arises: Which constituent of the complex splits the ATP, the myosin or the actin? We have no direct information on this problem. However, taking into account the fact that the complex does not seem to suffer any irreversible change after the sonication, it seems more probable that the ATP splitting is caused by the (F-)actin ATPase. If this is assumed to be the case, it follows that the accelerated phosphocreatine splitting during the sonication is a consequence of the increased rate of production of the ADP available to the creatine kinase system. As one possibility, we can expect that sonically activated F-actin ATPase itself is very much activated under influence of myosin. Its activity must be more than 100 times greater than that of the pure F-actin under the same conditions.

This work was supported by a grant No. A-4534 from the National Institutes of Health, U.S. Public Health Service.

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Received January 31st, 1963

Biochim. Biophys. Acta, 74 (1963) 142–144

SC 2236

Association of gluten proteins in solution

The presence of association–dissociation phenomena in solution has been well established for the complex seed proteins of the peanut[1,2], soyabean[3,4], and jack bean (urease[5]). This communication reports evidence for the existence of an aggregation equilibrium in solutions of a wheat protein fraction.

The acetic acid-soluble proteins of an Australian wheat (Gabo) flour, extracted by the method of COATES AND SIMMONDS[6], were chromatographed on a column of CM-cellulose according to the procedure described previously[7], but on a larger scale. The contents of the tubes corresponding to the required fraction (fraction B) were combined and subjected to the same chromatographic procedure several times, until traces of adjacent peaks could no longer be detected. Chromatographic patterns of fraction B and the parent acetic acid extract are compared in Fig. 1.

Fraction B dissolved readily and completely in the solvent used for ultracentrifuge experiments (0.09 M NaCl, 0.01 M sodium acetate, 1.0 M dimethylformamide, pH adjusted to 4.1 with acetic acid) to give a clear solution, which was then dialysed against the same buffer at 4° for 24 h. Lower concentrations were prepared by weight dilution of the stock solution, the concentration of which was determined colorimetrically[8]. Sedimentation-velocity experiments were performed at 59 780

rev./min in a Spinco model E ultracentrifuge equipped with a phase-plate schlieren diaphragm and R.T.I.C. unit.

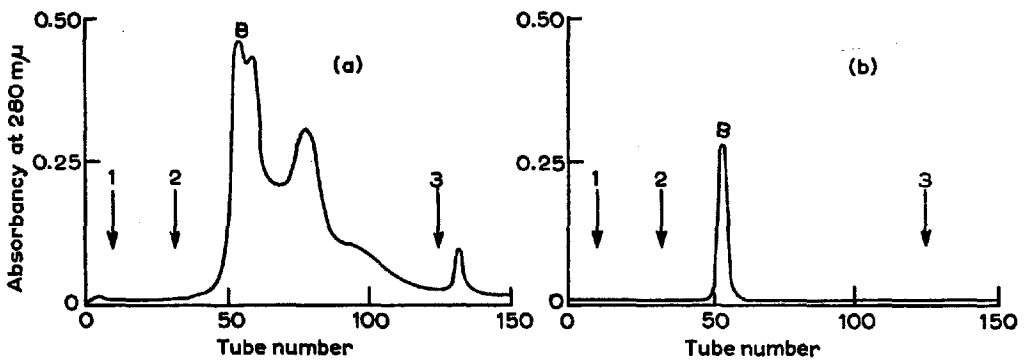

Fig. 1. Elution patterns obtained in the chromatography of (a) the whole acetic acid extract of flour, and (b) fraction B. Columns: 1.5 × 15 cm of CM-cellulose, equilibrated with 0.005 M acetate buffer (pH 4.1). Legend for elution procedure: (1) 0.005 M acetate buffer containing 1.0 M dimethylformamide (pH 4.1); (2) start of gradient to 0.2 M NaCl in acetate–dimethylformamide buffer; (3) 0.5 M NaCl in acetate–dimethylformamide buffer. Size of fractions: 10 ml.

Fig. 2 summarizes the data obtained for the concentration dependence of sedimentation coefficient, in which the square root of the second moment[9] has been used to locate the boundary position; the concentration values refer to the average concentration between the first and last exposures. The boundaries were generally fairly symmetrical, *e.g.* Fig. 3a, but in the experiment at the highest concentration, partial resolution of a second peak was clearly evident (Fig. 3b).

In studies of proteins positive concentration dependence of sedimentation coefficient, evident in Fig. 2, has generally been encountered only in interacting systems involving rapid association–dissociation equilibria[10] (*cf.* however, RUPLEY AND NEURATH[11]). The partial resolution of a second peak, as in Fig. 3b, has also been observed with other systems in rapid aggregation equilibrium, *e.g.* β-lactoglobulin[12,13], α-chymotrypsin[14] and the mercury derivative of papain[15]. Furthermore, from theoretical considerations of rapidly reacting systems of the type $nA \leftrightharpoons A_n$, GILBERT[16,17] has shown that for such resolution to occur trimers or larger aggregates must be present: no resolution will occur in a monomer ⇋ dimer system. Failure to observe a second peak at the lower concentrations does not, however, preclude the possibility that aggregate species other than dimers are present, since the GILBERT theory

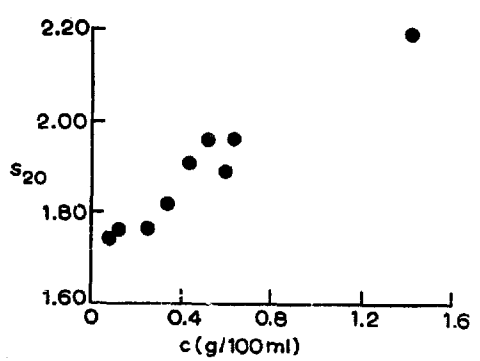

Fig. 2. The concentration dependence of the sedimentation coefficient of fraction B in acetate–chloride–dimethylformamide buffer (pH 4.1; *I* 0.10).

predicts that even for a system involving monomer $\leftrightharpoons$ hexamer equilibrium, a critical concentration must be exceeded for apparent resolution to occur.

