Preparation and fractionation of highly substituted hydroxyethyl derivative of acid-hydrolyzed potato starch

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A procedure of purification of acid-hydrolyzed potato starch was elaborated and its hydroxyethylation under various conditions has been studied. The products were fractionated and their physicochemical characteristics have been determined. While fractions obtained by precipitation of aqueous solutions of hydroxyethylstarch with acetone differed in their molecular weight, precipitation with methanol yielded fractions having different degree of substitution.

Был разработан способ очистки крахмала из кислотного гидролиза картофеля и было изучено его гидроксиэтилирование в разных условиях. Были определены физико-химические характеристики фракционированных продуктов. В то время как фракции, полученные осаждением из водных растворов гидроксиэтилированного крахмала, отличались своим молекулярным весом, осаждение с метанолом привело к фракциям с различной степенью замещения.

Recently, considerable effort has been directed towards the use of highly substituted, low-molecular weight hydroxyethylstarch as a substitute for blood plasma or as a cryoprotective agent of erythrocytes and other tissues [1].

For making hydroxyethylstarch of the required properties starch from waxy maize, containing more than 90% of the branched fraction (amylopectin) [2], has been used as the starting material. The common procedure involves graded hydrolysis with dilute hydrochloric acid and, after the products of side reactions had been removed, hydroxyethylation [3]. Several procedures of hydroxyethylation of native starch have been described in the literature [4—9]. When applied to acid-hydrolyzed potato starch the known procedures have been found unsatisfactory and, therefore, a modified procedure of purification of the starting material and of its hydroxyethylation has been elaborated.

Experimental

Instruments and methods

The molecular weight (M_w) was calculated from the values of the diffusion coefficient measured with Zeiss 35 electrophoresis instrument, from the sedimentation constants measured with an MOM ultracentrifuge, and from the partial specific volume α determined pycnometrically. The intrinsic viscosity for the solutions in water or 1 M sodium hydroxide was measured using a Ubbelohde viscometer as modified by Rafikov. Hydroxyethyl groups were determined by the method of Morgan as modified by *Lortz* [10], or by gas chromatography [11]. The content of amylose was determined iodometrically using biamperometric indication [12]. The potato starch was a commercial product.

Hydrolysis of potato starch with hydrochloric acid

a) In acetone

A suspension of starch (300 g, based on dry sample) in acetone (600 ml) was heated to 38°C and, after addition of concentrated hydrochloric acid (6 ml) the mixture was kept at the same temperature and stirred for 10, 20, 30, 45, 60, 80, and 120 min. Aqueous sodium acetate (1 M, 150 ml) was added to neutralize the acid, the mixture was filtered and the solid material was washed with water, acetone, and dried to give 298 g (99.3%) of a product.

b) In water, below the temperature of the formation of starch paste

A mixture of potato starch (450 g, based on dry sample) in water (1500 ml) was heated with stirring to 50°C and concentrated hydrochloric acid (60 ml) was added. The stirring at 50°C was continued for 9 h, the mixture was filtered through a fine-porosity sintered-glass funnel, the solid material was washed with water until neutral, then with acetone, and dried to give 427.5 g (95%) of a product.

Purification of the hydrolyzed starch

To a boiling suspension of the acid-hydrolyzed starch (400 g, based on dry sample) in 2-propanol (500 ml) aqueous sodium hydroxide (40% ; 20 ml) was added with stirring. The mixture was boiled with stirring for 1 h and filtered or, alternatively, 2-propanol was distilled off. The obtained brown alkalistarch, now containing the alkali-labile components in a water-soluble form, was added with stirring to 2 l of cold water. The mixture was filtered and washed with water until the filtrate was colourless. According to the extent of hydrolysis the loss of the material which had gone into the solution amounted to 20—30 g. The solid was then washed with 2-propanol and used for hydroxyethylation.

Hydroxyethylation

To a boiling suspension of the purified, acid-hydrolyzed starch, obtained as described above, in 2-propanol (400 ml) aqueous sodium hydroxide (40% ; 30 ml) was added with

stirring. After cooling to 5°C, the mixture was transferred into a pressurized vessel and, according to the desired degree of substitution and assuming 80% efficiency of the utilization of the reagent, ethylene oxide was added. The vessel was closed and the mixture was stirred at 40—45°C for 24 h. After cooling, the mixture was neutralized with dilute hydrochloric or acetic acid, filtered and the salts were removed by dialysis. The product was stirred with acetone, filtered and dried.

Fractionation of hydroxyethylstarch

The derivatized starch obtained as described above (5 g) was dissolved in hot water (40 ml) and precipitated by gradual addition of acetone or methanol (see Tables 2 and 3). The precipitate was collected by centrifugation and dried. The last fractions were obtained by concentration of the solution and freeze-drying.

Results

The results of acid hydrolysis are given in Table 1.

Regardless of the intrinsic viscosity of the starch used (within 2.3—2.8 dl/g), hydrolysis with 0.4 M hydrochloric acid in water gives products having intrinsic viscosity $[\eta] = 0.23$—0.26 dl/g.

Table 1

Effect of the duration of the hydrolysis (in acetone) upon the intrinsic viscosity (measured in H_2O) of products

τ/min	0	10	20	30	45	60	80	120
$[\eta]$/dl g^{-1}	3.36	2.34	2.23	2.02	1.97	1.74	1.45	1.32

Table 2

Fractionation of hydroxyethylstarch (5.0 g in 40 ml of water) with methanol

Fraction	Methanol added ml	Yield		$[\eta]$	M	Hydroxyethyl %	M. S.
		g	%				
I	60	0.58	11.6	0.264	40 200	4.95	0.19
II	50	0.79	15.8	0.250	94 900	11.10	0.46
III	100	0.93	18.6	0.240	45 300	17.93	0.80
IV	*a*	2.03	40.6	0.245	43 400	20.81	0.96
Total	210	4.33	86.6				

a) Fraction *IV* obtained on concentration.

