A STUDY OF THE POSSIBLE NATURE OF THE BOND OF THE CARBOHYDRATES TO THE PEPTIDE CHAIN OF GLUTELIN

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We have previously [1] reported the carbohydrate content of glutelin – an alkali-soluble protein from fodder yeast of the genus Candida, strain Kd-14. The impossibility of liberating the protein molecule from the carbohydrates by repeated reprecipitation, electrophoresis, and gel filtration compelled us to assign this glutelin to the complex proteins – glycoproteins. The composition of the suggested prosthetic grouping of the glutelin of Candida fodder yeast includes mannose and glucose [1]. To investigate their participation in the formation of a carbohydrate–protein bond, in the present work we have analyzed the nature of the kinetic curves of the carbohydrates and amino acids liberated from the glutelin when it is hydrolyzed with a 0.5 N solution of hydrochloric acid at 100°C. The carbohydrates of the glutelin are liberated and pass into the hydrolyzate at different times: the glucose on hydrolysis for 9 h and the mannose on hydrolysis for 6 h (Fig. 1). The nature of the kinetic curves apparently reflects differences in the strengths of the bonds of the glucose and the mannose to the protein part proper of the glutelin molecule.

The action of dilute mineral acid also causes the specific hydrolysis of the peptide chain [2], and therefore the amino acids were determined in the glutelin hydrolyzates obtained. The majority of them accumulate in the hydrolyzate in very small amounts even after reaction for 24 h. However, exceptions are formed by aspartic acid and tyrosine. Their amount in the hydrolyzate is extremely small during the first 9 h of hydrolysis and then there is an abrupt accumulation; i.e., this takes place after the complete liberation of the glucose and its passage into the hydrolyzate.

In view of literature information on the particular ease of cleavage of a peptide bond at aspartic acid [2] because of the participation of its β-carboxy group in this process and the interrelationship that we have observed between the liberation of the amino acid and of the glucose, it may be assumed that one of the forms of the carbohydrate–protein bond in the glutelin molecule is effected through the β-carboxy group of aspartic acid.

In view of the known forms of the carbohydrate–protein bond in glycoproteins [3, 4] and taking into account the absence of amino sugars from the fodder yeast glutelin investigated (Elson–Morgan reaction negative [5, 6]), we assume that one of the forms of the carbohydrate–protein bond in the glutelin may be an ester bond of the glucose and the β-carboxy group of the aspartic acid. Such an assumption explains the sequence of accumulation of glucose and aspartic acid in the hydrolyzate. To confirm this, we studied alkaline hydrolysis as a method of cleaving the suggested form of the bond. Hydrolysis with weak bases in combination with kinetic measurements is widely used [7-9] and has given interesting results in the investigation of O-glycosidic and O-acylglycosidic bonds.

Alkaline hydrolysis under the catalytic influence of a 0.05 N solution of sodium carbonate was performed at 70°C and the process was checked every hour by gel filtration on Sephadex G-100 and also by IR spectroscopy (Figs. 2 and 3). The carbohydrates were split off most completely from the protein part proper of the glutelin molecule in 4 h.

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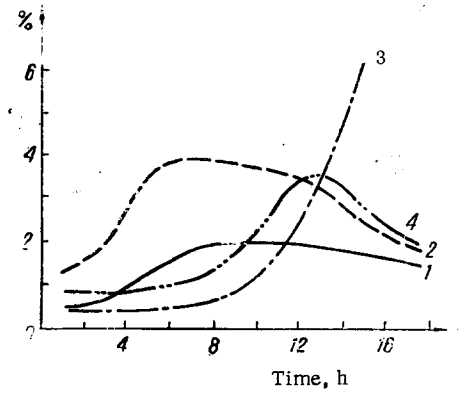

Fig. 1. Kinetic curves of the carbohydrates and the amino acids liberated in the hydrolysis of the glutelin by a 0.5 N solution of hydrochloric acid at 100°C: 1) glucose; 2) mannose; 3) aspartic acid; 4) tyrosine.

