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Abstract

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Eveleigh, D.E. and J. J. Child. Use of non-ionic substrates for the determination of cellulase (Cx).

Cellulose has been intensively studied in a wide range of organisms (Norkram 1967 Adv. Appl. Microbiol. 9:91), and has recently been investigated in Neurospora from both an industrial (Kuroda 1968 Chem. Abs. 68: 18955d) and a theoretical standpoint (Meyers and Eberhard 1966 Biochem. Biophyr. Res. Commun. 24: 782). Although there have been attempts to rationalize the cellulose assay (ß-1,4-glucanase = Cx) on on absolute basis (bonds broken per second = Almin and Eriksson 1968 Arch. Biochem. Biophyr. 124: 129) there is a lack of standardization between published methods. For example, carboxymethyl cellulose (CMC) has been used in several different states each with various degrees of substitution (DS) and of polymerization (DP), which control the rate of the reaction. Its ionic character limits its use in viscometric assays, as the viscosity is dependent on pH, ionic strength and polyvalent cation content of the assay medium. There limitations are magnified in the more enzymically reactive, lower substituted CMC's. Glycol cellulose (hydroxy ethyl cellulose = H EC) has be en proposed to replace CMC in order to circumvent there difficulties (Iwasaki et al. 1964 J. Biochem. (Tokyo) 55:30) but has been rarely used, presumably because there authors noted the laborious procedure of making this non-ionic substituted cellulose. A rigorously controlled range of H EC's is available commercially and thir allows the facile development of more standardized reductiometric and viscometric cellulose assays. For this purpose we hove used the readily soluble Natrosol 250 M H EC (4,500-6,500 centipoises at 2%, mean substitution 2.5, DS ca. 1.0, DP 565 = Hercules Powder Co., Wilmington, Delaware). Equivalent H EC's are available from Farbwerke Hoechst A. G., Frankfurt, Germany (Tylose H 4000) and U nion Carbide Corp., New York (Cellos' IZE WP 4400H). Viscometric assays were carried out using a Cannon-Fenske Viscometer (No. 200: efflux time of solvent 10.2 sec.) at 25°C with a reaction mixture of 7 ml 0.44% HEC (250 M Natrosol), 1 ml 0.5 M sodium acetate buffer pH 4.8 and 2 ml enzyme. The substrate was dissolved in water by shaking overnight at room temperature. Blending to aid the rapid solution of the substrate for as little as five seconds gave a much reduced viscosity level. Reductiometric analyses were performed using equivalent reaction mixture, reducing end groups being estimated by the ferricyanide method modification proposed by Pork and Johnson (1949 J. Biol. Chem. 181: 149). The colorimetric Somogyi-Nelson method could not be used as it caused precipitation of the substrate and concomitant absorption of the colored complex. Titrimetric analyses of the oligosaccharides by the Somogyi method or by direct reduction with alkaline iodine proved practical but tedious. Enzyme units ore defined: one reductiometric unit is that amount of enzyme which producer 1% degradation in 1 minute, under the above conditions, while a viscometric unit is that amount of enzyme which causes a change of Δf (specific fluidity) of 0. 10 in 10 minutes when incubated under the above conditions. Apparent zero order kinetics were maintained up to a change of Δf of 0. 10. It is important to note that substituted celluloses are degraded at a changing rate

throughout the reaction and hence it is necessary to define the reductiometric unit at some standard % degradation (1%). One reductiometric unit approximately equals 21 viscometric units, but varies with the particular enzyme under investigation.

A comparison has been made of the levels of Cx cellulose produced by Neurospora crassa STA4 (FGSC"262) and N. crassa ATCC 10355. The organisms were grown in Vogel's mineral medium N containing 0.5% glucose plus 1% "cellulose" (750 ml/2 l flask, 30°C with shaking). Celluloses used included: H EC (N atrosol 250 M), CMC (Cellofas BIO I.C. I. Ltd., England) and Avicel (Microcrystalline cellulose, FMC Corp., Marcus Hook, Pennsylvania). Relatively small amounts of enzyme were produced under there conditions (Table 1). For example, a crude commercial cellulose (Trichoderma viride) has a specific activity of 20.8 (viscometric). More enzyme was excreted into the medium than retained intracellularly but intracellular levels were measurable with there techniques. CMC proved a better inducer of cellulase than the other two substrates. Attempts to release additional bound cellulase by further incubation of the harvested mycelium or mycelial homogenates at pH 7.5 (P04) 16 hours (3°C) proved successful.

Table 1. The induction of cellulose by "celluloses" in two strains of Neurospora crassa (viscometric units).

	N. crassa 10355 (4 days)				N. crassa STA4 (4 days)				N. crassa STA4 (8 days)			
	Extracellular		Intracellular		Extracellular		Intracellular		Extracellular		Intracellular	
Inducer	µ/ml	SpAct	µ/ml	SpAct	µ/ml	SpAct	µ/ml	SpAct	µ/ml	SpAct	p/ml	SpAct
HEC	0. 14	0.72	0.30	0.21	0.28	2.55	0.12	0.06	0.17	1.70	1.16	0.39
CMC	0.68	4.86	0.11	0.06	1.20	14.20	0.64	0.42	1.46	13.2	0.94	0.40
AVICEL	0	▪	0	▪	0.10	1.25	0.01	0.18	0.42	4.2	0.47	0.66

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