2. Further evidence has been presented showing that sericin is probably a mixture of at least two simpler proteins, one of which is soluble and the other insoluble in cold water.

3. The study of silk proteins will be continued along various lines with special emphasis on the action of proteolytic enzymes on the various protein fractions.

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THE ISO-ELECTRIC POINTS OF GLIADIN AND GLUTENIN

By E. L. Tague

Received July 12, 1924 Published February 5, 1925

The characterization of proteins is a difficult matter. The ease with which the protein complex can be changed and the lack of knowledge concerning the manner in which the different groups are linked together, render the study of this group of compounds one of the most difficult in chemistry. In the preparation and purification of these compounds the iso-electric point of each individual protein is of prime importance.

In some protein investigations undertaken in this Laboratory considerable difficulty was experienced in obtaining preparations of gliadin from wheat (*Triticum vulgare*) which would agree with one another in their chemical characteristics. The same was true of glutenin also.

Since the chemical properties of both of these compounds have not been fully investigated, it was thought advisable to undertake a more thorough examination of them. The iso-electric point was one of the constants investigated.

The iso-electric point of ampholytes is in general determined in two ways: (1) by calculation from the ionization constants of the ampholyte as (a) an acid and (b) a base, and (2) by electrocataphoresis in a series of solutions whose hydrogen-ion concentrations differ from one another.

In some preliminary work on gliadin it was found that the electrocataphoresis method was unsatisfactory for two reasons: (1) the iso-electric point was so near the neutral point (P_H 7.00) that solutions of acids of the requisite concentrations had not sufficient conductivity, and (2) gliadin was so slightly soluble in buffer solutions that the ordinary protein tests failed, and consequently the direction of migration of the solute could not be determined in this way.

Investigations with the hydrogen electrode showed, however, that gliadin was sufficiently soluble in buffer solutions to cause an appreciable change in the hydrogen-ion concentration of such solutions, provided that the molar concentration of the salt was sufficiently small, on account of the precipitating action of the salt on the dissolved gliadin, and at the same

time large enough to have a constant buffer action. Mixtures of 0.01 *M* primary and secondary sodium phosphate solutions proved suitable for this purpose.

When gliadin was dissolved in one of these phosphate solutions whose hydrogen-ion concentration was greater than the iso-electric point of the gliadin, the dissolved gliadin behaved as a base and decreased the hydrogen-ion concentration of the phosphate solution. When the hydrogen-ion concentration of the phosphate solution was less than the iso-electric point, the dissolved gliadin behaved as an acid and increased the hydrogen-ion concentration of the phosphate solution.

Experimental Part

Preparation of Gliadin.—Well-washed gluten from a straight flour was used as a source of gliadin. The moist gluten was weighed and the water in the gluten estimated as two-thirds of the total weight. The moist gluten was cut into small strips and placed in a bottle of suitable size and to each portion of gluten was added twenty times its weight of dil. alcohol of such strength that, together with the water in the gluten, it formed an alcoholic solution containing 70% alcohol by volume. The bottle was tightly corked, placed on a shaking machine, shaken continuously for eight hours and then set aside and allowed to stand overnight. The supernatant liquid was siphoned off and filtered clear through asbestos and the filtrate was evaporated to a sirup under reduced pressure. At no time was the temperature allowed to rise above 65°. This sirup was poured into 100 times its volume of cold distilled water containing 10 g. of sodium chloride per liter. The precipitated gliadin was redissolved in dil. alcohol, the mixture filtered and the filtrate evaporated to a sirup, which was then poured into water as before. The re-solution and re-precipitation were repeated thrice more. Finally the sirup from the last evaporation was poured into a ten-fold volume of absolute alcohol. The precipitated gliadin was filtered off, washed thrice with dry ether and dried over sulfuric acid. This procedure gave a perfectly white, friable powder. Nitrogen determinations by the Kjeldahl-Gunning method gave 17.51% of nitrogen.

