognized. Purification by gel filtration stabilized the activity. Speed in filtration and application to the column was essential. Three day storage of purified rumen cellulase preparations was facilitated. The lyophilized enzyme preparation appears to be retaining full activity for indefinite periods of time.

The procedure appears lengthy when viewed in terms of time which elapses between application of the crude enzyme preparation and obtaining the purified enzyme solution, however, the actual working time in fact is small. The columns are of simple, inexpensive construction and a number of them can be set up requiring the part-time attention of a single operator only. Purification of the assay medium subsequent to incubation by ion-exchange chromatography or chemical manipulation of a much larger number of samples (*e.g.* assays are usually carried out in multiples) was found to be much more time consuming, and in the case of chemical manipulation, much less accurate. Besides, the advantage of stabilizing the cellulases from rumen fluid would not be realized.

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1 J. B. SUMNER AND E. B. SISLER, *Arch. Biochem.*, 4 (1944) 333.
2 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
3 H. G. WISEMAN AND W. C. JACOBSON, *J. Agr. Food Chem.*, 13 (1968) 36.
4 T. E. FRIEDMANN, C. W. WEBER AND N. F. WITT, *Anal. Biochem.*, 6 (1963) 504.
5 K. SELBY AND C. C. MAITLAND, *Biochem. J.*, 104 (1967) 716.
6 G. N. FELSTENSTEIN, *Biochem. J.*, 69 (1958) 562.
7 D. WYBENGA AND V. J. PILEGGI, *Clin. Chim. Acta*, 16 (1967) 147.
8 M. MANDELS AND E. T. REESE, *Develop. Ind. Microbiol.*, 5 (1964) 5.
9 T. S. NEUDOERFFER, unpublished results.

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