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Dimerization of the Myosin Heads in Solution[†]

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ABSTRACT: It is shown, by means of analytical ultracentrifugation, that skeletal myosin S-1 exists in the form of a monomer–dimer mixture, in rapid reversible equilibrium, sensitive to the hydrostatic pressure, the temperature, and the composition of the buffer (at least, pH, ionic strength, presence or absence of a Mg–(phosphate compound), and presence or absence of Mg^{2+}). The dimer is predominant at high pH, at low ionic strength, in the presence of a Mg–(phosphate compound), at high pressure, and at low temperature. The monomer is predominant in the reverse conditions. At atmospheric pressure and at room temperature, in a buffer having a composition close to that of the physiological medium, but containing no Mg–(phosphate compound), the monomer is largely predominant (more than 90% at 1 mg/mL S-1). At atmospheric pressure and at room temperature, in a buffer containing a Mg–(phosphate compound) and having a composition close to that of the physiological medium, S-1 exists in the form of a monomer–dimer mixture, with a noticeable proportion of dimer (more than 25% at 1 mg/mL S-1 in the presence of 2 mM MgADP and 3 mM Mg^{2+}). In such buffers, the monomer:dimer ratio is extremely sensitive to both the pH and the ionic strength. The sedimentation coefficients of the monomer and the dimer are respectively 5.05 ± 0.05 S and 6.05 ± 0.05 S. The two protomers making up the dimer are stuck together in an end-to-end arrangement. Both the monomer and the dimer are highly hydrated (about 0.9 g of water/g of protein for the monomer and probably more for the dimer).

Actin and myosin are the two principal proteins found in the contractile apparatus of muscle and nonmuscle motile cells. The myosin molecule contains two globular heads at one end of a long tail. The heads are extremely important, since they each bear an actin-binding site, an enzymatic site, and maybe a regulatory site (Yee et al., 1980). Furthermore, they are involved in the generation of the contractile force, either directly (Huxley, 1969) or indirectly (Morel et al., 1976). Thus, it is of major importance to know all their properties. Here we show they can form dimers in solution, and this finding adds a new property, leading most likely to a yet countless number of consequences.

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Until 1977, few experiments supported the possibility of head-to-head interactions in solution: "proteolytic degradation of myosin yields only isolated globules with no tendency toward dimer formation" (Margossian & Lowey, 1973). However, it was shown, by means of electron microscopy, that myosin treated with DTNB[1] may form head-to-head dimers (A. Elliott and G. Offer, unpublished observations). Schaub & Watterson (1976) reported evidence for head-to-head interactions in myosin filaments. In 1977, Margossian and Lowey reported that a reversible aggregation of isolated globules might occur in the presence of 0.1 mM Ca^{2+}. Barrett et al. (1978) claimed they had confirmed this result. A reversible aggregation of cardiac S-1 was also reported (Flamig & Cusanovich, 1980). However, we considered that the techniques used led to rather ambiguous results. Thus, we made new experiments, in order to have clear confirmation that S-1 can form aggregates and to determine some parameters influencing aggregation. After numerous experimental tests, we decided to measure the sedimentation coefficients of S-1, under various conditions, and to confirm our conclusions by sedimentation–diffusion equilibrium experiments.

Theory

Sedimentation Coefficients in the Case of a Rapid Monomer–Dimer Reversible Equilibrium. In the case of a monomer in rapid reversible equilibrium with a dimer, the substance (monomer + dimer) sediments with a single boundary (Svedberg & Pedersen, 1940; Fujita, 1962; Gilbert, 1963): "any monomer–polymer system in which chemical equilibria are attained instantaneously among all solutes is ultracentrifugally equivalent to a system of a single concentration-dependent solute" (Fujita, 1962). The total concentration profile is different from that corresponding to a single species, but its general shape is comparable (Fujita, 1962), and it is possible to determine an apparent sedimentation coefficient s^{app}, which is given by eq 4.20 in Fujita (1962). Each individual coefficient obeys the equation

$$s_{\mathrm{i}} = s_{\mathrm{i}}^0/(1 + k_{\mathrm{s}}c) \tag{1}$$

where the subscript "i" refers to either the monomer (m) or the dimer (d), c is the total protein concentration, expressed in milligrams per milliliter or grams per liter, and k_{s} is the concentration coefficient, assumed to be the same for the monomer and the dimer. The mathematical expression obtained by Fujita (1962) for s^{app} can be written, for a monomer–dimer equilibrium, as

$$s^{\mathrm{app}} = [s_{\mathrm{d}}^0 - (s_{\mathrm{d}}^0 - s_{\mathrm{m}}^0)(c_{\mathrm{m}}/c)]/(1 + k_{\mathrm{s}}c) \tag{2}$$

If C_{m} and C_{d} are respectively the molar monomer and dimer concentrations, C_{m}/C can be calculated from

$$C = C_{\mathrm{m}} + 2C_{\mathrm{d}} \qquad K^* = C_{\mathrm{d}}/C_{\mathrm{m}}^2 \tag{3}$$

