CHARACTERISTICS OF KAPPA-CASEIN IN THE PRESENCE OF VARIOUS DISSOCIATING AGENTS[1, 2]

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Kappa-casein, a principal component of the α-casein complex, is highly associated in neutral salt solutions, thus precluding molecular weight determinations of its monomeric form at values of pH normal to cows' milk (i.e. ≈pH 6.7). Consequently, molecular weight studies have been performed in the presence of various dissociating agents to ascertain the molecular weight of the basic structural unit. The results of these studies are reported. In addition, the amino acid composition of the k-casein preparation is given.

Analyses were performed in a Spinco Model-E analytical ultracentrifuge employing both the short column equilibrium technique described by Van Holde and Baldwin (1958) and the Archibald approach to equilibrium method as modified by Trautman (1956) and Erlander and Foster (1959). The dissociating solvents tried were 5.0 M guanidine · HCl, 7.0 M urea, 33% and 67% acetic acid in 0.15 M NaCl solution. Solvent densities were determined pycnometrically. The partial specific volume was calculated from the amino acid composition. The apparent molecular weights were not corrected for preferential interactions with solvent, concentration dependence, or charge effects.

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As indicated by the data in Table I, 67% acetic acid was the most effective dissociating agent for the protein interaction-complex. Guanidine · HCl (5 M) was superior to 7.0 M urea. The apparent molecular weight of the dissociated unit was of the order of 60,000 as indicated by the weight of the light component considerably larger than the previously reported values of 16,300 (Waugh, 1958), 26,000 (McKenzie and Wake, 1959) and 24,000 reported earlier by Swaisgood and Brunner (1962). However, these lower values were determined in phosphate buffer at pH 12. Since k-casein contains cystine (Jollès et al., 1962, and verified in this laboratory), it is suggested that the alkaline reaction may have catalyzed the cleavage of the disulfide bonds inherent to the basic structure. To test this hypothesis, equilibrium molecular weights were determined in 5.0 M guanidine · HCl and 7.0 M urea each containing the disulfied reducing agent 2-mercaptoethanol. The apparent molecular weight was ≈18,000 in the urea solution. Both the weight average and z-average molecular weight was ≈20,000 in guanidine · HCl solution.

Table I

Molecular Weight in Dissociating Solvents

Solvent	Concentration mg/ml	M_2^a	$\overline{M}^{\circ}_w{}^b$	$\overline{M}^{\circ}_z{}^c$
Guanidine · HCl, ~pH 5	7		88,000	102,000
Urea, ~pH 8.5	5	57,200	118,000[d] 108,000	144,000[d] 213,000
33% acetic acid 0.15 M NaCl	6	57,700		118,000[d]
67% acetic acid 0.15 M NaCl	7		88,000	86,000

[a] Molecular weight of light component determined from approach to equilibrium plots as described by Erlander and Foster (1959)
[b] Weight average molecular weight
[c] Z-average molecular weight
[d] Values determined from Trautman (1956) plots

Also, a straight line was obtained from a Trautman plot of the same protein preparation in phosphate buffer at pH 12, from which a molecular weight of 23,40(was calculated. Thus, on the basis of the data presented, it appears that the basic monomer of k-casein (≈60,000) consists of two or more polypeptide chains which are cross-linked by disulfide bonds.

The amino acid composition of the k-casein preparation was determined and compared with an analysis reported by Jollès et al. (1962), Table 2. Their analysis was made with k-casein prepared by the method of Mc Kenzie and Wake (1961). Our specimen was obtained as previously described (Swaisgood and Brunner 1962) but with additional purification and elution from a Sephadex G-75 column to eliminate contaminating quantities of λ-casein. Amino acid analyses were made with a Beckman analyzer on 24, 48 and 72 hr. acid hydrolysates. Based on a molecular weight of 60,000, our analyses show one cystine/mole of protein as compared to a value of 3.5 calculated from Jollès' data.

Table II

Amino Acid Composition of Kappa-casein

Amino Acid	This[1] study g/100g protein	Analysis of Jollès et al. (1962 g/100g protein
Asp	6.86	7.30
Thr	5.99	6.64
Ser	4.47	6.09
Glu	17.60	17.35
Pro	9.73	8.78
Gly	1.09	1.31
Ala	4.80	5.41
Cys	0.40	1.40
Val	5.60	5.10
Met	1.49	1.00
Ileu	6.31	6.14
Leu	5.43	6.08
Tyr	6.76	7.40
Phe	3.43	4.07
Lys	5.79	5.76
His	2.10	1.67
Arg	3.52	4.00
Try	--	1.05

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A partial specific volume of 0.729 was calculated using the values of Jollès et al. (1962) for tryptophan, galactose, N-acetylneuraminic acid, and galactosamine and our values for the remaining amino acids.

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