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THE MOLECULAR WEIGHT OF β-LACTOGLOBULIN

Sir:

The generally accepted molecular weight of β-lactoglobulin is in the vicinity of 35,000.[1,2,3,4] In the course of a detailed study of the association properties of this protein as a function of *p*H, it has been found that the weight of its disperse units is strongly dependent on *p*H.[5,6,7] In the *p*H regions below 3.5 and above 7.5, its sedimentation constant, measured at a protein concentration of 10 g./l., decreases from a value of $S_{20,w} = 2.85$ to $S_{20,w} = 2.25$, indicating that there occurs either a decrease in molecular weight or an expansion of the molecule, as in the case of serum albumin.[8,9,10,11,12]

In order to clarify this question, light scattering measurements were carried out on β-lactoglobulin as a function of *p*H. These show[7] that below *p*H 3.5 the molecule of β-lactoglobulin dissociates into units smaller than 35,000, probably into two portions of equal weight. These data are in qualitative agreement with the results of Rands and Tanford,[13] who also observed a similar decrease in the molecular weight of this protein at low *p*H. Because of the rapid re-equilibration of the dissociation with dilution, however, the light scattering data curve strongly upward in the low concentration range, and it is not possible to extrapolate them to a reliable value of the weight average molecular weight. As a result, ultracentrifugal determinations of the molecular weight were carried out by the Archibald technique,[14,15,16] under conditions of strong dissociation.

TABLE I

*p*H	Temp., °C.	Speed	Prot. concn., g./l.	Concn. at meniscus, g./l.	$\frac{1}{cx}\frac{dc}{dx}$ extrapd.	Mol. wt.
1.64	24.8	42,040	10.7	0.25	3.30 ± 0.20	16,900 ± 1000
				0.1	3.49 ± .20	17,900 ± 1000
2.01	23.7	39,460	13.2	2.8	3.75 ± .30	20,300 ± 1600
				0.36	3.23 ± .20	17,500 ± 1100
				0.1	3.22 ± .10	17,400 ± 500
2.08	25.0	20,410	12.1	5.4	1.047 ± .02	22,700 ± 500
				4.7	1.054 ± .03	22,900 ± 700

Solutions of β-lactoglobulin were prepared under the desired conditions of *p*H and ionic strength, and centrifuged in a Spinco Model E analytical ultracentrifuge. In each case, the protein solution was centrifuged in one Kel-F cell, while pure solvent was centrifuged in an identical cell used in place of the counterbalance. The menisci of the two were made to coincide by weighing in the proper amount of liquid into each cell, as suggested by Singer.[17] In this manner, the correct base line was obtained directly on each ultracentrifugal pattern. The weight average molecular weight at the meniscus was calculated at various times late in the run, so that the equilibrium be close to total dissociation. Some of the results obtained in a 0.1 Γ/2 NaCl-HCl medium are presented in the table.

These data show that the molecular weight of the dissociated species of β-lactoglobulin is in the vicinity of 17,500, or half of the normally accepted value. It is interesting to note that Bull has obtained a molecular weight of 17,000 from surface pressure measurements under a different set of conditions.[18] These results, together with the reports that β-lactoglobulin contains two identical amino[19] and carboxyl[19,20] end groups, strongly suggest that the molecular unit observed at isoelectric conditions is an aggregate of two identical protein units. It would appear, therefore, that the molecule of β-lactoglobulin consists of a single polypeptide chain with a molecular weight of *ca.* 17,500.

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