Carbohydrate Research, 33 (1974) 171–174

Note

The use of defatted cellulose for purification of amylopectin

NARAYAN B. PATIL*, SAROJ P. TASKAR, AND NARAYAN R. KALE†
Department of Chemistry, Division of Biochemistry, University of Poona, Poona 411007 (India)
(Received July 3rd, 1973; accepted for publication September 11th, 1973)

The experiments of Tanret[1], Gilbert *et al.*[2], and other authors[3] do not rule out the use of cellulose for the preferential adsorption of amylose but show it to be inefficient for the removal of amylose remaining in amylopectin prepared by the conventional methods[3–7] of fractionating starch. Meyer and Gibbons[8] have observed partial purification of amylopectin by removing the contaminating amylose as an insoluble, fatty acid complex. We now report on a more convenient method based on the selective, induced adsorption of amylose on to cellulose.

When a solution of starch in 2M urea was equilibrated with cellulose, there was no retention of amylose or amylopectin. However, it was observed that the introduction of ethanol into the system could induce reversible adsorption of the amylose on

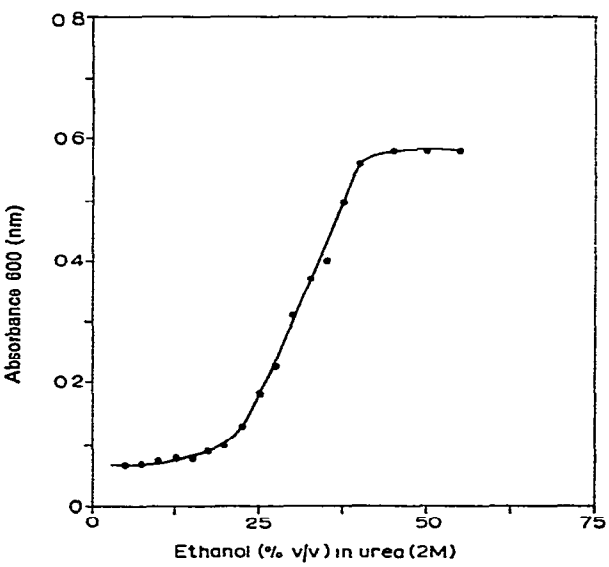

Fig. 1. Influence of the concentration of ethanol on the retention of amylose on cellulose (defatted).

*Present address: Department of Chemistry, Division of Biochemistry, Marathwada University, Aurangabad (Maharashtra), India.
†To whom enquiries should be addressed.

to cellulose. The optimal conditions for the ethanol-induced adsorption are illustrated in Fig. 1, from which it is seen that adsorption increases as the concentration of ethanol is increased from 19 to 35%; above this concentration, there is no further increase in adsorption. Complete precipitation of starch from solution in 2M urea is observed when the ethanol concentration is 38–40%. Therefore, an ethanol concentration of 32–35% is optimal for the adsorption of amylose. By repetition of the adsorption process, the amylose contamination of amylopectin can be reduced to a very low level.

The amylopectin isolated by the above procedure was readily soluble in water and had the following properties indicative of high purity: an intrinsic viscosity[14,15] (η) in 0.5M potassium hydroxide at 30° of 1.49, iodine-binding capacity 0.21 (determined by a potentiometric method[5,11]), blue value 0.16 (determined by a spectrophotometric method[12]), beta-amylolysis limit[13] of 50–51%.

EXPERIMENTAL

Defatting of starch and cellulose. — Cellulose powder (Whatman No. 1) was exhaustively defatted first by refluxing with light petroleum (b.p. 30–40°) to remove loosely bound, lipid material, followed by hot extraction with 85% methanol as described by Schoch[6], and then dried. Potato starch was defatted by the same procedure.

Starch solutions. — The defatted, wet, potato-starch granules (20 g) were dispersed in 8–10M urea[9,10] (1 litre, buffered to pH 6.2) by prolonged, mild stirring at room temperature (26–28°) to avoid degradation. The dispersed starch was precipitated by adding an equal volume of ethanol (95%). This precipitate, collected by centrifugation (2500 *g*), was easily dissolved in 2M urea (pH 6.2) and preserved at 26–28°.

Influence of ethanol concentration on the adsorption of amylose. — Mixtures containing 1 ml of a solution of starch (20 mg) in 2M urea, 4 ml of a slurry of defatted cellulose (12.5%) equilibrated in 2M urea, and 5 ml of ethanol (10–100%) in 2M urea were incubated at 30 $\pm$0.1° for 3 h with intermittent shaking. The cellulose was then collected by centrifugation (2500 *g*) and the supernatant was removed by decantation. The residue was washed with the respective ethanol–urea system (3 $\times$ 5 ml). The adsorbed amylose was eluted quantitatively by treating the residue with 5 ml of 2M urea and determined spectrophotometrically[12] at 680 nm after treatment with iodine (KI_3). The results are illustrated in Fig. 1.