K^* is the equilibrium constant, in M^{-1}, and C is the total protein concentration, expressed in moles per liter of protomer. Therefore, we have

$$C_{\mathrm{m}}/C = 2/[(1 + 8K^*C)^{1/2} + 1] \tag{4}$$

The expression of s^{app} becomes

$$s^{\mathrm{app}} = [s_{\mathrm{d}}^0 - 2(s_{\mathrm{d}}^0 - s_{\mathrm{m}}^0)/[(1 + 4Kc)^{1/2} + 1]]/(1 + k_{\mathrm{s}}c) \tag{5}$$

In this equation, we have $K = 2K^*/M_{\mathrm{m}}$ (M_{m} = the molecular mass of the monomer, in daltons), if c is expressed in milligrams per milliliter or grams per liter, as is usual. c is the concentration in the working zone in the cell and is related to the initial concentration c_0 by the radial dilution law $c = c_0(r_0/\bar{r}_{1/2})^2$. r_0 is the radial distance from the axis of rotation to the meniscus, $r_{1/2}$ is the radial position of the maximum in the refractive index gradient curve, and $\bar{r}_{1/2}$ is the arithmetic mean of the values of $r_{1/2}$ for the sedimentation photographs taken at the beginning and at the end of the particular sedimentation experiment (Williams, 1972). If the working zone lies between r_1 and r_2, we have $\bar{r}_{1/2} = (r_1 + r_2)/2$.

In the case of very slight degrees of aggregation ($4Kc \ll 1$), eq 5 reduces to $s^{\mathrm{app}} \sim s_{\mathrm{m}}^0/(1 + k_{\mathrm{s}}c)$. For very strong aggregation ($4Kc \gg 1$), eq 5 leads to $s^{\mathrm{app}} \sim s_{\mathrm{d}}^0/(1 + k_{\mathrm{s}}c)$. Thus, as pointed out by Gilbert (1963), in both cases it is difficult to draw a clear conclusion, since the system apparently behaves as a nonaggregating one. Between these extremes lies a region where aggregation affects the "s^{app} vs. c" curve (Gilbert, 1963). This is the case when $4Kc$ is of the order of magnitude of, say, 0.1–10. In these conditions, according to eq 5, the value of s^{app} is s_{m}^0 at $c = 0$. When c increases, an increase in s^{app} is expected, followed by an asymptotic decrease along the curve $s_{\mathrm{d}}^0/(1 + k_{\mathrm{s}}c)$. Furthermore, all the parameters which alter K must modify s^{app}. As we shall see below, S-1 belongs to this third class of aggregating substances.

Sedimentation–Diffusion Equilibrium in the Case of Ideal Solutes. For a single solute at concentration c, the translational diffusion coefficient is given by (Tanford, 1967; eq 21.8)

$$D_{\mathrm{t}} = RT[1 + c\partial(\ln y)/\partial c]/(N_{\mathrm{A}}f) \tag{6}$$

where y is the activity coefficient. RT has the usual meaning. On the other hand, the sedimentation coefficient is given by (Tanford, 1967; eq 22.5)

$$s = M(1 - \rho\bar{V})/(N_{\mathrm{A}}f) \tag{7}$$

where s is expressed in seconds, ρ is the specific gravity of the buffer, and $\bar{V}$ is the specific volume of the solute. In both eq 6 and 7, the frictional coefficient f is a function of c, which can be written $f(c) = f(0)(1 + B'c)$ (Herbert & Carlson, 1971). Thus, by combining with eq 7, we get $s = s^0/(1 + B'c)$, with $s^0 = M(1 - \rho\bar{V})/[N_{\mathrm{A}}f(0)]$ (ρ is assumed to be independent of c, which is justified for the usual values of c). By identifying with eq 1, we get $B' = k_{\mathrm{s}}$. Now, in most cases found in practice, the dependence of D_{t} on c is weak for single solutes, and this means that $\partial(\ln y)/\partial c$ is of the order of magnitude of B', i.e., k_{s}. Since k_{s} is extremely weak for S-1 (see Results), the activity terms can be neglected over a wide range of S-1 concentrations. The same conclusion has been drawn some years ago by Lowey et al. (1969) and confirmed by Margossian et al. (1981). Therefore, S-1 approximately behaves as an ideal solute and eq 6.22 in Tanford (1967) becomes

$$\mathrm{d}(\ln c)/\mathrm{d}r^2 = M_{\mathrm{w}}(1 - \rho\bar{V})\omega^2/(2RT) \tag{8}$$

where M_{w} represents the molecular weight of the species and ω the angular velocity. As we shall show below (Results), the S-1 monomer on the one hand and S-1 dimer on the other hand obey eq 8; i.e., ln c vs. r^2 is a straight line in both cases.