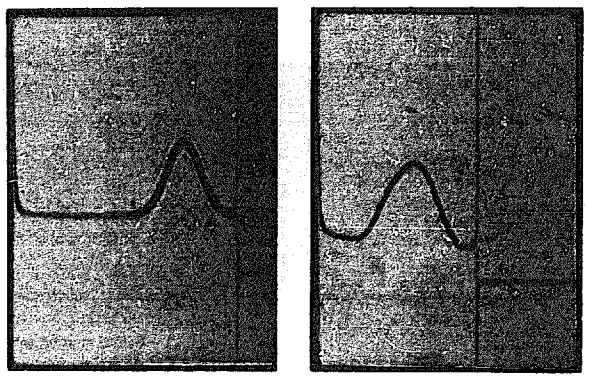

Fig. 3. Ultracentrifuge patterns of fraction B in acetate–chloride–dimethylformamide buffer (pH 4.1, I 0.10), after 100 min at 59780 rev./min. (a) 0.57%; (b) 1.49%. Sedimentation is from right to left.

Further evidence of rapid, reversible association was provided by boundary analysis measurements on the ultracentrifuge patterns[9]. The presence of a chemically reacting system causes additional boundary spreading, and hence the apparent diffusion coefficient is larger than that calculated from a diffusion experiment[18,19]. That gluten fraction B belongs to this category is clearly evident from the results presented in Fig. 4 (D = slope/2): curve A was obtained by analysis of ultracentrifuge patterns, while curve B summarizes the data calculated from a diffusion run at the same concentration in the ultracentrifuge at low speed.

FIELD AND OGSTON[19] have developed a theoretical procedure for boundary analysis which takes into account the spreading of a chemically reacting system of the type $nA \leftrightharpoons A_n$. Application of the method to results obtained with haemoglobin under dissociating conditions[18] showed that the plot of $\sigma^2_{corr.}$ *versus* t is linear provided

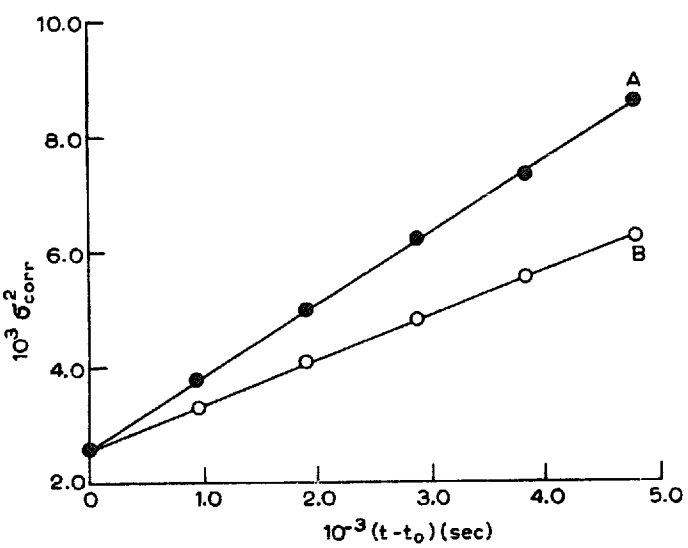

Fig. 4. Boundary analysis of patterns obtained in the ultracentrifuge: ●—●, measurements from experiment performed at 59780 rev./min, corrected for centrifugal but not concentration-dependence effects; O—O, measurements from run performed at 9945 rev./min. Initial concentration in each experiment was 0.57%.

equilibrium attainment is rapid. Although the results of this study are qualitatively open to this interpretation, a critical test by the FIELD AND OGSTON theory cannot be made, since no data on the concentration dependence of sedimentation coefficient under non-associating conditions or the diffusion coefficient are available. In this connexion it should be pointed out that the boundary spreading measurements on the patterns obtained in the low-speed run (or indeed from a classical diffusion experiment) contain a contribution from the equilibrium reaction, and therefore do not give a true measure of the diffusion coefficient.

The existence of rapid, reversible aggregation in solutions of gluten proteins has not been previously reported, although there are several studies in which some features of the results may be interpreted in terms of association–dissociation phenomena, *e.g.* the non-ideal osmotic pressure data for gliadin dispersed in 6.6 M urea[20], and the anomolous light-scattering behaviour of gliadin in 0.1 M acetate–3 M urea[21]. Concentration-dependent aggregation was not evident, however, in other studies, *e.g.* the positive, linear dependence of Hc/τ values for gliadin dissolved in dil. HCl[21], and the failure to observe variations of molecular weight at different concentrations of gluten fractions in 0.017 M aluminium lactate (pH 3.2) as solvent[22]. It would therefore appear that the association phenomenon detected in this study does not exist in all solutions of gluten proteins, but that it may well be a property of gluten dispersions containing reagents capable of hydrogen-bond rupture.

I wish to thank Professor A. E. ALEXANDER, University of Sydney, for providing the opportunity to carry out the ultracentrifuge runs, and Mr. A. NETSCHEY for technical assistance with these experiments. I am grateful to Dr. L. W. NICHOL of Clark University for advice on the interpretation of sedimentation experiments which involve associating systems.

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Received November 30th, 1962