Sedimentation of myosin in urea solutions

Studies of myosin in urea solutions were first reported by SNELLMAN[1], and later in some detail by TSAO[2]. More recently KOMINZ *et al.*[3] reported the isolation of a small fragment from urea-treated myosin. The consensus of these studies is that myosin breaks up in urea to yield fragments smaller than the original molecule, presumably with concomitant loss of three-dimensional structure.

In the experiments here reported, three distinct peaks were observed in solutions of rabbit skeletal myosin in 8 *M* urea, studied in the velocity ultracentrifuge (Fig. 1). We designate them in order of increasing sedimentation rate as the α, β and γ peaks.

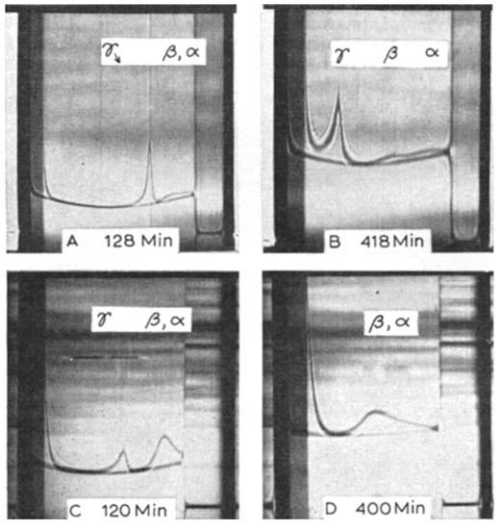

Fig. 1. A and B: Sedimentation of freshly-prepared myosin (c, 0.85 %) at 50,740 rev./min in solvent: 8.0 *M* urea, 0.37 *M* KCl. Double-sector cell shows solvent baseline. C and D: Sedimentation of iodoacetamide-treated myosin after one month in 8 *M* urea. Speed, 47,660 rev./min; solvent and protein concentration as in A and B. Sedimentation proceeds from right to left. Diaphragm angles: A, 70°; B, 40°; C, 65°; D, 52.5°.

Use of a double-sector cell proved indispensable in resolving the minor peaks. The γ peak comprises about three quarters of the total area under the peaks, the α and β peaks comprising about one eighth each. The relative areas do not appear to change with total protein concentration. To test whether any of the peaks could be artefacts produced, for example, by a redistribution of urea in the solution, we carried out a control sedimentation on bovine serum mercaptalbumin in the same solvent. At every stage of the sedimentation of the albumin (duration 10 h at 59,780 rev./min) only a single peak was observable, and no anomaly appeared (see also ref. 4). Plots of log peak position *vs.* time were linear both in the albumin and the myosin solutions. This relationship seems surprising in view of the obvious formation of a urea gradient during the run, but it does provide a convenient empirical description of the system.

Reporting the sedimentation coefficients poses a problem, since the usual assumptions about the density and viscosity relationships may lose significance in such a mixed solvent. For this reason, we report in Table I the actual rate of

sedimentation, $S'' \equiv \frac{dr/dt}{\omega^2 r}$, as well as the apparent sedimentation coefficient $S'_{20,w}$ obtained by making the conventional density and viscosity corrections. For the solvent used in these experiments $\varrho_{20} = 1.133$ and $\left(\frac{\eta_{20}}{\eta_{20,w}}\right) = 1.69$. Note that whereas the α and β peaks show little or no concentration dependence in their sedimentation coefficients, a large effect is seen for the γ peak. Such an extreme concentration dependence probably reflects a marked molecular asymmetry; it does not seem to be consistent with a random-coil model. It appears that the component corresponding to the γ peak may be roughly the same molecular weight as the parent myosin molecule.

TABLE I

SEDIMENTATION COEFFICIENTS OF MYOSIN COMPONENTS IN UREA

Component	*Total protein* concentration (%)*	S''	$S'_{20,w}$
α	0.85	0.4	1.2 ± 0.3
	0.43	0.6	1.6 ± 0.3
β	0.85	1.2	3.0 ± 0.3
	0.43	1.2	3.3 ± 0.3
γ	1.29	0.9	2.3
	1.29	0.8	2.1
	0.62	1.7	4.7
	0.39	2.3	6.1
	0.22	3.4	9.2
	0.12	4.3	11.4
	0.06	4.3	11.6

* Corrected, in the case of the γ-component, by a mean radial-dilution factor, $\frac{r^2 \text{ men.}}{\bar{r}^2}$.

