ON THE SUBUNIT OF α_{s1}-CASEIN

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It is well known that casein splits into smaller subunits under certain conditions such as a high urea concentration or high alkalinity of the medium (BURK and GREENBERG, 1930; VON HIPPEL and WAUGH, 1955). The present communication deals in particular with the dissociation of the α_{s1}-casein component at high pH. The transition of the sedimentation coefficient between pH 6 and 12 is shown in figure 1. The pH of half transition suggests that the dissociation of α_{s1}-casein is governed by the acid-base equilibria of the hydroxyl groups of tyrosine and the ε -amino groups of lysine. Both amino acids are amply found in α_{s1}-casein (DE KONING and VAN ROOIJEN, 1965). It is obvious from figure 1 that the dissociation becomes complete at pH 10.5, the sedimentation coefficient reaching the value of 1.3 S (not corrected to zero concentration). There exists considerable confusion about the corresponding molecular weight. Values between 13,000 and 27,600 have been reported from physical investigations (VON HIPPEL and WAUGH, 1955; McKENZIE and WAKE, 1959; WAUGH et al., 1962; DREIZEN et al., 1962; SCHMIDT and PAYENS, 1963). The molecular weights estimated from amino acid and end group analysis range from 26,000 to 35,000 (MANSON, 1959, 1961; WAUGH et al., 1962; THOMPSON et al., 1963, 1964; GORDON et al. 1963, 1965; KALAN et al., 1964; DE KONING and VAN ROOIJEN, 1965). Recently the values of

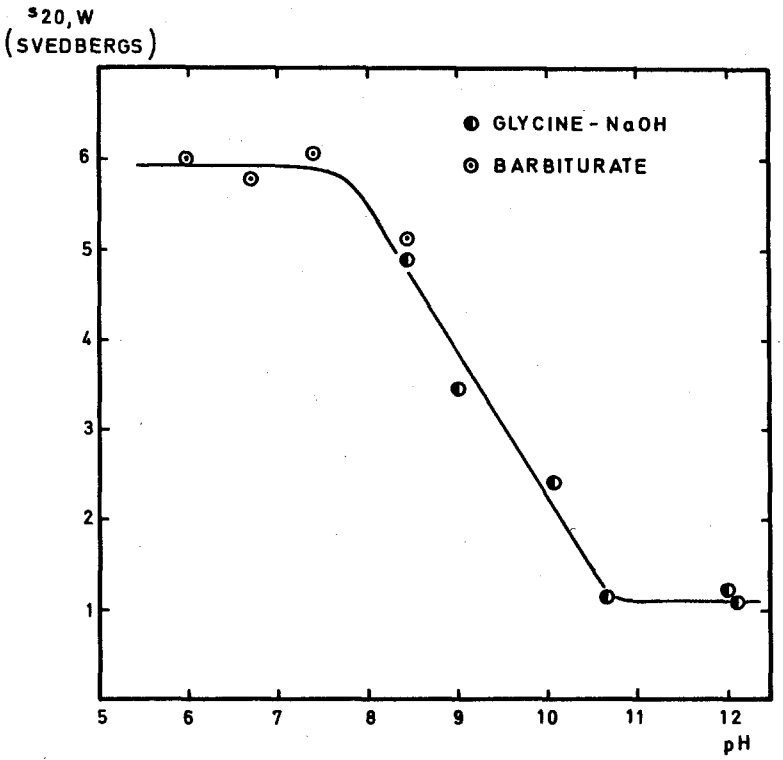

Fig. 1. The sedimentation coefficient of α_{s1}-casein B, C as a function of the pH. Protein concentration 7.0 x 10^{-3} g/ml, temperature 2 °C.

barbiturate - HCl buffers of ionic strength 0.2;

glycine - NaOH buffers of ionic strength 0.2.

23,600 and 24,600 were reported from ultracentrifuge measurements (CHUN, 1965; NOELKEN, 1966). This large scattering prompted us to reinvestigate the molecular weight of the α_{s1}-casein subunit by ultracentrifugation and light scattering. Supplementary viscosity measurements have given insight into the molecular configuration of the subunit at pH 12.