Characterization of amylopectin. — The analytical methods developed by Bates *et al.*[11], Lansky *et al.*[5], Bourne *et al.*[12], and Bernfeld[13] were applied. The intrinsic viscosity of amylopectin in 0.5M potassium hydroxide was determined according to the method of Schoch[14] and the experimental details outlined by Greenwood[15]. The total carbohydrate content was determined by the thymol–$FeCl_3$–HCl procedure[16].

Isolation and purification of amylopectin[17]. — A 2% solution (1 litre) of starch in

2M urea (pH 6.2) was equilibrated at 30°, and ethanol was added at 10 ml/min, with vigorous stirring, until a concentration of 33% was reached. The turbid solution was then allowed to equilibrate for 18–20 h with mild stirring. The precipitate, mainly amylose, was collected by centrifugation (13,200 *g*). The ethanol concentration of the supernatant, which contained amylopectin contaminated with amylose, was raised to 35% and defatted cellulose powder (10 g) was added with mild stirring. The mixture was allowed to equilibrate at 30° for 2 h to remove traces of amylose by adsorption. The cellulose was removed by centrifugation (1400 *g*), and the supernatant was re-treated with further batches (3–4) of cellulose powder in order to completely remove traces of amylose from amylopectin (Table I). The efficiency of the method is increased if the solution of amylopectin is passed through a cellulose column equilibrated with 32–35% ethanol in 2M urea[18]. This procedure is applicable to other tuber and cereal starches.

TABLE I

CHARACTERIZATION OF AMYLOPECTIN AFTER TREATMENT WITH DEFATTED CELLULOSE, AND ADSORBED AMYLOSE AFTER ELUTION[9,17]

	Amylose	*Supernatant (Amylopectin)*	*Ttreatment with cellulose*			
			1	*2*	*3*	*4*
Iodine-binding capacity[a] (IBC)	19 3 – 19.7	2.29	1.33	0.56	0.21	0.21
Blue value (BV)	1.16 – 1.28	0.36	0.32	0 28	0.16	0.16
Beta-Amylolysis limit	97.5 – 98.2	58.1	54.0	53.5	52.0	51 0 – 50 4
Intrinsic viscosity (η)	—	—	—	—	—	1.49

[a]Expressed as mg of iodine bound per 100 mg of polysaccharide.

The amylopectin, precipitated by increasing the concentration of ethanol to 38–40%, was a gum which was converted into powder form by repeated treatments with cold (−10°) ethanol. The amorphous, white powder (14 g) was repeatedly washed with ethanol to remove urea and dried over phosphorus pentaoxide at 26–28°.

ACKNOWLEDGMENTS

The authors thank Professors S. K. K. Jatkar and H. J. Arnikar for their interest and help, and the Council of Scientific and Industrial Research and the University Grants Commission for the awards of Junior Research Fellowships to S. P. T. and N. B. P.

REFERENCES

1 C. TANRET, *Compt. Rend.*, 158 (1914) 1353; *Bull. Soc. Chim. Fr.*, 4, 17 (1915) 83.
2 G. A. GILBERT, C. T. GREENWOOD, AND F. J. HYBART, *J. Chem. Soc.*, (1954) 4454.

3 J. W. MULLEN AND E. PACSU, *J. Amer. Chem. Soc.*, 63 (1941) 1168; M. E. BALDWIN, *ibid.*, 52 (1930) 2907.
4 H. BAUM AND G. A. GILBERT, *Chem. Ind. (London)*, (1954) 489.
5 S. LANSKY, M. KOOI, AND T. J. SCHOCH, *J. Amer. Chem. Soc.*, 71 (1949) 4066.
6 T. J. SCHOCH, *J. Amer. Chem. Soc.*, 64 (1942) 2957; *Cereal Chem.*, 18 (1941) 121.
7 P. J. KILLION AND J. F. FOSTER, *J. Polym. Sci.*, 46 (1960) 65.
8 K. H. MEYER AND G. C. GIBBONS, *Helv. Chim. Acta*, 33 (1950) 210.
9 N. B. PATIL, Ph.D. Thesis, University of Poona, 1971.
10 N. B. PATIL AND N. R. KALE, *Biochem. J.*, 128 (1972) 61P.
11 F. L. BATES, D. FRENCH, AND R. E. RUNDLE, *J. Amer. Chem. Soc.*, 65 (1943) 142.
12 E. J. BOURNE, W. N. HAWORTH, A. MACEY, AND S. PEAT, *J. Chem. Soc.*, (1948) 924.
13 P. BERNFELD, *Methods Enzymol.*, 1 (1955) 149.
14 T. J. SCHOCH, *Methods Enzymol.*, 3 (1957) 5.
15 C. T. GREENWOOD, *Methods Carbohyd. Chem.*, 4 (1964) 179.
16 N. B. PATIL, S. V. BHIDE, AND N. R. KALE, *Carbohyd Res.*, 29 (1973) 513.
17 S. P. GUPTE, Ph. D. Thesis, University of Poona, 1964.
18 N. B. PATIL AND N. R. KALE, *J. Chromatogr.*, 84 (1973) 75.