In the case of an ideal monomer–dimer mixture in reversible equilibrium, eq 8 is also valid, with the only modification that M_{w} is given by

$$M_{\mathrm{w}}/M_{\mathrm{m}} = 2/[1 + (1 + 4Kc)^{-1/2}] \tag{9}$$

Equation 9 can be easily obtained, since we have $M_{\mathrm{w}} = (M_{\mathrm{m}}c_{\mathrm{m}} + M_{\mathrm{d}}c_{\mathrm{d}})/c$ (Svedberg & Pedersen, 1940, for instance) and therefore $M_{\mathrm{w}} = M_{\mathrm{m}}(2 - c_{\mathrm{m}}/c)$. c_{m}/c is given by eq 4, except

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[1] Abbreviations: HMM, heavy meromyosin; S-1, myosin subfragment 1; LC, S-1 light chain (LC2 = DTNB light chain); DTNB, bis(5-carboxy-4-nitrophenyl) disulfide; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; P_i, inorganic phosphate; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

that C_m and C are here replaced by c_m and c, respectively, and K^* by K. Let us now put

$$\sigma_m = M_m(1 - \rho\bar{V})\omega^2/(RT) \qquad (10)$$

If we put $x^2 = (1 + 4Kc)$, eq 8 can be written as

$$dx/(x - 1) = (\sigma_m/2)\, dr^2 \qquad (11)$$

This equation can be easily integrated, and we get

$$(1 + 4Kc)^{1/2} - 1 = [(1 + 4Kc^0)^{1/2} - 1] \exp[\sigma_m(r^2 - r_0^2)/2] \qquad (12)$$

where r_0 is the radial position of the meniscus and c^0 the total (monomer + dimer) concentration at the meniscus. Since the curve giving c vs. $(r^2 - r_0^2)$ is experimentally known, eq 12 is extremely interesting in the case where σ_m, i.e., M_m, is known. In fact, the least-squares method permits the calculation of K for each loading concentration. If the same values of K are obtained in any case, one can conclude that a reversible equilibrium exists between the monomer and the dimer. For more details on the usual approach of the problem, see Figure 4. In the case of an ideal monomer–dimer mixture, eq 12 gives the most rapid and the best results.

Materials and Methods

Chymotryptic S-1 from rabbit skeletal myosin was prepared according to the standard procedure of Weeds & Pope (1977). The preparations were characterized by $NaDodSO_4$ gel electrophoresis (Figure 2B). We studied the S-1 solutions by means of sedimentation velocity measurements and sedimentation–diffusion measurements. Before use, all the S-1 solutions were centrifuged at 40 000 rpm for 1 h, in order to remove lipids (which remain at the meniscus) and heavy materials (which fall to the bottom of the tubes). We took the S-1 samples in the medium region of the tubes.

For sedimentation velocity analyses, we paid particular attention to the choice of the working zone in the cell (see Discussion). For loading concentrations c_0 above 2 mg/mL, the schlieren method was used. For c_0 between 0.8 and 1.3 mg/mL we made use of UV absorption and for c_0 lower than 0.7 mg/mL of UV scanning.

The sedimentation–diffusion equilibrium experiments were carried out in a usual Yphantis six-channel cell, and we made use of the Rayleigh interference optical system.

In all cases, we paid attention to the presence or absence of a Mg–(phosphate compound) in the buffer. We chose MgADP in order to obviate the problem of hydrolysis, which occurs with MgATP.

Results

Starting from the above theoretical conclusions, we made three sets of sedimentation velocity experiments, which we confirmed by sedimentation–diffusion equilibrium experiments.

Variations of the Sedimentation Coefficients with the S-1 Concentration. In a first set of experiments, we studied the variations of $s_{20,w}^{app}$ with the S-1 concentration. The experimental results are shown in Figure 1. As is the case for all our sedimentation velocity experiments, we observed that the protein sedimented with a single boundary. Figure 1 indicates a steep dependence of $s_{20,w}^{app}$ on c; when c increases, $s_{20,w}^{app}$ first increases and then slightly decreases, as expected from eq 5. "So far as reported data are concerned, sedimentation coefficients for binary systems (solvent + one solute) are either constant or decreasing functions of solute concentrations" (Fujita, 1962). This is not the case here, and Figure 1 (together with the single boundary) undoubtedly proves that we are in the presence of a monomer–dimer mixture in rapid reversible equilibrium (see Theory). It appears that the values of $s_{20,w}^{app}$ also depend on the presence or absence of MgADP under given conditions (compare curves a and b). More precisely, Figure 1 shows that $s_{20,w}^{app}$ is higher in the presence of MgADP. This means that K is higher in the presence of MgADP than in its absence (see eq 5) and also that the two globules making up the dimer are more tightly bound in the presence of MgADP.

