HENRYK KOSTYRA[1], ROBERT PANASIUK[1], ELŻBIETA KOSTYRA[2]

CAN STARCH BE A REAGENT IN NON-ENZYMATIC GLYCOSYLATION OF PROTEINS?

Abstract

Non-enzymatic glycosylation of proteins plays an important role in the development of the diabetic complications and the aging processes. In nature, starch is the lipid-metallo-protein complex. The starch-protein complexes may serve as the source of the amino and aldehyde groups.This investigation was carried out to test the hypothesis that the starch can be a reagent in non enzymatic glycosylation of proteins in the physiological pH. Two suspensions were incubated: (1) Potato starch in the phosphate buffer at pH 6.0, 7.0 and 8.0, at 37°C for 24 h. (2) Potato starch in the phosphate buffer a leucine solution added. Amino nitrogen and glucose were determined. The decrease in the content of the amino groups during the incubation was observed for all starch suspensions. The highest decrease of the amino groups occurred at pH 6.0, and the lowest at pH 8.0. The addition of leucine to the starch suspensions intensified a decrease in amino group amount during the incubation. The content of aldehyde groups increased mostly during the incubation at pH 6.0 and decreased at the higher pH. The liberation of aldehyde groups from the starch suspension was not influenced by the addition of leucine. The increase the aldehyde group number during the incubation proves that starch was hydrolysed. However, the increase of amino points to the Maillard reactions which can include different components of the starch complex. Finally, the results seem to confirm our hypothesis, that starch can react on the non-enzymatic glycosylation of proteins at physiological pH.

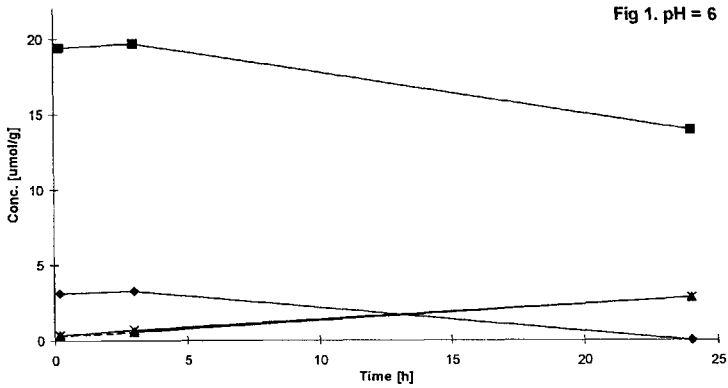
Introduction

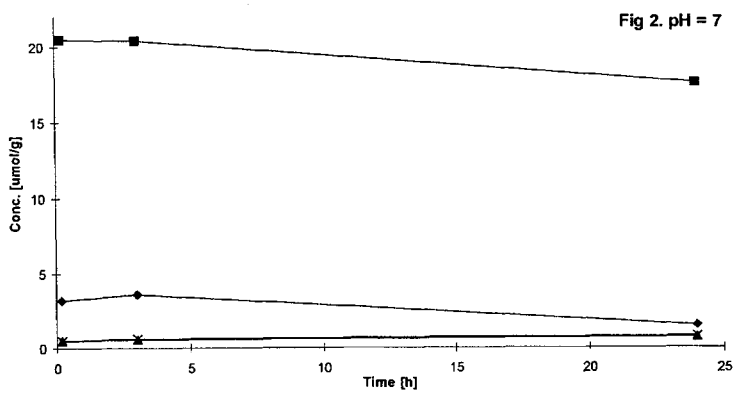
Non enzymatic glycosylation of proteins plays an important role in the development of the diabetic complications and the aging processes [1, 2]. The starch – protein complexes may serve as the source of the amino and aldehyde groups. Then, the incubation of these complexes with amino acids, peptides, proteins or reducing sugars can lead to the Maillard components. From the theoretical and practical points of view it is interesting, whether these reactions can occur in the physiological conditions. This

[1] *Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland*

[2] *Department of Chemistry and Biochemistry, High Pedagogic School, Olsztyn, Poland*

investigation was carried out to verify the hypothesis that starch can react on the non-enzymatic glycosylation of proteins at physiological pH.





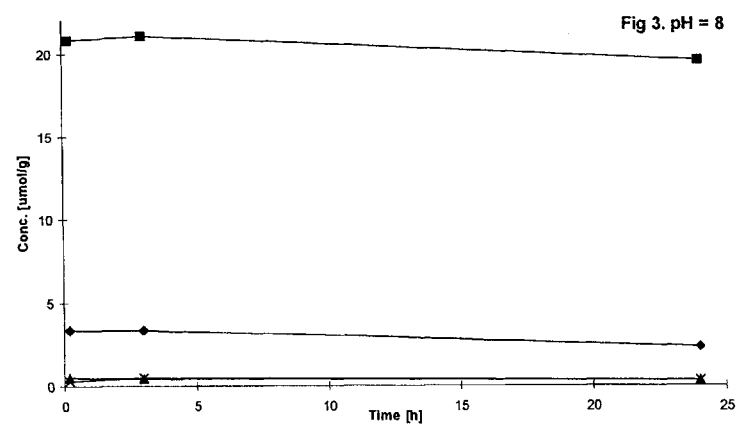


Fig. 1-3. Changes in the content of the free amino and aldehyde groups in the starch suspensions during the incubation with phosphate buffer at pH 6.0, 7.0 and 8.0, expressed in μmol/g starch. With leucine: ■ – (-NH_2), ⧓ – (-CHO), without leucine: ◆ – (-NH_2), ▲ – (-CHO).