When the hydrolysis is carried out in acetone the average content of amylose present in the starting native starch remains unchanged (21.1%). In the presence of water, the hydrolysis at 50°C is more extensive and after 9 h a slightly increased content of amylose, to the average value of 21.6 %, was observed. When the present low-molecular weight material, that reacts with iodine at its higher concentration during the determination [13, 14], was taken into account the found average content of amylose was 24.7%. The acid-hydrolyzed (9 h, 50°C, 0.4 M hydrochloric acid) hydroxyethylstarch showed the following characteristics: $[\eta] = 0.249$, $M = 45\ 900$, hydroxyethyl content = 16.27%, M. S. = 0.715. The analytical data for the obtained fractions are given in Tables 2 and 3.

Table 3

Fractionation of hydroxyethylstarch (5.0 g in 40 ml of water) with acetone

Fraction	Acetone added ml	Yield		$[\eta]$	M	Hydroxyethyl %	M. S.
		g	%				
	50	0	0	–	–	–	–
I	5	2.55	51.0	0.280	62 800	16.23	0.710
II	5	1.21	24.2	0.230	35 200	16.29	0.717
III	*a*	0.97	19.4	0.160	21 600	16.28	0.714
Total	60	4.73	94.6				

a) Fraction *III* obtained on concentration.

Discussion

The known methods of hydroxyethylation of native starch have been found unsatisfactory with low-molecular weight products obtained from potato starch by acid hydrolysis. When the older procedures were tested the following observations were made:

a) When gaseous ethylene oxide was allowed to react with the alkali salt of native starch [4], starch paste was formed at the early stages of hydroxyethylation, the material formed a homogeneous pasty mass allowing only topochemical reaction to take place while the inner portion of the mass remained unchanged. The procedure became even less workable when hydrolyzed starch was used as the substrate. When liquid ethylene oxide was added to a suspension of powdered alkalistarch unspecific, strongly exothermic reactions took place.

b) Under the conditions of triethylamine-catalyzed hydroxyethylation in 2-propanol [5] most of the reagent used polymerized to poly(ethylene glycols). Thus, the observed degrees of substitution of the products were much lower than those expected.

c) Hydroxyethylations in an aqueous solution [6—8] are rather impractical in that they require relatively large reaction vessels (for 5 g of hydroxyethylstarch a 100 ml autoclave is needed). In addition, the efficiency of derivatization is only 15% since most of ethylene oxide reacts with water to give poly(ethylene glycols), and the isolation of the product is difficult. The use of less basic catalysts (*e.g.* sodium carbonate instead of sodium hydroxide) does not give higher, but lower yields of the desired products.

d) The necessity to include in the overall process of hydroxyethylation a purification step arose when the hydroxyethylation procedure carried out with native starch in 2-propanol and in the presence of aqueous sodium hydroxide [9] was applied to acid-hydrolyzed starch [15]. Apart from the desired product, acid hydrolysis of starch yields also alkali-labile substances which undergo degradation in alkaline medium during the following hydroxyethylation. The degradation products not only affect negatively the course of hydroxyethylation but cause also discolouration of the product and its other unwanted properties. Since it is difficult to remove the coloured material from the final product it is essential that the substrate to be derivatized be purified prior to the hydroxyethylation step. According to the literature [3] this is done by reduction of the alkali-labile substances with sodium borohydride. The purification, as we have found, can be accomplished by treatment of the suspension of the acid-hydrolyzed starch in boiling 2-propanol with aqueous sodium hydroxide. In this way the alkali-labile substances are converted to water-soluble products and can easily be removed from the substrate by washing with water. The hydroxyethylation can then follow. The procedure does not require a large reaction vessel (a 5 l autoclave is sufficient for the preparation of 1 kg of the product) and less than 20% of ethylene oxide is lost in unwanted reactions.

The viscosity measurements showed that when the depolymerization is carried out in acetone only mild hydrolysis takes place. On the other hand, hydrolysis performed in the presence of a large amount of water is more extensive and the product contains a larger proportion of the linear component. This can be explained by the location of the linear component of starch in the crystalline region of the starch grains. Thus, below the temperature of the formation of starch paste the linear component is less accessible to the action of the acid. This explanation is supported by the fact that although the hydrolysis product has considerably lower molecular weight than the starting starch, the starch grains are compact and easy to filter through a fine-porosity sintered-glass funnel, and after washing with acetone a powdered product is obtained.

Fractionation with methanol and acetone of an aqueous solution of highly substituted, low-molecular weight hydroxyethylstarch gives fractions of different degree of substitution and different molecular weight, respectively (Tables 2 and 3). We explain these phenomena by higher solubility (or more pronounced

solvation) of the highly substituted starch in methanol, but not in acetone. Therefore, during the fractionation with methanol the first fractions precipitated contain molecules having low degree of substitution, irrespective of their molecular weight.

In conclusion, some comment is desirable on the advantages of potato starch as a raw material for hydroxyethylation over the traditional starch from waxy maize and sorghum. Firstly, potato starch does not contain proteins and thus, no deproteination step has to be involved in the processing of this raw material. Secondly, the purification of the acid-hydrolyzed product prior to hydroxyethylation is easier to perform with potato starch than with starch of other origin. Of some disadvantage is the high content of the linear portion resulting in high degree of crystallinity of potato starch grains and, consequently, in heterogeneous hydroxyethylation. However, fractionation, an operation unavoidable also with waxy starch hydroxyethyl derivatives that are meant for pharmaceutical use, is less labourious in the case of hydroxyethyl derivative of potato starch than in the case of native starch.

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