

Application of the pH-Stat for the Measurement of Proteolysis

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The methods ordinarily used to follow the opening of peptide bonds in proteins often fail in cases of limited proteolysis due to lack of sensitivity. Provided that the enzymatic reaction takes place at pH-values which are so high that most of the liberated amino groups exist in the unionized form, it is, however, possible to follow the opening of the peptide bonds by continuous titration at a constant pH by means of a Jacobsen-Léonis pH-stat. Different constructions of this device will be discussed. Using the pH-stat it is possible at pH 8 to follow the opening of a single peptide bond in the conversion of ovalbumin to plakalbumin. It has been attempted to correlate the opening of this bond with the change in solubility and crystal form. It was further observed that the salt-free ovalbumin solutions need a small continuous addition of alkali in order to keep pH constant, and the rate of this alkali addition seems to depend on the pH at which the ovalbumin was stored previously to the experiment.

Studies on a Myosin Fragment Obtained by Ammonium Sulphate Treatment of Myosin

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Some preliminary data about the fragmentation of myosin with neutral ammonium sulphate have been given in another communication¹.

By treatment of the myosin with ammonium sulphate at 20° C two different fragments are obtained in nearly equal amounts by weight. Further studies have now been performed on the water-soluble fragment (H₂₀). This fraction precipitates in the preparation process between 40—50 % saturation of ammonium sulphate but cannot, after having been redissolved, be precipitated again with ammonium sulphate. It does not show any ability to asso-

ciate with actin. The fragment is unstable and with time it is transformed to a large extent into dialysable pieces. During the deterioration process many different ultraviolet spectra can be obtained by different treatments. The fraction seems at first to break into three different parts as indicated by paper-chromatographic analysis. The final product obtained is dialyzable and has a high positive charge. Amino acids can no longer be obtained from it by acid hydrolysis.

Different means have been tried to stop the deterioration of the H₂₀ fragment when it just has been obtained by the ammonium sulphate treatment. It has hereby been found that a treatment of the newly formed fragment with calcium hydroxide can stop the deterioration and a product is obtained which has the ability to associate with actin with an ensuing viscosity increase. This actin complex responses to ATP with a viscosity decrease independent of the salt concentration, and the viscosity increases again after a certain time to the normal level. A treatment with potassium oxalate of the calcium hydroxide-treated H₂₀ fragment does not alter this effect. If the calcium hydroxide is added in a later step when the deterioration has begun, no effect of it will be found. As yet no other means have been found to restore the activity. The protein treated with calcium hydroxide shows an UV absorption spectrum with a maximum at 260—270 m μ .

From different experiments it is quite obvious that the myosin molecule must possess a unique structure. Probably some compound containing calcium is of importance in the reactions of the myosin, and when the calcium ions are removed this compound is very labile.

1. Snellman, O. *Biochim. et Biophys. Acta* (In press).

Studies on Human Acute Phase Protein (C-reactive Protein)

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The fractionation of a sample containing sera from two persons in the acute phase of streptococcal pharyngitis and maxillary sinusitis, respectively, has been reported by Hed-

lund and Brattsten¹. The fractionation was carried out by continuous zone electrophoresis² in a starch supporting medium at pH = 8.5 and the ionic strength = 0.03. The presence of acute phase protein in the fractions was determined by the capsular swelling reaction (Löfström³). Pneumococci suspensions of type 23 B were used. Reactive substance was found in the γ -globulin fractions exclusively, the capsular swelling titration curve showing a rather striking conformity with the γ -globulin distribution curve.

In order to check these results the same fractions were afterwards analysed against a pneumococci suspension of type 27, which was even more sensitive than type 23 B, and by precipitation with C-reactive protein antiserum (obtained from Schieffelin & Co., New York). Both tests confirmed the findings reported.

In the fractions obtained in the electrophoretic separation the reactive substance was present in mixture with other proteins of the γ -globulin type. This material has been studied as to its solubility in the presence of ammonium sulfate. As judged from the capsular swelling analyses, the reactive substance remains in solution at half saturation of this salt, but some precipitation occurs as the salt content is further increased. However, complete precipitation could not be achieved this way; even at saturation of ammonium sulfate the supernate showed a relatively strong activity reaction.

The initial fractionation experiment also showed that adequate isolation of acute phase protein could be obtained at a considerably lower total resolution than that used. Hence the procedure could be simplified by increasing the rate of fractionation. In subsequent work a quantity corresponding to 1.5 ml of undiluted serum has been fractionated per hour. In all, 65 ml of serum has been worked up. This includes material from three different cases of pneumonia in the acute stage. In order to establish a possible influence of the ionic strength on the mobility of the active material two fractionations have also been carried out at the ionic strength 0.06, other conditions remaining unchanged. After enrichment of the proteins, the fractions have been analysed by the capsular swelling reaction using type 23 B and 27 pneumococci suspensions and the C-reactive protein antiserum test. In all instances complete qualitative agreement with the primary result was obtained, thus showing that acute phase protein, developed in the diseases stated, resembles the γ -globulins in electrophoresis under the reported conditions.

The capsular swelling reaction was found to be more sensitive than the antiserum reaction.

Six months of storage in the frozen state (at -10°C , in Ringer's solution) did not affect the titer of the active substance.

1. Hedlund, P. and Brattsten, I. *Scand. J. Clin. Lab. Invest.* 7 (1955) 99.
2. Svensson, H. and Brattsten, I. *Arkiv Kemi* 1 (1949) 401; Brattsten, I. *Continuous zone electrophoresis by crossed velocity fields in a supporting medium*. Diss. Uppsala 1955.
3. Löfström, G. *Acta med. Scand.* Suppl. 141 (1943).

On the Localization and Composition of Phosphoprotein in *Escherichia coli B*

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In an earlier communication the preparation of a new phosphorylated amino acid in *Lactobacillus casei* has been reported^{1,2}. Several peptides containing this compound were also found. In attempts to prepare more high molecular phosphoproteins the following experiments were carried out with *E. coli B*.

The bacteria was cultivated on Friedleins synthetic medium containing Na-lactate as the carbon source and NH_4Cl as nitrogen source³. To this medium was added 1 mC radioactive phosphate per liter. Procedures for cultivation and separation of bacteria have been described by Palmstierna and Holme^{4,5}. In this experiment the initial bacterial density was 1.25×10^9 cells/ml. At the end after 120 minutes of incubation the density was 1.95×10^9 cells/ml. 24 g of freeze-dried bacteria was obtained.

Suspensions containing 0.5 g dry weight bacteria/20 ml in 0.04 M Tris-buffer were shaken in the Raytheon disintegrator for 30 minutes during continuous cooling. The cell walls possibly contaminated with some cytoplasmatic debris⁶ were removed from the lysed solution by centrifugation at 40 000 r.p.m. One part of