Materials and Methods

Two suspensions were incubated on mechanical stirring: (1) Potato starch (30g) in 150 ml of the phosphate buffer (0.2 M) 15 ml water added pH 6.0, 7.0 and 8.0 were maintained at 37°C for 24 h, (2) Potato starch in 150 ml of the phosphate buffer 15 ml of leucine solution (30 mM.) added. Samples were collected at 0.6, 3 and 24 h intervals. The samples were centrifuged at 4000 g for 10 min. The supernatants were kept at -25°C followed by the determination of the amount of amino nitrogen and glucose immediately after thawing.

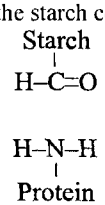
Amino nitrogen was determined by the Alvarez-Coque et.al. method [3]. An aliquot of samples (0.25 ml) was taken for analysis. The absorbance was measured at 340 nm. The calibration curve was obtained by using the leucine solutions with the concentrations ranging from 0 to 10 μmol/ml.

The concentration of glucose was determined by the Davis et.al. method [4]. The 1 ml samples were analysed. The absorbance was measured at 420 nm. The calibration curve was obtained from the glucose solutions of the concentrations ranging from 0 to 2.0 μmol/ml. The glucose solution was stabilized with 0.25 % benzoic acid.

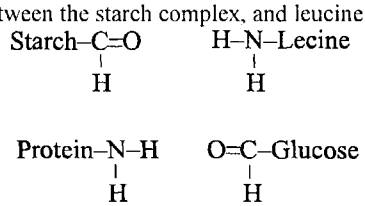
Results and Discussion

The decrease of the amino group content during the incubation was observed for all starch suspensions. The highest decrease occurred at pH 6.0, and the lowest at pH 8.0. The addition of leucine to the starch suspensions intensified the amino group ceasing on the incubation. The content of aldehydo groups increased mostly during the incubation at pH 6.0 and decreased at the higher pH. The liberation of aldehyde groups from the starch suspension was not influenced by the addition of leucine. In nature, starch is the lipid-metallo-protein complex. Thus, protein is the source of the free amino groups in this complex and the terminal glucose units deliver the aldehydo groups. The increase in the aldehydo group population during the incubation proves the starch hydrolysis. However, the increase of the amino groups points to Maillard reactions which involve various components of the starch complex, as follows:

a/ within the starch complex



b/ between the starch complex, and leucine and glucose



The highest decrease in the amino group content at pH 6.0 proves that the top reaction rate of carbonyl-amine condensation is reached in the moderately acidic medium.

General conclusion

The critical evaluation of the aldehydo groups determination suggests that glucose can partly be liberated from amylose by thermolysis. Thus, the method requires modification. However, the results seem to confirm our hypothesis, that starch can react on non-enzymatic glycosylation of proteins at physiological pH.

REFERENCES

[1] Horiuchi S., Araki N.: Gerontology, **40** (suppl 2), 1994, 10-15.
[2] Lyons J.L.: Contrib Nephrol. Basel, Karger, **112**, 1995, 1-10.
[3] Alvarez-Coque M.C.G., Hernandez M.J. M., Camanas R.M.V., Fernandez C.M.: Analytical Biochemistry, **180**, 1989, 172-176.
[4] D.K. Davies.: Am. J. Clin. Path. , **36**, 1966, 775-779.

CZY SKROBIA MOŻE BYĆ REAGENTEM W NIEENZYMATYCZNEJ GLIKOZYLACJI BIAŁEK?

Streszczenie

Nieenzymatyczna glikozylacja białek odgrywa ważną rolę w komplikacjach zdrowotnych u diabetyków i w procesach starzenia ludzi. Skrobia jest de facto kompleksem lipo-metalo-proteino-polisacharydowym. Fakt ten stał się powodem postawienia hipotezy, że może ona być reagentem w reakcji nieenzymatycznej glikozylacji białek przy fizjologicznych wartościach pH. Inkubację skrobi ziemniaczanej z dodatkiem i bez dodatku leucyny prowadzono przy pH 6.0, 7.0 i 8.0. Badano zmianę zawartości wolnych grup aminowych i aldehydowych w czasie inkubacji. Obniżenie zawartości grup aminowych występowało przy wszystkich wartościach pH, z tym że największe było przy pH 6.0. Podobnie przy wszystkich wartościach pH następował wzrost wolnych grup aldehydowych. Na podstawie uzyskanych wyników stwierdzono, że przy wszystkich wartościach pH skrobia ulegała częściowej hydrolizie. Natomiast wolne grupy aminowe pochodzące od białka związanego ze skrobią lub od leucyny wchodziły w reakcje Maillarda. Generalnie uznano zasadność postawionej hipotezy, że skrobia może uczestniczyć w reakcjach nieenzymatycznej glikozylacji białek w fizjologicznych warunkach pH.