Preparation of Glutenin.—The residues from the alcohol extraction of the gluten were extracted repeatedly with 70% alcohol until no more gliadin was dissolved. They were then dried at ordinary temperatures, ground very finely and thoroughly extracted with ether. The residual ether was allowed to evaporate from the residues. Next the dried residues were dissolved in 0.2% sodium hydroxide and the solution filtered by suction through asbestos. The glutenin was precipitated by careful neutralization, with very dilute hydrochloric acid. The precipitate was washed several times with water by decantation. The precipitate was redissolved in 0.2% sodium hydroxide, re-precipitated by dil. hydrochloric acid and washed as before. This purification was repeated four times. Finally the precipitated glutenin was dehydrated very thoroughly with absolute alcohol and dried over sulfuric acid. This procedure gave a fine, white powder, not so friable as the gliadin. This preparation gave 17.39% of nitrogen by the Kjeldahl-Gunning method.

Determination of the Iso-electric Point

Gliadin.—Mixtures of 0.01 *M* solutions of primary and secondary sodium phosphates were used as solvents. These solutions had sufficient buffer action to give constant Sörensen (P_H) values with the hydrogen

electrode, and at the same time gliadin was sufficiently soluble in them to cause an appreciable change in the hydrogen-ion concentration of the solvent on either side of the iso-electric point.

After considerable preliminary work the following method was adopted. Mixtures of the phosphate solutions in different proportions were made and the Sörensen value of each was determined by means of the hydrogen electrode. A 500-cc. portion of each solution was pipetted into a bottle and 0.5 g. of gliadin added. Three drops of toluene for each 100 cc. of the solution were added in order to prevent bacterial action. The bottles were corked tightly, placed on a shaking machine and shaken constantly for eight hours. The mixtures were allowed to stand over-

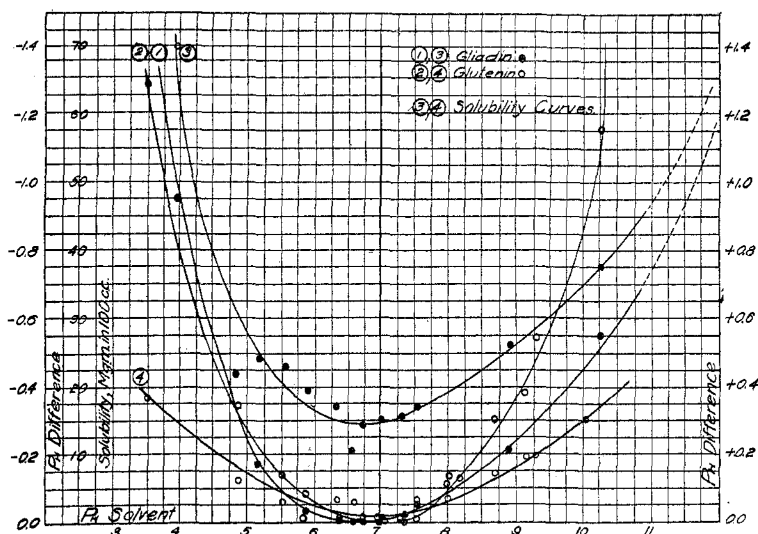

Fig. 1.

night, then filtered and the Sörensen values of the filtrate determined again with the hydrogen electrode. A comparison of these values with these originally obtained showed the influence of the dissolved gliadin on the hydrogen-ion concentration and also whether the gliadin acted as an acid or a base. The results obtained are indicated in Curve 1 of Fig. 1.

As a further source of information nitrogen determinations were made on each of the resulting solutions by the Kjeldahl-Gunning method. One hundred-cc. portions were taken excepting at or near the iso-electric point, where large volumes were necessary owing to the slight solubility of gliadin in this region. All of the results, however, are on a 100-cc. basis. The nitrogen was computed to gliadin, using the factor 5.70. These results, also, are set forth in Curve 3.

The hydrogen-ion determinations were made with the same apparatus

and by the same method as those described in a former paper,[1] excepting that the saturated potassium chloride cell was used. The measurements were made at a constant temperature of 25° and read to 0.5 millivolt. The Sörensen values were obtained from the tables of Schmidt-Hoagland.[2] The value of the saturated potassium chloride cell as compared with the normal cell was taken from the work of Fales and Mudge.[3]

In Curves 1 and 2 the negative sign before the difference in Sörensen values indicates merely that the dissolved protein decreased the original hydrogen-ion concentration of the solvent, that is, the protein behaved as a base, while the plus sign indicates that the protein increased the hydrogen-ion concentration of the solvent, that is, it behaved as an acid.

Glutenin.—The iso-electric point of glutenin was determined in the same way. The results are indicated in Curve 2.