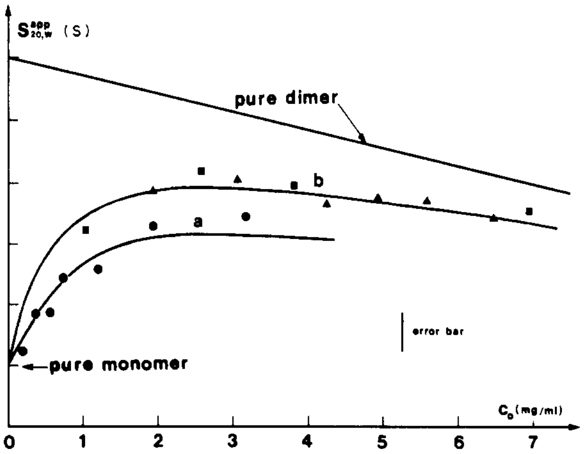

FIGURE 1: Variations of $s_{20,w}^{app}$ vs. the initial concentration c_0 of S-1. The actual concentration in the working zone is given by $c = 0.94c_0$ (see Theory and Discussion). Temperature 20 °C. (●) Rotor speed 42 040 rpm; 130 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, pH 6.9; (▲) same composition as (●), but with adjunction of 2 mM ADP; (■) rotor speed 59 780 rpm; 155 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, pH 7.3. The straight line representing the pure dimer was deduced from $s_{d,20,w}^0 = 6.05$ S (Figures 2 and 3) and from $k_s = 0.010$ mL/mg (see Results). The two curves were numerically calculated by using eq 5, with $c = 0.94c_0$. Curve a gives the best fit with the experimental points (least-squares method) and corresponds to $s_{m,20,w}^0 = 5.00$ S and $K = 1.0$ mL/mg $= 5.4 \times 10^4$ M^{-1}. Curve b gives $K = 4.0$ mL/mg $= 2.1 \times 10^5$ M^{-1}, by taking $s_{m,20,w}^0 = 5.00$ S. Note that the experimental points (■) and (▲) lie, in the limit of the experimental error, on the same curve (b), but this is purely coincidental.

Figure 1 also suggests that, in the absence of MgADP, $s_{20,w}^{app}$ might depend on the rotor speed and the composition of the buffer. However, both the composition of the buffer and the rotor speed were varied simultaneously, and we made two further sets of experiments, in order to clarify these preliminary observations.

Dependence of the Sedimentation Coefficients on KCl, pH, and MgADP. In a second set of experiments, we studied the influence of the KCl concentration, the pH, and the presence or absence of MgADP on $s_{20,w}^{app}$, at a fixed rotor speed. The experimental results are shown in Figure 2. At KCl concentrations lower than 90 mM, $s_{20,w}^{app}$ exhibits a plateau independent of pH and MgADP and corresponding to a pure dimer. At moderate pH (6.9), and in the absence of MgADP, $s_{20,w}^{app}$ steeply decreases with KCl concentration (curve a in Figure 2). Under the same conditions, but in the presence of MgADP, $s_{20,w}^{app}$ is significantly higher than in its absence (for KCl concentration > 90 mM). At a given concentration of KCl (>90 mM), $s_{20,w}^{app}$ increases with pH. Although we cannot entirely rule out the possibility that the variations in $s_{20,w}^{app}$ might be due, at least partly, to changes in the shape and/or degree of hydration of S-1, we think the interpretation of the present behavior is straightforward. In fact, we have seen that $s_{20,w}^{app}$ depends on the equilibrium constant K (eq 5). The simplest explanation of Figure 2 is that K depends on the ionic strength, the pH, and the presence or absence of a Mg–(phosphate compound) (here MgADP). The dimer is predominant at low ionic strength, at high pH, and in the presence of MgADP. This behavior might be compared with the steep decrease in

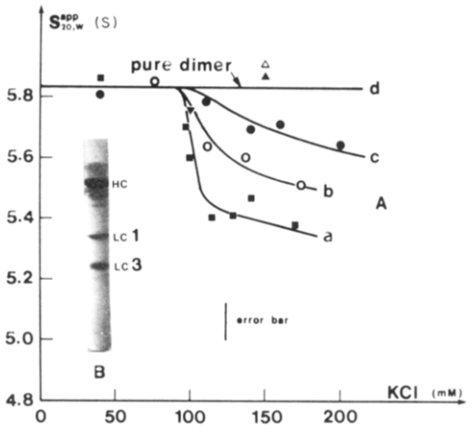

FIGURE 2: (A) Values of $s^{app}_{20,w}$ vs. KCl concentration. Temperature 20 °C; S-1 initial concentration $c_0 = 3.8$ mg/mL; actual concentration in the working zone $c = 0.94c_0 = 3.6$ mg/mL; rotor speed 42 040 rpm. (■) 5 mM $MgCl_2$, 50 mM imidazole, pH 6.9; (○) same composition as (■), but with adjunction of 2 mM ADP; (●) same composition as (■), but pH 7.3. In this figure, we have also represented some experimental points obtained by other authors, under different conditions, in order to have a comparison between our own results and other published results. (▲) Rotor speed 59 780 rpm; pH 7.5 (Jones & Perry, 1966); (▼) rotor speed 59 780 rpm; pH 7.7 (Guillain, 1977); (△) rotor speed 59 800 rpm; pH 7.0; temperature 4 °C; S-1 concentrations up to 18 mg/mL (Mendelson & Kretzschmar, 1980). It appears that these supplementary points lie approximately on the same horizontal straight line (d) as our three points at <90 mM KCl. Therefore, the mean of the seven upper points represents the maximum value of $s^{app}_{20,w}$, irrespective of the experimental conditions, and corresponds to a pure dimer at $c = 3.6$ mg/mL. Since $k_s = 0.010$ mL/mg (see Results), we get $s^0_{d,20,w} = 6.03$ S, a value which is in full agreement with those obtained on Figure 3. (B) $NaDodSO_4$ gel electrophoresis on the S-1 preparations used in the present study. Concentration gel 3%; separation gel 12%.