As a result of the alkaline hydrolysis, the carbohydrate moiety of the initial glutelin (Fig. 2a) was split off and, as a component of lower molecular weight, appeared after the protein component on gel filtration (Fig. 2b). The presence of several fractions in the carbohydrate moiety split off shows either a series of carbohydrate chains in the composition of the prosthetic grouping of the glutelin or the degradation of the carbohydrate moiety during the alkaline hydrolysis. The almost complete elimination of the carbohydrate moiety from the glutelin molecule under mild alkaline hydrolysis confirms the assumption of the ester nature of one of the carbohydrate–protein bonds.

On comparing the IR absorption of the initial glutelin (Fig. 3a) and that which had been subjected to mild alkaline hydrolysis (Fig. 3b) the following facts can be observed. The initial fodder yeast glutelin has absorption bands at 1740-1690 cm^{-1} and 1300-1200 cm^{-1}, which are characteristic [10, 11] for ester bonds of aspartic acid.

On alkaline hydrolysis (Fig. 3b), the absorption maximum at 3600-3000 cm^{-1}, reflecting the presence of hydroxy groups and also indicating the formation of salts in the liberation of the amino acid groups [12] rises considerably. The absorption maxima at 1440 and 1390 cm^{-1} increase, which shows the presence of dissociating carboxy groups [12]. Simultaneously, the absorption in the 1740-1690 cm^{-1} region falls sharply and becomes unclear and diffuse. It is likely that on alkaline hydrolysis the destruction and disappearance of the ester bonds in glutelin take place.

Thus, in the mild alkaline hydrolysis of the glutelin, the nature of the kinetic curves of the accumulation of carbohydrates and aspartic acid, permitting the assumption that one of the possible types of carbohydrate–protein bonds is an ester bond, has obtained unambiguous confirmation by the use of alkaline hydrolysis with monitoring by the gel filtration and IR absorption methods.

On the basis of the kinetic curves reflecting the mild acid hydrolysis of glutelin it can be seen that in addition to the liberation of carbohydrates and aspartic acid there is an increase in the amount of free tyrosine. These two amino acids are apparently participants in the carbohydrate–protein bond.

On considering the possible role of tyrosine in the formation of the bond with the carbohydrates, assuming the participation of the phenolic hydroxyl, we expected a shift in the short-wave part of the UV spectrum. The absorption curve in the UV region has a very flat maximum at 285 nm. This gives no clear idea of the possibility of a bond between the phenolic hydroxyl and the carbohydrates. The nature of the absorp-

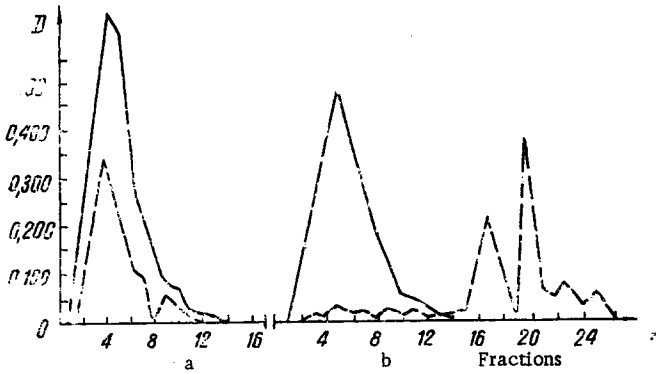

Fig. 2. Gel filtration on Sephadex G-100 of the initial glutelin (a) and its alkaline hydrolyzate (b): 1) protein by Lowry's method; 2) carbohydrates by the anthrone method.

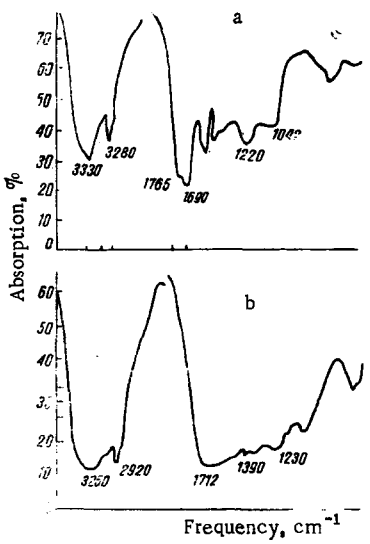

Fig. 3. IR spectra of the initial glutelin (a) and of the glutelin subjected to alkaline hydrolysis for 4 hours (b).

tion in the UV spectrum permits the assumption of an extended structure of the glutelin studied [13].