Pure α_{s1}-casein was prepared as described previously (SCHMIDT and PAYENS, 1963), starting from the milk of individual cows homozygous

for either the B or C variant. No differences were found in the molecular weight of the two variants at pH 12. The majority of the following experiments was carried out with α_{s1}-casein B, the most frequent variant. The protein was dissolved in glycine-NaOH buffer of pH 12. If desired the ionic strength of the buffer was adjusted by addition of NaCl. Sedimentation experiments were performed in a PHYWE air-driven ultracentrifuge at 25 °C and at an ionic strength 0.5. Sedimentation equilibrium was achieved in 1 mm columns after 4 to 6 hours at 18,000 x g. The molecular weight at different concentrations was calculated by method III of VAN HOLDE and BALDWIN (1958). The partial specific volume of α_{s1}-casein was determined pycnometrically, yielding 0.710 ml/g (S = 0,008; φ = 3).

The reciprocal molecular weights from sedimentation equilibria at different concentrations are given in figure 2. By extrapolation to zero concentration a molecular weight of 22,600 (S = 700; φ = 3)

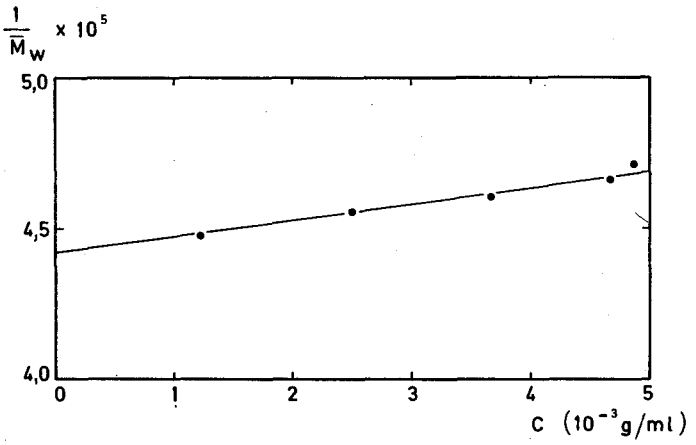

Fig. 2. Reciprocal molecular weight of α_{s1}-casein B versus the concentration as determined from sedimentation equilibrium at pH 12.
Temperature 25 °C, glycine - NaOH buffer of ionic strength 0.5.

is obtained. This value is related to the true molecular weight M by (MIJNLIEFF, 1963)

$$M_{ext} = M\left\{1 - \frac{\partial F_{el}}{\partial \mu_s}\left(\frac{1-\bar{v}_s\rho}{1-\bar{v}_p\rho}\right)\right\} \qquad (1)$$

In eqn (1) $\partial F_{el}/\partial \mu_s$ represents the change of the free energy of the electrical double layer with the chemical potential of the salt; $\bar{v}_s$ and $\bar{v}_p$ are the partial specific volumes of the salt and protein respectively and ρ is the density of the solvent.

Light scattering measurements were carried out at room temperature in a CENCO-TNO apparatus using cylindrical cells with a diameter of 3 cm. Unpolarized incident light of wave length 5461 Å was used and the intensity of the scattered light was determined with the turbidity of benzene as a reference. A value 15.6 x 10^{-6} cm^{-1} was accepted for the Rayleigh Ratio R 90 (KRATOHVIL et al., 1962).

The dissymmetry of the scattering pattern, being always less than 1.05 was neglected. No corrections were made for the depolarization of the scattered light. The protein solutions were rendered dust free by filtration through milipore filters with a pore size of 100 mμ.

The refractive index increment was determined with a Zeiss interferometer. The measurements were done at constant chemical potential of the salt in order to avoid electrical charge effects (VRIJ and OVERBEEK, 1962), yielding 0.190 ml/g (S = 0.004; φ = 19).