In the chart the change brought about in the Sörensen value of the solvent by dissolving the protein in it, is plotted against the Sörensen value of the solvent alone. That portion of each curve on the left of the chart shows the influence of the dissolved protein in question when acting as a base, while that portion on the right indicates the influence as an acid.

Discussion of Results

An inspection of the curves in Fig. 1 shows that gliadin and glutenin are very similar in regard to their acidic and basic characteristics. Their iso-electric points are very close together. If we assume that these proteins, prepared as outlined above, are pure, the resemblance is all the more remarkable. The changes brought about in the Sörensen value of the solvent by dissolved proteins depend principally on their solubilities and their strengths as acids or bases, respectively. The curves show that both gliadin and glutenin are more soluble in acids than in alkalies of the same normality, if the buffer action of the phosphate solutions is ignored. Since flour contains inorganic phosphates, the curves show exactly the influence of the hydrogen-ion concentration in flour solutions.

The curves also show that the iso-electric point of gliadin is about P_H 6.5, while that of glutenin is about P_H 6.8–7.0. In the method of preparation, absolute alcohol was used to dehydrate both proteins, as well as to separate them. The influence of alcohol upon the acidic and basic properties of these proteins has never been investigated, so that the method of preparation described above may not give the pure compounds. This point is under investigation in this Laboratory.

Since the minimum solubilities of ampholytes occur at their iso-electric points, nitrogen determinations should afford additional evidence of the

[1] Tague, This Journal, **42**, 173 (1920).

[2] Schmidt-Hoagland, *Univ. California Pub. in Phys.*, **5**, No. 4 (1919).

[3] Fales and Mudge, This Journal, **42**, 2434 (1920).

correctness of the above method[4] for determining the iso-electric points. The nitrogen determinations for both gliadin and glutenin show full agreement with the electrometric method.

Summary

The iso-electric points for gliadin and glutenin, respectively, have been determined, and have been found to be *P*H 6.5 for gliadin and *P*H 7.0 for glutenin.

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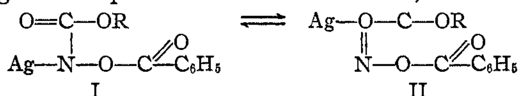
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CINCINNATI]

SOME NEW HYDROXY-URETHANS AND CHROMO-ISOMERIC SILVER SALTS OF THEIR ACYL DERIVATIVES. II[1]

BY RALPH E. OESPER AND WALTER A. COOK

RECEIVED JULY 31, 1924 PUBLISHED FEBRUARY 5, 1925

A previous paper with the above title by L. W. Jones and one of us[2] contained an account of some preliminary studies of the white and yellow forms of the silver salts of benzoylated carbalkoxy-hydroxamic acids. It was suggested that these salts, which are reversibly transformable into each other, might correspond to the isomeric forms, I and II, or they might



be polymers of either I or II. Molecular-weight determinations were inconclusive, while the evidence deduced from hydrolysis of the products obtained by replacing the silver atom by alkyl groups indicated that the yellow forms reacted as though they possessed Structure I. The behavior of the white forms under similar conditions was not studied in detail. This study has now been made, and the results lead to the conclusion that when direct replacement of the silver by alkyl occurs, both the yellow and white forms have Structure I. It has been pointed out by several investigators of similar cases[3] that this assumption is not always warranted and, furthermore, the media in which the reactions occurred may induce partial reversion of one form of the silver salt to the other until an equilibrium is reached, the latter condition then being disturbed by the interaction with the alkyl halide. No visible evidence of such transformation

[4] Michaelis, "Wasserstoffionen Konzentration," Julius Springer, Berlin, **1914**, p. 40.

[1] This communication is an abstract of a thesis submitted by Walter A. Cook in partial fulfilment of the requirements for the degree of Doctor of Philosophy, at the University of Cincinnati.

[2] (a) Jones and Oesper, THIS JOURNAL, **36**, 2208 (1914); (b) **36**, 726 (1914).

[3] Sidgwick, "Organic Chemistry of Nitrogen," Oxford Clarendon Press, **1910**, pp. 103, 81. Tafel and Enoch, *Ber.*, **23**, 1550 (1890). Comstock, *Am. Chem. J.*, **13**, 514 (1891). Lander, *J. Chem. Soc.*, **83**, 418 (1903).