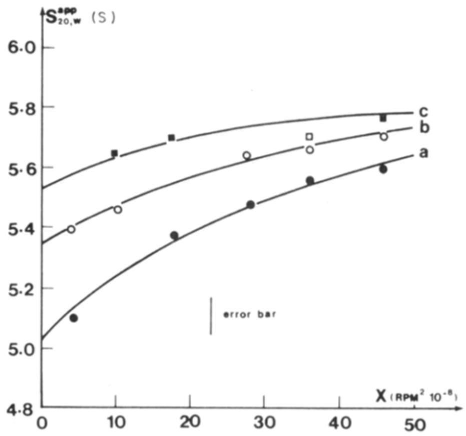

FIGURE 3: Values of $s^{app}_{20,w}$ vs. the square of the rotor speed. Temperature 20 °C; initial S-1 concentration $c_0 = 3.8$ mg/mL ($c = 3.6$ mg/mL). (●) 115 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, pH 6.9; (○) same composition as (●), but with adjunction of 2 mM ADP; (■) 95 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, pH 7.2; (□) usual point obtained by many authors at $c = 3.6$ mg/mL. Curve c was drawn by eye. Curves a and b were calculated as proposed in the Appendix. Curve a gives the following values: $s^0_{d,20,w} = 6.09$ S; $s^0_{m,20,w} = 5.06$ S; $K_0 = 0.08$ mL/mg = 4.3×10^3 M^{-1}; $\Delta\bar{V} = -0.016$ mL/g. Curve b gives the following values: $s^0_{d,20,w} = 6.03$ S; $s^0_{m,20,w} = 5.10$ S; $K_0 = 0.4$ mL/mg = 2.1×10^4 M^{-1}; $\Delta\bar{V} = -0.014$ mL/g. The value of 0.08 mL/mg is extremely low and in full agreement with the study of Margossian et al. (1981), who have shown that, in a buffer comparable to ours and at approximate atmospheric pressure, the monomer is largely predominant (see also legend to Figure 4). For curve b, K_0 is significantly higher, and we find once more that the two protomers making up the dimer are much more tightly bound in the presence of MgADP (see also Figures 1, 2, and 4).

V_{max} for the Ca-ATPase of S-1 vs. the KCl concentration at a fixed pH and the steep increase of V_{max} vs. pH at KCl concentration fixed (Guillain, 1977). Our results are very likely related with variations in the monomer:dimer ratio (see above), and the results of Guillain (1977) might be interpreted in terms of cooperative effects in the dimer.

Variations of the Sedimentation Coefficients with the Hydrostatic Pressure. In a third set of experiments, we studied the influence of the rotor speed on $s^{app}_{20,w}$. When the rotor speed increases, the hydrostatic pressure also increases. Now, according to Harrington & Kegeles (1973) and Marcum & Borisy (1978), the equilibrium constant at pressure P (measured from 1 atm) is given by

$$K_p = K_0 \exp[-P\Delta\bar{V}/(RT)] \tag{13}$$

where K_0 is the equilibrium constant at atmospheric pressure and $\Delta\bar{V}$ the variation of the specific volume for the reaction monomer → dimer. Since $\Delta\bar{V}$ is only exceptionally zero and since a value of only 0.001–0.010 mL/g is sufficient to induce large variations in K_p with the values of P reached in the ultracentrifuge, a variation of $s^{app}_{20,w}$ vs. the square of the rotor speed is expected. The results of such experiments are shown in Figure 3. It appears that $s^{app}_{20,w}$ is indeed a function of the square of the rotor speed. However, one must be cautious with the interpretation of these results. The variations of $s^{app}_{20,w}$ might be due to variations in k_s with P. This is not the case. In fact, we have studied $1/s^{app}_{20,w}$ vs. c in the conditions of curve c on Figure 3, at 31 410 rpm (almost pure dimer), and we have found $k_s = 0.010$ mL/mg, which is quite comparable with the value obtained at 60 000 rpm (0.011 mL/mg; Yang & Wu, 1977). Furthermore, an orientation of the globules is not possible, since S-1 is too small sized to undergo such a phenomenon (Svedberg & Pedersen, 1940). However, here again, the variations of $s^{app}_{20,w}$ might be due to variations in the shape and/or degree of hydration of S-1. We do not at all favor this possibility, since the results presented in Figure 3 are consistent with those presented in the other figures. Therefore, we consider that the variations of $s^{app}_{20,w}$ with rotor speed are due to variations in K_p. According to Figure 3, the dimer is predominant at high hydrostatic pressure. This means that K is an increasing function of P and that $\Delta\bar{V}$ is negative (~ −0.015 mL/g; Figure 3). A negative value of $\Delta\bar{V}$ is rather unexpected for a protein, but this particularity might be related with the unusual high degree of hydration of S-1 (see Discussion). As it is the case for Figures 1 and 2, Figure 3 shows that K is higher in the presence of MgADP than in its absence.

Sedimentation–Diffusion Equilibrium Experiments. Although our sedimentation velocity measurements lead to the conclusion that a S-1 dimer exists, in rapid reversible equilibrium with the monomer, we made sedimentation–diffusion equilibrium experiments in order to definitely support our conclusions.

