CLXIV. THE MOLECULAR WEIGHT OF CRYSTALLINE MYOGEN¹

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BARANOWSKI [1939] has recently obtained from rabbit muscle extract a crystalline protein, which he has named myogen A. As this is one of the few tissue proteins ever crystallized, it was of particular interest to investigate its molecular weight and its homogeneity by ultracentrifugal methods [Svedberg, 1939].

Experimental. The sedimentation constant of crystalline myogen was determined in the ultracentrifuge at 69,000 r.p.m., corresponding to a force of about 350,000 times gravity. The ordinary scale method of Lamm [1937] was used for the observations. For refractometric determinations of the protein concentration during the centrifuge runs it was necessary to know the refractive index increments dn/dc of the myogen solutions. These were measured in a differential double prism with pure water as reference liquid. For a 2.09 % solution dn/dc was found to be 1.86×10^{-3} at $\lambda = 436 \text{ m}\mu$, and 1.80×10^{-3} at $\lambda = 546 \text{ m}\mu$.

All centrifuge experiments were carried out in NaCl solutions, in general 0.2 *M*, to depress the electrostatic effects. To obtain different pH values buffer salts were added according to Table I, which also gives the results.

Table I. *Sedimentation velocity measurements*

Exp. no.	Salts	Total salt molarity	pH	Protein con- centration, %	s_{20}^*
3	NaCl	0.18	—	0.09	8.29
16	NaCl	0.20	—	0.51	7.91
2	NaCl	0.18	—	0.54	7.85
1	NaCl	0.20	—	1.03	7.93
18	NaCl	0.23	—	1.49	7.64
9	NaCl	0.20	—	2.04	7.52
6	NaCl	0.20	—	6.0	5.81
7	NaCl, NaOAc, HOAc	0.25	4.5	0.85	—†
19	NaCl, NaOAc, HOAc	0.25	4.6	1.0	—†
13	NaCl, NaOAc, HOAc	0.30	4.6	1.05	8.1, 2.6†
5	NaCl, NaOAc, HOAc	0.25	5.0	0.99	7.99
4	NaCl, Na ₂ HPO ₄ , KH ₂ PO ₄	0.24	6.0	1.04	7.97
8	NaCl, Na ₂ HPO ₄ , KH ₂ PO ₄	0.25	8.0	1.06	8.13
10	NaCl, Na ₂ B ₄ O ₇ , KH ₂ PO ₄	0.25	9.0	0.95	7.83
11	NaCl, Na ₂ B ₄ O ₇ , Na ₂ CO ₃	0.25	9.5	1.08	7.61
12	NaCl, Na ₂ HPO ₄ , NaOH	0.25	10.5	1.01	6.88
14	NaCl, NaOH	0.24	12	0.98	1.56†

* Sedimentation constants are given in units of 10^{-13} .

† Many dissociation products of different sizes.

‡ Probably homogeneous.

The figures given in the table for concentrations are analytically determined. (The solutions were weighed out from a stock solution, the concentration of which was determined by evaporation to dryness.) The concentrations were

¹ After this paper had been submitted an article was published by Baranowski (*Hoppe-Seyl. Z.* 1939, 260, 43) in which mention is made of some of our results.

also calculated refractometrically from the sedimentation diagrams, and agreed within a few per cent. with those analytically found except in the runs at pH 4.5-4.6, where the calculated values were definitely lower.

The sedimentation constant depends very little on concentration in the range 0-2 %, although there was a small decrease with increasing concentration. The average s_{20} is 7.86.

Between pH 5 and about 9.5 the sedimentation constant is independent of acidity. The diagrams show only one peak, most probably representing a homogeneous substance. Above and below this range the molecules are not stable. At pH 4.5 a number of different molecular species exist, as revealed by the several peaks in the sedimentation diagram. The same phenomenon was also observed by Deuticke [1934] in his investigation on muscle proteins. The lower limit of the pH stability range is just below 5, as indicated by the fact that in one experiment at pH 4.6 a main component with $s_{20}=8.1$ was obtained, obviously unchanged myogen. The upper limit of the stability range is not so well defined. Deuticke found reduced stability above pH 6.5, but we have not observed any decrease in s_{20} or any inhomogeneity below pH about 9.5.

Diffusion measurements were carried out according to Lamm's [1937] method. The curves obtained were all symmetrical and almost exactly ideal distribution curves, which supports the assumption that the protein is homogeneous. Table II gives the results.

Table II. *Diffusion measurements*

Exp. no.	Salts	Total salt molarity	Protein concentration, %	Time sec.	D ₂₀ [*]	Average
1	NaCl	0.2	0.82	76,020 159,900	4.76 4.68	4.72
2	NaCl	0.2	0.65	78,900 166,080	4.89 4.71	4.80
3	NaCl Na ₂ HPO ₄ , KH ₂ PO ₄	0.25	0.92	86,340 167,520	4.79 4.82	4.81
4	NaCl	0.2	0.27	88,740 145,140	4.90 4.72	4.81
Average						4.78

* Diffusion constants are given in units of 10^{-7} .