The nature of the prosthetic grouping of the glutelin was investigated by the methylation method. In each individual case the achievement of the maximum degree of methylation was determined from the IR spectrum.

The nature of the accumulation of methoxy groups as a function of the method of methylation of the glutelin (% on the dry substance) was as follows:

Method	Number of methylations	Content of $-OCH_3$ groups
Haworth	5	7.00
Purdie	3	7.73
Hakamori	3	8.30

Below we give information on the identification of the methylated monosaccharides:

Methylated monosaccharide	R_g according to the authors	R_g according to the literature
2,3,4,6-O-Tetramethylglucose	1.00	1.00 [14]
2,3,4,6-O-Tetramethylmannose	0.95	0.96 [15] 0.94 [16]
2,4,6-O-Trimethylglucose	0.76	0.76 [14]
3,4-O-Dimethylmannose	0.54	0.54 [16]

The results of the identification of the methylated monosaccharides show that the carbohydrate moiety of the prosthetic grouping is relatively little branched. The presence of 1,6 and 1,3 bonds between the monosaccharide residues in the prosthetic grouping may be assumed.

EXPERIMENTAL

Absorption in the UV spectrum was determined on an SF-4a instrument. The IR spectra were taken on an IKS-14 spectrograph in carbon tetrachloride as solvent (possibility of its use in the range from 900 to 5000 cm^{-1} [17]).

The isolation and fractionation of the glutelin was performed by a method described previously [1] from Candida fodder yeast of strain Kd-14 obtained from the inoculators of the Krasnodar Chemical Combine. After extraction of the glutelin with 0.2% sodium hydroxide solution, precipitation at pH 4.5, and repeated reprecipitation to constant composition, the substance was fractionated preparatively on a column of Sephadex G-100. The rate of elution was 0.5-0.7 ml/min. The protein fraction was determined by Lowry's colorimetric method and the carbohydrate fraction by the anthrone method.

The homogeneity of the glutelin fraction isolated was determined by independent methods – gel filtration on Sephadex G-100 and electrophoresis on paper in an ÉFA-1 instrument using borate buffer, pH 10.8, ionic strength 0.05, potential gradient 12-15 V/cm, and current strength 25 mA.

The composition of the prosthetic grouping was determined after hydrolysis in the presence of a 0.5 N solution of hydrochloric acid by paper chromatography [1]. A difference from the method indicated was the use in the quantitative determination not of standard curves but of a comparison of the spot of the hydrocarbon under investigation with the spot of a model solution of similar optical density. The prosthetic grouping was characterized by methylation according to Haworth's method [15], Purdie's method (in N. P. Elinov's modification) [16], and Hakamori's method [18]. The completeness of methylation was checked from the accumulation of methoxy groups both by titration [19] and by IR spectroscopy [17] in carbon tetrachloride. The methylated monosaccharides, after the hydrolysis of the whole of the methylated glutelin [14], were separated by paper chromatography in the butanol–ethanol–water (4:1:5) system. The methylated monosaccharides were identified by the R_g values of the spots observed relative to 2, 3, 4, 6-O-tetramethylglucose and by qualitative reactions characterizing the positions of the free hydroxy groups [20].

The degradation of the glutelin was studied by using mild acid and alkaline hydrolysis.

Mild acid hydrolysis was effected under the catalytic influence of 0.5 N hydrochloric acid solution at 100°C for 1-24 h. The hydrolyzates obtained were characterized with respect to the presence of amino acids and carbohydrates by paper chromatography [21] and by the plotting of kinetic curves.

Mild alkaline hydrolysis was performed under the catalytic influence of a 0.05 N solution of sodium carbonate at 70°C. The hydrolyzates obtained were studied by gel filtration with the colorimetric determination of the proteins by Lowry's method and of the carbohydrates by the anthrone method.

The determination of the amino sugars was performed by the Elson–Morgan method [5, 6] using as markers amino sugars of the firm California Corporation of Biochemical Research, Los Angeles.

SUMMARY

The prosthetic grouping of the glutelin of Candida fodder yeast is relatively little-branched. One of the forms of its bond with the protein part proper of the molecule of the glutelin is an ester bond.

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