First, we tried to confirm that a S-1 dimer exists, especially at low KCl concentration, at high pH, and in the presence of MgADP. In order to minimize the effects of the hydrostatic pressure, we made use of extremely short column lengths (1 mm). The other conditions for the experiments were the following: temperature 4 °C; rotor speed 10 000 rpm (duration of the experiment 24 h); loading S-1 concentration 0.38 mg/mL; composition of the buffer 20 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, 2 mM ADP, and 2 mM DTT, pH 7.5. As expected from eq 8 (valid for a single ideal solute), we found that the curve ln c vs. r^2 was a straight line over the entire column length, corresponding to a molecular weight of 215 000 (with $\bar{V}_d = 0.725$ mL/g; see Figure 3). This value corresponds to a pure dimer, and we confirm here our above conclusions. However, in order to check that this result was not due to artifactual phenomena, we did the following experiment. In

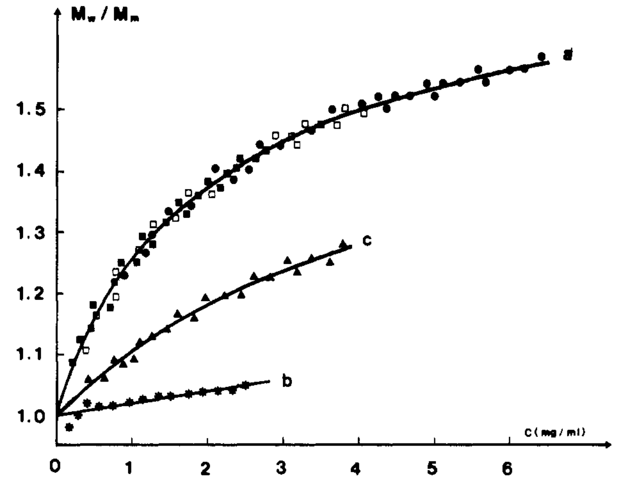

FIGURE 4: M_w/M_m vs. the local cell concentration in sedimentation–diffusion equilibrium experiments. The value of M_m is 107 000 (see Results). M_w was computed from both slopes of the curves, ln c vs. r^2 and $\int c\, dr^2$ vs. c (Teller, 1973). Composition of the buffer: 115 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, ADP (0 mM for curve c; 2 mM for curve a), 2 mM DTT, pH 6.9. For curve a, three loading concentrations were used: 0.5 (■), 1.0 (□), and 1.5 mg/mL (●). Temperature 4 °C; column length 2.5 mm; rotor speed 10 000 rpm; duration of the experiment 40 h. Solid line a corresponds to the best fit and gives $K = 0.5$ mL/mg = 2.7×10^4 M^{-1}. The same value was obtained by using eq 12. The value of 0.5 mL/mg is of the order of magnitude of K_0 for curve b in Figure 3. However, here K is significantly higher and we see that dimerization is higher at 4 °C than at 20 °C (see also below). (*) Experimental points redrawn from Figure 2 in Margossian et al. (1981). In this case, K is close to zero and eq 9 reduces to $M_w/M_m = 1 + Kc$. A linear regression on these points leads to $M_m = 108\,000$ and $K = 0.02$ mL/mg (curve b). The correlation coefficient is $r = 0.891$; the minimum value of r, which shows that the correlation is significant, is $r_{min} = 0.582$. Thus, in contrast with the opinion of Margossian et al. (1981), there is a small, but significant, amount of dimer in these experiments (~5% at 2 mg/mL). Moreover, we find that the true molecular weight for EDTA·S-1 is 108 000, i.e., exactly the same value as ours (107 000; see Results). In Figure 2 in the paper of Margossian et al. (1981), the experimental points representing M_n are systematically below those representing M_w. This is another argument in favor of the presence of a dimer. Note that the same characteristic trait occurs for Mg·S-1, and this fact confirms that dimerization is not related with the presence or absence of the LC-2 chain (see also Conclusion). (▲) Experimental points obtained in our buffer, but in the absence of ADP, with a loading concentration of 1.0 mg/mL (the experimental points for the other loading concentrations are not represented, but they superimpose on those represented here). We have, in the present case, $K = 0.14$ mL/mg. This value is significantly higher than that for K_0 for curve a in Figure 3, which confirms that K increases when the temperature decreases.

a second channel of the Yphantis cell, we introduced the same loading concentration of S-1 in a benign buffer, in which S-1 was expected to be in the form of a pure monomer. For that purpose, we made use of the following buffer: 115 mM KCl, 50 mM imidazole, and 2 mM DTT, pH 6.9. We found once more that ln c vs. r^2 was a straight line over the entire column length, but the slope corresponded to a molecular weight of 107 000 (with $\bar{V}_m = 0.740$ mL/g), i.e., exactly the value for a pure monomer (see Figure 4).

Second, we tried to confirm that the monomer and the dimer are in reversible equilibrium. For that purpose, we made use of benign buffers and three loading S-1 concentrations, as shown in Figure 4. This figure clearly confirms that the monomer and the dimer are in reversible equilibrium, since the experimental points for the three loading concentrations superimpose in any buffer [according to Teller (1973) and Margossian et al. (1981) this is a criterion of reversibility]. As for this equilibrium, we have seen above that S-1 sediments with a single boundary in all conditions, and this is a good criterion to conclude that the equilibrium is rapid. However, more precise experiments would be necessary to measure the rates of association and dissociation.