The excess scattering was determined in solutions of ionic strength 0.01, 0.04, 0.1, 0.4, 0.9 and 1.3. Typical results are collected in figure 3. The extrapolated value of C/R 90 at zero concentration yields a molecular weight of 23,500 (S = 2,090, φ = 47) with no significant dependence on the ionic strength. The second virial coefficient, however, increased from 0.67 x 10^{-3} to 6.80 x 10^{-3} ml.mol.g^{-2} when the ionic strength was varied from 1.3 to 0.01. This increase should be explained by the combined effects of intra- and intermolecular electrical repulsion.

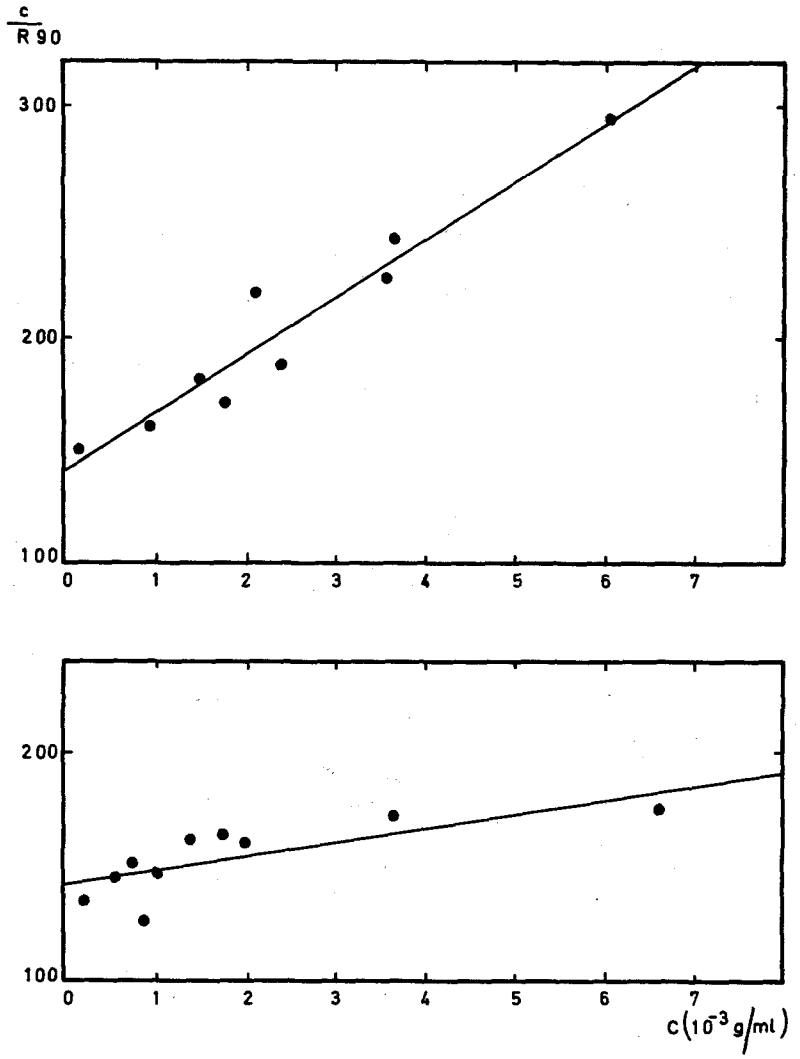

Fig. 3. Graphs of C/R 90 as a function of the concentration for α_{s1}-casein B at pH 12. Upper figure: glycine - NaOH buffer of ionic strength 0.04; lower figure: glycine - NaOH buffer of ionic strength 0.4. Room temperature.

The values of the extrapolated molecular weights at zero concentration as determined by ultracentrifugation and light scattering do not differ significantly. Apparently the factor $\partial F_{el}/\partial \mu_s$ in eqn (1) is within the experimental error, which

is not unreasonable in view of the high ionic strength used in the sedimentation experiments. We conclude therefore that the minimum molecular weight of the α_{s1}-casein is 23,000 $\pm$ 2,000.