The partial specific volume of an impure myogen preparation in water was found by Weber [1927] to be equal to 0.74. For the dialysed salt-free solution of the crystalline myogen used in this study it was measured pycnometrically at 20° and was found to be 0.734 ± 0.002 for a 6.07 % solution and 0.735 ± 0.005 for a 2.09 % solution. It is thus independent of concentration within experimental error, and the value 0.735 was accepted as an average, in good agreement with Weber's result.

The molecular weight of myogen A is calculated to be 150,000, using the formula

$$M = \frac{RT s_{20}}{D_{20} (1 - V\rho)}.$$

The ratio of the observed molecular frictional constant to that of a spherical, unhydrated molecule of the same weight, f/f_0 , is 1.26.

The molecular weight was also determined by the sedimentation equilibrium method, using a 1 % solution buffered at pH 7.0. Two cells were used, at different distances from the centre of rotation, and the equilibrium was established

at 4500 r.p.m. The calculations showed no drift in the molecular weight value along the cells. This is a good proof for homogeneity. The values obtained are given in Table III.

Table III. *Sedimentation equilibrium measurements*

Cell I		Cell II	
Distance from centre of rotation cm.	Mol. wt.	Distance from centre of rotation cm.	Mol. wt.
4.70	139,000	5.45	(154,000)
4.75	139,000	5.50	137,000
4.80	139,000	5.55	136,000
4.85	137,000	5.60	137,000
4.90	137,000	5.65	136,000
4.95	136,000	5.70	135,000
5.00	135,000	5.75	134,000
5.05	134,000	5.80	133,000
5.10	134,000	5.85	133,000
5.15	134,000	5.90	132,000
Average	136,000		135,000

The difference between these values and the one obtained by the sedimentation-diffusion method may possibly be explained by experimental errors which cause large absolute errors in the equilibrium method. The value 150,000 is certainly more reliable.

DISCUSSION

The crystalline myogen A is certainly identical with the fastest-sedimenting component found by Deuticke [1934] in extracts from rabbit muscle. He reported a sedimentation constant of 7.70, which is a little lower than that found for crystalline myogen A. The difference may be explained by the fact that Deuticke always had slower-sedimenting molecules present, causing an increase of the viscosity of the medium in which the myogen sedimented. Deuticke also reported a sedimentation constant of the same order of magnitude for a protein in frog muscle extract.

Stöver [1933] has made osmotic measurements on impure myogen and reported a molecular weight of 81,000. The ultracentrifugal methods are certainly more accurate. Stöver found dissociation in urea and association in thiocyanate solutions. In 6*M* urea we found the sedimentation constant to be 1.9 and the diffusion constant 2.4. The myogen was not quite homogeneous in this medium, and the values given are averages. Assuming that the partial specific volume is not changed, a molecular weight of 72,000 is obtained. It is therefore probable that urea splits the molecules into halves. Stöver's figures also indicate such a splitting. Ammonium thiocyanate caused precipitation of most of the protein. Ultracentrifugal analysis showed that the substance left in solution was very heterogeneous with respect to molecular weight, and both association and dissociation seemed to have occurred.

It is of interest to note that myogen, the albumin of the muscle, has about the double molecular weight of serum albumin [70,200, Svedberg, 1939], and that myogen can be split by urea into molecules of this size. It is obvious that Svedberg's multiple rule [1939] is applicable. In the same multiple group of molecular weights ($8 \times 17,600$) are hitherto known only proteins of the globulin type (serum globulins, antipneumococcus serum globulins, and a phycocyan dissociation component). All these proteins have a molar frictional ratio f/f_0 of 1.4–1.5, which is definitely higher than that of myogen, 1.26. The myogen

molecule is therefore more symmetrical or less hydrated than the globulins. Quite possibly both these effects are involved.

The frictional ratio of the myogen molecules split by urea is 3.2, indicating a much more extended shape of the particles. Possibly some intramolecular linkages are split, permitting some sort of unfolding of the molecules.

SUMMARY

1. The following physico-chemical constants for the crystalline myogen A from rabbit muscle were determined:

$$dn/dc = 1.86 \times 10^{-3} \text{ for } \lambda = 436 \text{ m}\mu, \text{ and } 1.80 \times 10^{-3} \text{ for } \lambda = 546 \text{ m}\mu.$$

$$s_{20} = 7.86.$$

$$D_{20} = 4.78.$$

$$V = 0.735.$$

Mol. wt. = 150,000 (136,000 by the sedimentation equilibrium method).

$$f/f_0 = 1.26.$$

2. By different methods of observation (sedimentation, diffusion, sedimentation equilibrium) the protein was found to be homogeneous with regard to molecular weight.

3. Within a pH range from 5 to about 9.5 the molecule is stable. Outside these limits dissociation occurs.

4. Concentrated urea solutions cause dissociation of the molecules into halves.

5. The molecular weight of myogen A falls into the Svedberg multiple system.

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