Curves b and c of Figure 4 show that the monomer is largely predominant in a buffer having a composition close to that of the physiological medium, but in the absence of a Mg–(phosphate compound): this result confirms our sedimentation velocity analyses. In sharp contrast, curve a of Figure 4 confirms that, in the presence of MgADP, the proportion of dimer is considerably enhanced. In this context, we may here propose a rough quantitative approach for the values of the different equilibrium constants. Figure 4 shows that both Mg^{2+} and MgADP increase the proportion of dimer. Let us assume that, in the absence of these compounds, S-1 is in the form of a pure monomer in a benign buffer (this is justified by our above finding that S-1 is in the form of a pure monomer in these conditions; see above). Moreover, let us assume that the two dimerization reactions may be written as

$$2M + Mg^{2+} \rightleftarrows D \qquad (14)$$

$$2M + MgADP \rightleftarrows D^* \qquad (15)$$

where M is the S-1 monomer and D and D* the dimers. By writing these reactions, we explicitly assume that only one Mg^{2+} or MgADP can bind to two monomers to make up a dimer. The two equilibrium constants can be written as

$$K_{Mg^{2+}} = [D]/([M]^2[Mg^{2+}]) \qquad (16)$$

$$K_{MgADP} = [D^*]/([M]^2[MgADP]) \qquad (17)$$

The equilibrium constant K measured in the present study is defined by

$$K = ([D] + [D^*])/[M]^2 = K_{Mg^{2+}}[Mg^{2+}] + K_{MgADP}[MgADP] \qquad (18)$$

In the study of Margossian et al. (1981), we have [MgADP] = 0, $[Mg^{2+}] \sim 0.8$ mM, and $K = 0.02$ mL/mg = 1.1×10^3 M^{-1} (see Figure 4), at 5 °C. In this case, we get $K_{Mg^{2+}} \sim 1.4 \times 10^6$ M^{-2}. In our conditions and in the absence of MgADP, we have $[Mg^{2+}] = 5$ mM and $K = 0.14$ mL/mg = 7.5×10^3 M^{-1} (see Figure 4), at 4 °C. Thus, we get $K_{Mg^{2+}} = 1.5 \times 10^6$ M^{-2}. These two values of $K_{Mg^{2+}}$ have been obtained in comparable conditions of buffers and temperatures, and they are almost identical. Therefore, our eq 14 and 15 are most likely correct.

In the presence of 3 mM Mg^{2+} and 2 mM MgADP, we have $K = 0.5$ mL/mg = 2.7×10^4 M^{-1}, and we get from eq 18, by replacing $K_{Mg^{2+}}$ with its calculated value, $K_{MgADP} = 11 \times 10^6$ M^{-2}; in our buffer and at 4 °C, we have, hence, $K_{MgADP}/K_{Mg^{2+}} = 7.3$. If the same calculation is made at 20 °C and at atmospheric pressure ($X = 0$; see Figure 3), we obtain a ratio of 11. Although our approach is extremely simplified, it appears that K_{MgADP} is about an order of magnitude greater than $K_{Mg^{2+}}$. Thus, both the sedimentation velocity and the sedimentation–diffusion equilibrium experiments show that the two protomers making up the dimer are much more tightly bound in the presence of MgADP than in the presence of Mg^{2+}, and obviously than in the absence of Mg^{2+}.

In conclusion of these experiments, we have shown that both the sedimentation velocity and the sedimentation–diffusion equilibrium experiments lead to the conclusion that an S-1 dimer exists, in reversible equilibrium with the monomer. However, we consider that the sedimentation analyses are more informative than the other types of experiments. For instance, they permit the calculation of the frictional coefficients and other related parameters (approximate degree of hydration of the monomer and approximate shape of the dimer; see Discussion).

Discussion

Preliminary Remarks. Siemankowski & Dreizen (1975) have reported that below ~50 μg/mL soluble myosin loses its light chains. It is probable that the same phenomenon occurs for S-1 concentrations < 50 × (110 000/470 000) = 12 μg/mL. Now, we have not measured $s_{20,w}^{app}$ for S-1 concentrations below 200 μg/mL. Thus, the value of $s_{m,20,w}^{0}$ obtained in the present study cannot be mistaken for the sedimentation coefficient of LC-free S-1. Our results might be related to a heterogeneity of the preparations, arising from the presence of species other than well-defined monomers and dimers. This is not the case, since *all* the S-1 species present a high degree of homogeneity, under conditions where the monomer is greatly predominant (low hydrostatic pressure; pH 7.0; 100 mM KCl, 1 mM $MgCl_2$, and 0.3 mM EGTA; low S-1 concentrations; Margossian et al., 1981).