We have not been able to reproduce the apparent ideal behaviour and the lower molecular weight of 16,500 communicated earlier (SCHMIDT and PAYENS, 1963). NOELKEN (1966) has suggested that a lower molecular weight might be the result of degradation in the glycine buffer. However, in the present study we have not observed a decrease of the molecular weight in this medium, even not after 48 hours of equilibrium centrifugation. At least a partial explanation of the discrepancy is afforded by a reinvestigation of the buoancy term $(1 - \bar{V}_p \rho)$, which was found to be 0.280 $\pm$ 0.008.

It is worthwhile to comment on the molecular configuration of the subunit at pH 12. From optical rotatory measurements, HERSKOVITS (1966) concluded that α_{s1}-casein in solution exists as random coils rather than compact particles with much intramolecular organization. This makes a description of the molecule by Oncley's treatment for impenetrable ellipsoids of revolution unrealistic (YANG, 1961). An alternative approach was offered by SCHERAGA and MANDELKERN (SCHACHMAN, 1959, YANG, 1961) who characterized the molecule by a function β defined as

$$\beta = \frac{N s [\eta]^{1/3} \eta}{100^{1/3} M^{2/3} (1 - \bar{V}\rho)} \qquad (2)$$

where N is Avogadro's number, S the sedimentation coefficient in sec^{-1}, M the molecular weight and η the viscosity of the solvent. The intrinsic viscosity $[\eta]$ at pH 12 and an ionic strength 0.5 was established to be 19.5 ml/g ($S = 0.5$; $\varphi = 4$). Substitution of the experimental data of S, M, $[\eta]$ and $(1 - \bar{V}_p \rho)$ in eqn (2) yields $\beta = (2.24 \pm 0.21) \times 10^6$. For flexible, partially drained coils MANDELKERN and FLORY (YANG, 1961) derived that β should be a constant

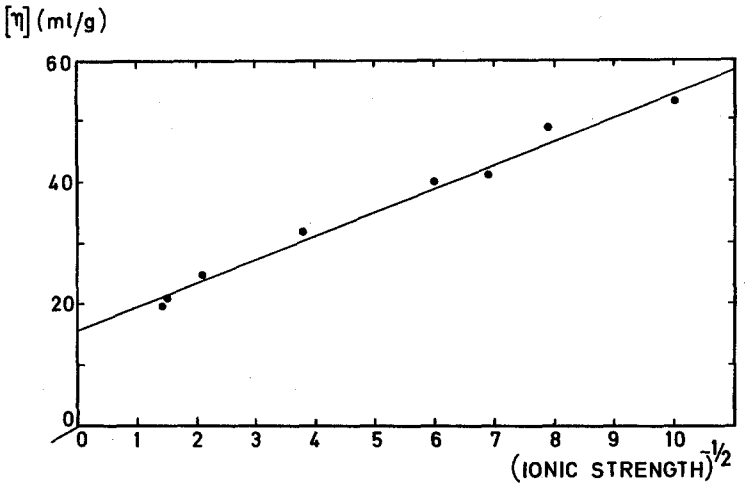

Fig. 4. Dependence of the intrinsic viscosity of α_{s1}-casein B on the reciprocal square root of the ionic strength; glycine - NaOH buffers, 25 °C.

with the value 2.10×10^6. For synthetic polymers experimental values of β of about 2.5×10^6 are reported. This shows that the β-value of α_{s1}-casein is well within the possible range to be expected for flexible coils. Strong support for a flexible structure is obtained from the decrease of the intrinsic viscosity with increasing ionic strength. As is shown in figure 4 the intrinsic viscosity depends linearly on the reciprocal square root of the ionic strength as is commonly observed for flexible polyelectrolytes. Preliminary calculations (SCHMIDT, to be published) show that this dependence can be accounted for satisfactorily by the theory of polyelectrolytes as developed by RICE and HARRIS (1954).

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