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STARCH FLUORESCEIN COMPLEXES

Abstract

Starch with its hydroxylic groups can excite fluorescein. This dye, red when solid and fluorescing when hydrated in aqueous solution exhibited a yellow fluorescence when sorbed on starch. Comparison of the properties of starch - KI_s, starch - fluoresceine, and starch - KI_s - fluoresceine complexes led to the conclusion that fluorescein formed surface complexes and also entered the amylose helix.

Introduction

Solid fluorescein is a red to brown powder. It develops a bright yellow color with a strong green fluorescence on activation by hydration in an aqueous solution [1]. We have assumed that also the hydroxyl groups of starch could activate fluorescein.

Starch is known for its ability to accept selectively various dyes. This selectivity results from the starch granule structure, i. e. starch morphology as well as from the dye nature (see review by Tomasik and Schilling [2] and references therein). According to Kobamoto [3] basic dyes adsorb to a greater extent on larger starch granules whereas acid dyes perform better on small granules. This observation does not apply to potato starch [4, 5]. The sorption of certain dyes on starch allows to distinguish between crystallographic patterns of starch (Acridine Orange) [6], to determine the distribution of positive and negative charges in starch granules (Acridine Orange, Erythrosine) (7), determination of α-amylose in starch (Congo Red) [5, 8] and {2-[2-(4-hydroxy-6- methylpyrimidylazo-4-sulfochlorido)]-1-naphthol} [9]. The sorption of dyes on starch changes some properties of dyes. Thus, the fluorescence of Acridine Orange becomes concentration dependent [10], and Methylene Blue could be protected from fading [11]. The sorption heats of Direct Scarlet B and Chrysophenine G on starch are hardly 29.3 and 62.8 k J/mol, respectively [12]. Nevertheless, the sorption of certain dyes on starch is sufficiently strong for application of such complexes for coloring of artificial threads as shown for several sym-triazine dyes [13].

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The mode of sorption of dyes on starch is only partly recognized. It is suggested that the sorption involves not only surface of granules but also capillaries [14]. Fluorescence and optical rotatory dispersion studies carried out with Bengal Rose and amylose strongly suggest that sorption and helical inclusion complexes are formed [15]. The Pal [16, 17] studies on induced dichroism of starch-iodine-Methylene blue and starch-iodine- Acridine Orange complexes have led to the conclusion that starch-iodine helical complex is wrapped inside of an additional helix formed by these dyes.

In this paper the studies on the sorption of fluoresceine on potato starch i. e. on a combination of amylose and amylopectin are presented.

Materials and methods

Materials

Potato starch manufactured in Niechlów Potato Enterprise in Poland in 1994. Fluorescein, the product of B.D.H.

Ethanol 99.9% manufactured by Polmos Cracow.

Methods

Starch - fluoresein complexes

They were prepared by an immersion of starch (1 g) in ethanolic solutions (100 cm3) containing subsequently 0.010, 0.050, 0.100, 0.250 and 0.500 g of fluorescein and refluxed for 8 hrs. After cooling to room temperature the reaction mixture was filtered off and filtrate of known volume was analyzed for the noncomplexed fluorescein content. The UVVIS, UV2101PC Shimazu spectrophotometer was used. The absorbance was measured at 496 nm. The calibration curve was drawn based on the absorbance estimations for 10^{-3}, 10^{-4}, $2 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, 10^{-5}, $2 \cdot 10^{-5}$ and $5 \cdot 10^{-5}$ M ethanolic fluorescein solution.

Starch - iodine complex

It was prepared according to Pal and Pal [17].

Starch-iodine-fluoresceine complex

Starch-iodine complex (7 g) was refluxed for 5 hrs in 10^{-4} M ethanolic fluorescein solution (100 cm^3). Cold reaction mixture was filtered off and dried in the air.

Fluorescence at elevated temperature

Samples of starch - fluorescein complexes were heated at 150 and 250°C for up to 3 h, and at 300°C for 1 h and their fluorescence was checked with UV lamp at 254 and 366 nm.

Thermal analysis

All samples of plain potato starch and its complexes (200 mg in every case) were thermally analysed in the air in the range of 20 to 400°C with corrundum (8 mm) as the standard. The Paulik-Paulik-Erdey 1500 instrument made in Hungary was used.

Results and discussion

The experiments showed that starch formed complexes with fluorescein but the guest molecule (fluorescein) uptake by starch reached around 10% of the amount available for the complexation (see Table 1).

Table 1

Attempted and achieved composition of starch - fluorescein complexes

Amount of fluorescein in 1 g of starch	
Attempted	Achieved
in 10^{-3}M	
15.40	3.12
7.67	1.91
3.04	1.09
1.62	0.20

The complexes were bright yellow demonstrating that fluorescein in the complex was in its excited state. Temperature tests were carried out in order to distinguish between water and the hydroxylic groups of starch glucose units capable of the excitation of fluorescein. The bright yellow fluorescence of the complexes started to vanish just on either 3 or 1 hour heating at 250°C and 300°C, respectively. The samples turned gradually brown and brown regions of the complex did not fluoresce. It is commonly accepted and experimentally proved [18] that starch maintained at 130°C for 2 hours was free of adsorbed water. Thus, the parameters at which the fluorescence of complexes ceased eliminated the participation of water in the excitation of fluorescein.