The sedimentation patterns of Fig. 1 (A and B) are qualitatively unchanged over a pH range from 3.3 to 10.5, and are also unaffected by short periods of heating (1 h at 50°) at both these extremes of pH. The pattern is further indifferent to the addition of an excess (over the $^-$SH content) of iodoacetamide or *p*-chloromercuribenzoate. Preliminary observations of the time stability of myosin in 8 *M* urea at 25° indicated no change over a period of several days. The sedimentation pattern of such a myosin solution after two weeks at room temperature is qualitatively unchanged, but the sedimentation rate of the γ peak increases, in the presence or absence of thiol reagents. After one month in 8 *M* urea at room temperature, myosin shows a markedly altered sedimentation pattern (Fig. 1 C and D). The β peak grows at the expense of the γ peak, suggesting a simple transformation of the latter to the former. Such a process also offers an explanation for the increase of the sedimentation rate of the γ peak on shorter periods of ageing, in view of its demonstrated concentration dependence. The slowness of this change is consistent with the observations of TSAO[2]. A single experiment suggests that the transformation is more rapid at 4° than at room temperature.

Following an interchange of correspondence with W. F. HARRINGTON, two double-sector sedimentations with myosin in 5 *M* guanidine hydrochloride were run. These show no γ peak, a large β peak, and a typical small α peak. Although the relative

areas under the two peaks were difficult to measure, we estimate that the α peak contains about 10% of the total area. The following values for the β peak were determined: at 0.96% myosin, $S'' = 0.7_4$ and $S'_{20,w} = 1.7$; at 0.48% myosin, $S'' = 1.0_0$ and $S'_{20,w} = 2.3$. This present finding of the heterogeneity in 5 M guanidine hydrochloride is in contrast with the conclusions of KIELLEY AND HARRINGTON[5], who deduce that myosin in this solvent dissociates into three identical subunits. Trial calculations show, however, that the heterogeneity will probably not greatly al er the weight-average molecular weight found by these authors.

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[1] O. SNELLMAN AND M. TENOW, *Biochim. Biophys. Acta*, 2 (1948) 650.
[2] T-C. TSAO, *Biochim. Biophys. Acta*, 11 (1953) 368.
[3] D. R. KOMLNZ, W. R. CARROLL, E. N. SMITH AND E. R. MITCHELL, *Arch. Biochem. Biophys.*, 79 (1959) 191.
[4] R. TRAUTMAN AND C. F. CRAMPTON, *J. Am. Chem. Soc.*, 81 (1959) 4036.
[5] W. W. KIELLEY AND W. F. HARRINGTON, to be published in *Biochim. Biophys. Acta.*

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Manganese content and changes in light absorption during photosynthesis in green algae

Studies on the effect of manganese deficiency on photoreduction and photosynthesis in green algae have led to the conclusion that manganese is specifically involved in photosynthetic O_2 evolution[1–4]. This assumption received further support from measurements of delayed light emission and fluorescence in manganese-deficient algae[5] and from work on the dependence of the HILL reaction on the manganese content of algae and higher plants[6]. In order to obtain further information concerning the role of manganese in photosynthesis, we have studied the effect of manganese deficiency on the changes in light absorption during illumination of green cells.

Four types of changes in light absorption have been observed during photosynthesis[7–10]. Those of Type 2 are obviously connected with the splitting of water. Their amplitude is independent of temperature, and they are not influenced by inhibition of the other basic processes of photosynthesis (*i.e.*, O_2 evolution, photosynthetic phosphorylation, and CO_2 reduction)[7,11].

Ankistrodesmus braunii (strain Marburg) was grown in culture media with and without addition of manganese[5]. After about 3 weeks, the photosynthetic activity of the manganese-deficient cells had dropped to one-fourth of that observed with normal algae. Fig. 1 shows that the changes in light absorption of Type 2, induced by short (10^{-4} sec) flashes of light[11], are not influenced at all by manganese deficiency.