The liberation of acid and base binding groups on denaturation of ovalbumin

KAUZMANN and co-workers have shown[1,2,3] that ovalbumin in the presence of urea or guanidinium chloride undergoes time dependent changes which may be profitably studied by the techniques of optical rotation and viscosity. These studies demonstrate that urea and guanidinium ion probably unfold the ovalbumin molecule by simultaneously rupturing a small number of hydrogen bonds in the secondary structure. We have recently observed that the denaturation of ovalbumin in the presence of guanidinium chloride is accompanied by the liberation of both acid and base binding groups which may be measured in the JACOBSEN-LÉONIS[4] auto-titrator. We report some of our findings here since the method would appear to have general application in protein denaturation studies[5,6], and since the results point to the importance of polar group hydrogen bonding in stabilizing the secondary structure of the native protein.

At pH values below 7 acid must be added to a solution of ovalbumin in the presence of guanidine to maintain a constant pH; at pH values above 7 base is required. The rate of acid or base consumption, as recorded by the auto-titrator is not first order but appears, as in the optical rotation studies of SIMPSON AND KAUZMANN[1], to consist of two reactions which we will denote as primary and secondary. If the uptake of acid (or base) by the slow, secondary reaction is subtracted from the overall process the primary reaction has apparent first order dependence over the pH range investigated (pH 3–11).

The denaturation process has been followed at various pH values and at a constant concentration of guanidinium chloride of 2.59 *M*. Fig. 1 presents the measured consumption of acid (below pH 7) and base (above pH 7) at infinite time by the primary reaction at each pH. The plot may be considered as the titration curve of the liberated groups and is consistent with the liberation of 8 carboxylate groups in the acid range and 8 tyrosine or lysine groups in the alkaline region. The calculated p*K*'s correcting for the salt effect[7] are 4.9 and 9.8 respectively.

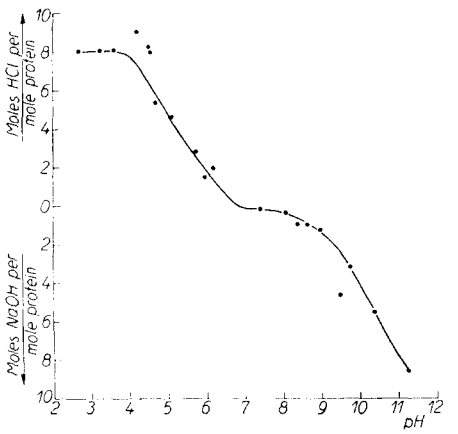

Fig. 1. Liberation of acid and base binding groups from ovalbumin (primary reaction) in 2.59 *M* guanidine hydrochloride. Temp. 15.4° C.

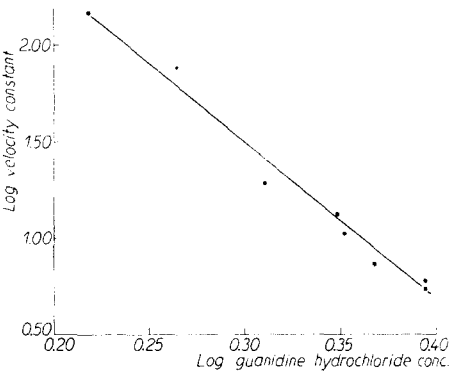

Fig. 2. Dependence of velocity constant of group liberation on guanidinium chloride concentration at 15.4° C.

A series of runs were made at a constant pH (4.71) and at differing concentrations of guanidinium chloride. The logarithm of the velocity constant of the primary reaction is plotted against the logarithm of the guanidinium chloride concentration in Fig. 2. The slope of the resulting line was 7.8 and therefore the order of the reaction with respect to guanidinium ion, n^*, is undoubtedly close to 8.

In terms of the ideas of SIMPSON AND KAUZMANN this would mean that the simultaneous attack of 8 guanidinium ions is required to unfold the helical hydrogen bonded fabric of the molecule.

It may be recalled that the spectrophotometric work of CRAMMER AND NEUBERGER[7] revealed 7–8 non-ionizing tyrosine residues in native ovalbumin and that these authors suggested the possible involvement of these missing tyrosines in intramolecular hydrogen bonding. The evidence presented in Figs. 1 and 2 suggests that 8 tyrosyl-carboxylate hydrogen bonds are ruptured in ovalbumin through a concerted attack by 8 guanidinium ions. This process may be responsible

for the unfolding of certain regions of the secondary structure. It will appear that this interpretation places emphasis on the importance of the postulated tyrosyl-carboxylate linkage in stabilizing significant areas of the secondary structure[8,9]. We may infer that such a stabilizing bridge could be important in other proteins such as serum albumin[10], lysozyme[11], and ribonuclease[12] where non-ionizing phenolic hydroxyl groups have been demonstrated in the native protein.

A detailed report of this work will appear later in *Comptes rend. trav. lab. Carlsberg, Série chim.*

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Early changes of myosin in vitamin E-deficient rabbits

The early onset of creatinuria in animals with nutritional muscular dystrophy has been explained as being due to incapacity of muscle to retain creatin after incorporation from the blood stream[1,2]. On the other hand, MENNE[3] reported experiments indicating that myosin acts as an apo-enzyme in creatine synthesis from arginine, histidine and choline. Similar evidence is showed by the recent experiments of NEKHOROCHEFF[4].

Researches in our laboratory on contractile muscle proteins in vitamin E-deficiency show very early myosin alterations in this condition which are probably in strict connection with the onset of creatinuria.

Rabbits were fed on Houchin and Mattill diet. Myosin was prepared according to Mommaerts and Parrish procedure. We began to study the biochemical properties of myosin of these rabbits after the first week of diet (8th and 11th day), when histological findings and polarized microscopy are completely negative.

Even during the myosin preparation we had already observed that after a longer period of diet (2 weeks), a large part of the myosin precipitated at 0.28 *M* KCl. Starting from rabbits kept for one week on diet, myosin precipitation curves were carried out at variable KCl concentrations and it was observed that dystrophic myosin very early loses its full solubility at 0.1 *M* KCl, pH 6.8, while normal myosin shows a sharp increase in solubility from 0.05 to 0.1 *M* KCl. Viscosity measurements, carried out with an Ostwald viscosimeter at 0° C, show a decrease in $Z\eta$ values from 0.2, which is the normal value for myosin, down to 0.13.

More detailed accounts of the present experiments will be published elsewhere.

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