Preliminary Notes

PN 1313

Polydisperse ultracentrifugal patterns of ovalbumin in presence of aluminium

Unexpected polydispersity of crystalline ovalbumin during ultracentrifugal studies in dilute acetate buffer (pH 4.59) has been reported by CREETH and coworkers[1]. They ascribed the asymmetry and occasional resolution of the pattern into two peaks ($s_{20,w}$ = 3.6 S and 6.1 S) to the effect of high pressure, but noted that the polydispersity was not reproducible.

We observed a similar polydispersity during ultracentrifugation of an isoelectric solution of four times crystallized ovalbumin in 0.1 M NaCl. The effect only appeared when we used a particular Dural (aluminium alloy) centerpiece. Solution left in contact with this centerpiece for 24 h led to the formation of whisker-like growths originating from the wall surface, suggesting corrosion in defective spots of the anodized surface to be responsible for the effect. In view of this we decided to study the effect of adding $AlCl_3$ to unbuffered ovalbumin solutions before centrifuging them in plastic (Kel-F) centerpieces.

The effect of adding increasing concentrations of $AlCl_3$ to a 0.25 mM solution of ovalbumin (1.1 %) in 0.1 M NaCl (pH 5.0) is shown in Fig. 1. A molar ratio of aluminium to protein of 1:10 (Fig. 1A) or 1:2.5 (1B) did not result in polydispersity ($s_{20,w}$ = 3.3 S), although a small shoulder can be discerned in the pattern of Fig. 1B. At a molar ratio of 1:1 (Fig. 1C) or 4:1 (Fig. 1D) about 25 % or 65 %, respectively, of the total ovalbumin was observed as a fast component ($s_{20,w}$ = 6.1 S). A further increase in aluminium concentration resulted in a diffuse sedimentation pattern and a cloudy solution, making the interpretation of this experiment uncertain. Addition of aluminium to protein solutions immediately before centrifugation resulted in patterns which were indistinguishable from those obtained 24, 48 and 72 h after mixing, indicating the reaction involving aluminium and ovalbumin to be completed in less than 1 h.

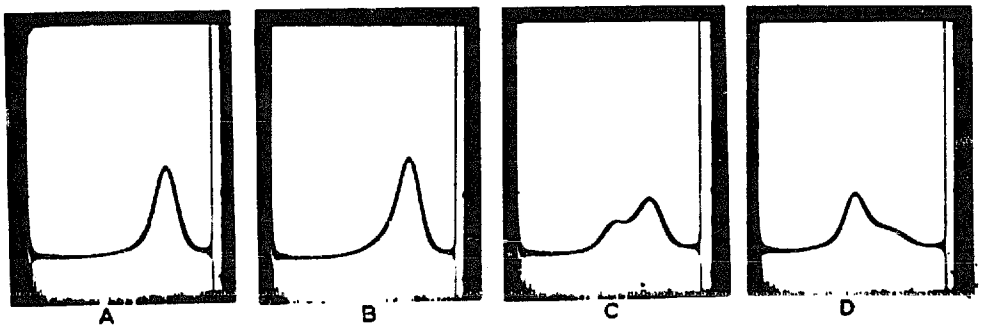

Fig. 1. Representative ultracentrifugal pattern of 0.25 mM ovalbumin (1.1 %) in 4 different concentrations of $AlCl_3$ (pH 5.0): 0.025 mM (A), 0.1 mM (B), 0.25 mM (C), 1 mM (D). An equal volume of $AlCl_3$ was slowly added to 0.5 mM ovalbumin and the solution adjusted to pH 5.0. Centrifugation in Kel-F centerpiece, at rotor temperature 20° ± 0.1, 59780 rev./min. Centrifugation is from right to left and all exposures are made about 65 min following speed; bar angle 60°.

The ratio of 4 aluminium atoms per ovalbumin molecule (Fig. 1D) was selected to study the effect of pH on the extent of association. As seen in Fig. 2, the association reached a maximum of 65–70 % and it was independent of pH in the range of 4.5–6.2, while complete dissociation into monomer occurred within 0.5 pH unit to either side of this range. These experiments, at a protein concentration of 1.1 % indicate a complex unit to be present having a sedimentation constant of $s_{20,w} = 6.1 \pm 0.1$ S.