The excitation of fluorescein in contact with starch strongly suggested that surface sorption complexes were formed. However, such circumstance did not eliminate a possibility of the parallel formation of helical complexes. Polewski and Maciejewska [15] proved the formation of such starch complexes with Bengal Rose being structurally close to fluorescein. Therefore, the formation of the complex of starch blue the helical starch - $KI_3.I_2$ complex [2] with fluorescein was attempted. The contact of starch blue with fluorescein gave a brown solid. The appearence of the product sug-

gested that the fluorescence of fluorescein ceased due to common heavy atom effect on the fluorescence [19]. It is likely that such effect could be observed provided that iodine was pushed by fluorescein out of the helical envelope and formed a brown surface complex with starch [20]. Such possibility was examined based on thermograms (TG, DTG, DTA) of plain starch, starch- fluorescein complexes, starch - KI_5 complex, and starch - KI_5 - fluorescein ternary complex. The complexation of fluorescein with starch did not affect the thermogram of plain starch. The decomposition peaks of fluorescein were hidden under strong peaks of starch. Contrary to it fluorescein strongly affected the thermogram of starch blue (Fig. 1).

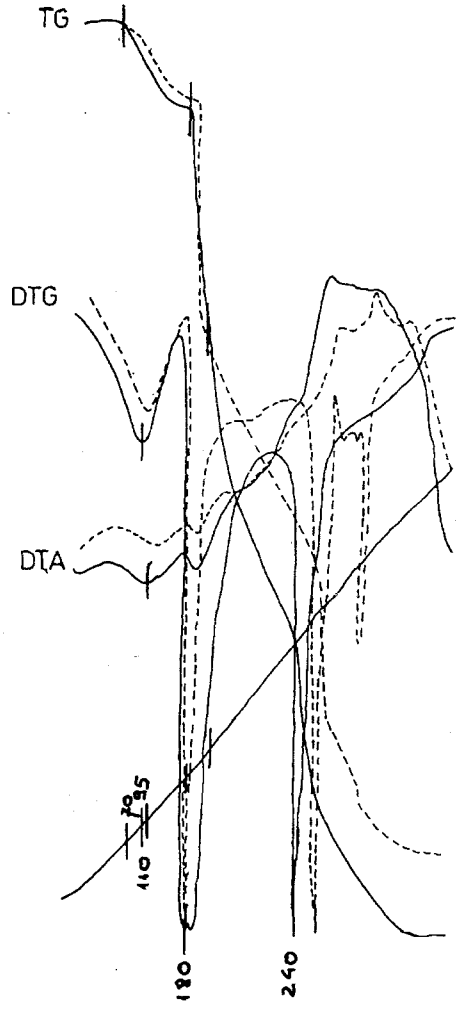

Fig. 1. Thermograms of starch - KI_5 complex, solid lines, and starch - KI_5 - fluorescein complex, broken lines). Thermograms of plain starch and its complexes with fluorescein are identical.

Iodine within the amylose helix decreases the thermal starch stability. Addition of fluorescein increased this stability, although the latter is lower than that of the starch fluorescein complex as shown also in Table 2.

Table 2

Thermal analysis, TG, DTG, DTA, of starch-fluorescein, starch - iodine and starch - iodine - fluorescein complexes, and fluorescein

Complex	Characteristics[a]
Starch - fluorescein[b]	TG: 70 (beginning of the weight loss); 70-145, (-15%); 145-235 (-15.5%); 235-265 (-65%); 265-365 (-87%). DTG: 95s↓; 240vs↓; 355sh,w; 395s↓. DTA: 110b,w; 180vw; 195vw; 215vw; 240w.
Starch- KI_5	TG: 60 (beginning of the weight loss); 60-155 (-8.5%); 155-165 (-35%); 165-330 (-66%); 330-350 (-78%); 350-400 (-87%). DTG: 95s↓; 155vs↓; 340vs↓; 370w↓; 400s↓. DTA: 110b,w; 160w; 205vw; 245sh; 260sh; 295vw; 370w; 400sh.
Starch - KI_5 - fluorescein	TG: 60 (beginning of the weight loss); 60-150 (-10%); 150-315 (-69%); 315-460 (-93%). DTG: 95s↓; 165vs↓; 315vs↓. DTA: 110b,w; 165w; 225sh; 270sh; 280sh; 295sh.
Fluorescein	TG: 275 (beginning of the weight loss); 275-360 (-8.5%). DTG: 295w↓; 350vs↓. DTA: 250b,w; 295w; 350sh; 390vw.

[a] The data for TG, DTG and DTA are given in °C. The arrow down denotes the endothermic process. The values in parentheses following the TG data show the total weight loss up to the end of the indicated interval. The other symbols introduced are as follows: s - strong; vs - very strong; w - weak; vw - very weak; b - broad; sh - a shoulder.

[b] The thermograms are identical for all starch - fluorescein complexes regardless their fluorescein content.

Also the differences in the course of the weight loss (the TG-curve on Fig. 1) suggests that the iodine was liberated from the complex on fluoresceine addition. In the latter case the weight loss is easier and faster.

Conclusion

Starch formed with fluorescein surface and helical complexes. Due to excitation with the starch hydroxylic groups complexed fluorescein fluoresce.

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KOMPLEKSY SKROBI Z FLUORESCEINĄ

Streszczenie

Skrobia może wzbudzać fluoresceinę swymi grupami hydroksylowymi. Barwnik ten jest czerwony w fazie stałej, ale fluoryzuje po zhydratowaniu i wykazuje żółtą fluorescencję po osadzeniu na skrobi. Porównanie właściwości kompleksów skrobi z KI_5, skrobi z fluoresceiną i skrobi z fluoresceiną KI_3 prowadzi do wniosku, że fluoresceina tworzy kompleksy sorpcyjne i wchodzi też do wnętrza heliksu amylozy.