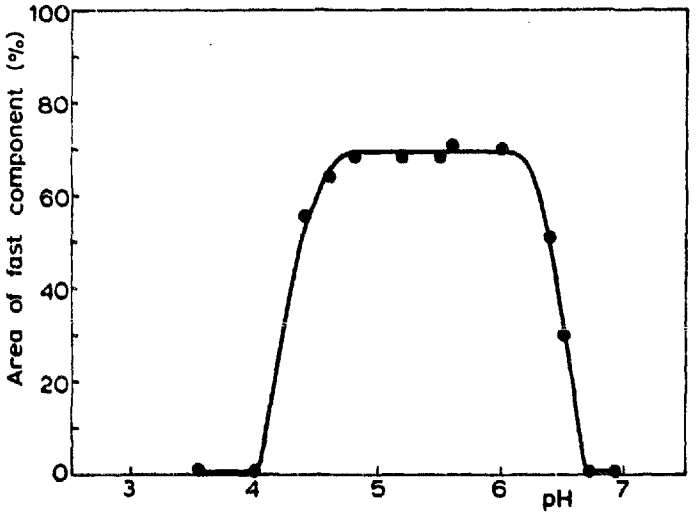

Fig. 2. The dependence of ovalbumin association in $AlCl_3$ on pH. A stock solution containing 0.25 mM ovalbumin and 1 mM $AlCl_3$ in 0.1 M NaCl was adjusted to the desired pH with 0.1 N NaOH or 0.1 N HCl. Conditions of ultracentrifugation as in Fig. 1; area measurements were made by planimetry.

Parallel studies of these protein solutions showed no significant variation in the optical rotation and the viscosity ($[\alpha]^{20°}_{578\,m\mu} = -32° \pm 2$; b_0 in MOFFITT equation[2] $= -163 \pm 3$; $\eta_{sp}/C = 0.053 \pm 0.004$ dl/g). Therefore the association reaction upon the addition of aluminium does not appear to be accompanied by any significant denaturation of the ovalbumin molecule. Assuming the intrinsic viscosity and the partial specific volume to be identical for monomer and complex we might use the SCHERAGA–MANDELKERN equation[3] to obtain an estimate of the molecular weight of the complex. If the axial ratio is relatively unchanged by the association, this equation indicates that the complex ($s_{20,w} = 6.1$ S) corresponds to a protein trimer. The effect of protein concentration and equilibrium sedimentation studies are necessary to verify this value.

The association of 0.25 mM ovalbumin 1 mM $AlCl_3$ was found to be reversed by the addition of fluoride, phosphate, citrate, or oxalate at pH 5.0 at a concentration of 4 mM. However, even extensive dialysis against 0.016 M acetate buffer–0.1 M NaCl (pH 5.0) did not reverse the association.

Among the metal salts tested at pH 5.0, only trace amounts of ovalbumin associated in the presence of $CdCl_2$ and $FeCl_3$ at 1 mM. As identical polydisperse patterns were obtained in $Al_2(SO_4)_3$, alum, and $AlCl_3$ solution, the reaction appears specific for aluminium at pH 5.0.

A survey of other proteins at pH 5.0 (established with 0.02 M acetate buffer) revealed that a 1 % solution of bovine plasma albumin, β-lactoglobulin, and plak-albumin associated in the presence of $AlCl_3$ while chymotrypsinogen, RNAase (EC 2.7.7.16), and trypsin (EC 3.4.4.4) did not.

The mechanism by which ovalbumin associates in the presence of $AlCl_3$ is not yet clear. The involvement of carboxylate groups on the protein is suggested by the acid limb of the pH profile, the reversal of the association by polycarboxylic acids, and the strong binding of aluminium to oxygen[4]. The failure of acetate to interfere with the association might suggest that chelation of the metal by the protein is necessary. However, simple trivalent aluminium only exists below pH 4.0 and a hydrolytic polymer $Al_8(OH)_{20}^{4+}$, is postulated in the range pH 4–7 by MATIJEVIC and coworkers[5]. It appears that there is a progressive addition of hydroxide ions to the polymer and the eventual dissolution of the polymer into $Al(OH)_3$ at alkaline pH. It is possible then, that the ovalbumin complex is dependent on the size and electrical state of the aluminium polymer.

In any case, it appears that polydisperse patterns of some proteins previously exposed to aluminium can, under certain conditions, be attributed to the presence of aluminium alone.

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Magnetische Untersuchung einer reversiblen Myoglobin-Denaturierung

Anionische Netzmittel genügender Kettenlänge bewirken eine Denaturierung von Hämoglobin zu denaturiertem Globin-Hämochrom und von Myoglobin zu denaturiertem Myoglobin-Hämochrom. In einer vorangegangenen Arbeit (KROMPHARDT UND LÜBBERS[1]) wurde der Umwandlungsprozess mit optischer Methodik untersucht und erkannt, dass er sich nach einem Alles-oder-Nichts-Gesetz vollzieht. Verdünnungsversuche liessen sich am besten so deuten, dass im Gegensatz zum Hc die Bildung des Mc beider Wertigkeitsstufen revertierbar sein kann. Da dieser Befund die Frage nahelegte, ob die Denaturierung zum Mc(II) wirklich in einer derartig vollständigen Umbildung der Bindung der prosthetischen Gruppe zum Eiweiss besteht wie bei dem

Abkürzungen: Hc(II), Denaturiertes Globin Ferrohämochrom; Hc(III), Denaturiertes Globin Ferrihämochrom; Mc(II), Denaturiertes Myoglobin Ferrohämochrom; Mc(III), Denaturiertes Myoglobin Ferrihämochrom.