Tinoco et al. (1978) give the following formula for the molecular weight of a protein (if $s_{20,w}^{0}$ is expressed in svedbergs):

$$M = 770(s_{20,w}^{0})^{3/2}[\eta]^{1/2}[(f_0/f)\nu^{1/3}]^{-3/2}(1 - \rho\bar{V})^{-3/2} \qquad (19)$$

$[\eta]$ is the intrinsic viscosity, and $(f_0/f)\nu^{1/3}$ is a shape factor independent of the degree of hydration. For the chymotryptic S-1 monomer, we have $M_m = 107\,000$ (see Results), $\bar{V} = 0.740$ mL/g (Yang & Wu, 1977), and $(f_0/f)\nu^{1/3} \sim 1.38$, irrespective of the exact shape of S-1 (Tinoco et al., 1978). Furthermore, $[\eta] \sim 6.5$ cm^3/g in the case where the monomer is almost pure [atmospheric pressure, no Mg^{2+}, no Mg–(phosphate compound), pH 7.0, 100 mM NaCl; Yang & Wu, 1977]. From eq 19 we deduce $s_{m,20,w}^{0} \sim 5.17$ S, a value which is in good agreement with our experimental value (Figures 1 and 3).

Choice of the Working Zone in the Cell (Sedimentation Analyses). Since we now know that s^{app} depends on the hydrostatic pressure and since this pressure increases from 1 atm at the meniscus to several hundred atmospheres at the bottom, it is imperative to have a good and invariable choice for the working zone in the cell. We have seen that the equilibrium constant K is an increasing function of the hydrostatic pressure P. Thus, K increases with the position $r_{1/2}$ of the boundary. Furthermore, the dilution law gives $c(r_2) = c(r_1)(r_1/r_2)^2$, where r_1 and r_2 are two values of $r_{1/2}$. The product Kc in eq 5 is hence a function of $r_{1/2}$, and a variation in s^{app} vs. $r_{1/2}$ is expected. However, a judicious choice of the working zone in the cell allowed us to simultaneously minimize the effects of the hydrostatic pressure and to get constant values of s^{app}. We chose for the minimal radial position $r_1 = 6.10 \pm 0.02$ cm (position of the meniscus $r_0 = 6.00 \pm 0.02$ cm) and for the maximum position $r_2 = 6.28 \pm 0.02$ cm. With these values, the variations of s^{app} with $r_{1/2}$ are of the order of magnitude of the experimental error on the determination of s^{app}. In fact, if we neglect $k_s c$ ($\ll 1$), the variations in s^{app} between r_1 and r_2 are given by

$$\Delta s^{app} = 2(s_d^0 - s_m^0) \times [1/[1 + (1 + 4Kc)^{1/2}]_{r_1} - 1/[1 + (1 + 4Kc)^{1/2}]_{r_2}] \qquad (20)$$

The maximum value of Δs^{app} is obtained at 67 770 rpm, in the conditions of curve a in Figure 3. By using eq 3 of Marcum & Borisy (1978) (with $K_0 = 0.08$ mL/mg and $\Delta\bar{V} = -0.015$ mL/g; Figure 3), we find $\Delta s^{app} \sim 0.34$ S, i. e., a maximum incertitude on s^{app} of ±0.17 S, which is of the order of magnitude of the experimental error on the measurement of s^{app}. At 40 000 rpm, the incertitude is only ±0.08 S. Thus, our choice for r_1 and r_2 is suitable. We must point out that this choice is *critical* for the present type of study. If the working zone is too far from the meniscus, only the dimer is observed (pressure effect; Figure 3). Furthermore, values of s^{app} independent of $r_{1/2}$ are obtained only if the working zone is sufficiently narrow. In fact, with the exception of the experiments in which the dimer was predominant even at atmospheric pressure, we observed a slight upward curvature in the curve ln $r_{1/2}$ vs. t, over the entire column length. This curvature is not attributable to radial dilution, since k_s for S-1 is extremely low (see Results), and a difference of less than 1% in the curvature should be expected between the meniscus and the bottom of the cell [see Meyerhof (1963) for the formula]. The observed upward curvature can be only attributed to an increase in K with the hydrostatic pressure, as already found in the results. Moreover, in our chosen working zone we could not detect any significant curvature.

Approximate Shapes and Degrees of Hydration of the Monomer and Dimer. The exact shape of the S-1 monomer is probably rather complicated, and we are now studying this problem by making use of the procedures of Yang & Wu (1977) and Mendelson & Kretzschmar (1980) and by taking into account the fact that dehydrated S-1 is pearlike shaped (Elliott & Offer, 1978). For this reason, we shall not develop here the problem, since it merits an extensive treatment. However, we think it is interesting to give some indications in this field.

Elliott & Offer (1978) have found that the myosin heads are elongated (about 19 nm long and 6.4 nm in diameter at the widest part). Thus, if we rule out the possibility of considerable distortions in the head shape by dehydration, it seems appropriate to consider that the S-1 monomer in solution can be modulated, as first approximated, by a prolate ellipsoid. Furthermore, we shall take an axial ratio of 4, which is close to the values obtained by Mendelson et al. (1973) and Kretzschmar et al. (1978). We are conscious this is an oversimplification, but we shall see in the following that this choice is sufficient for our present purpose.

According to Tinoco et al. (1978), we have for a 1:4 prolate ellipsoid $f/f_0 = 1.182$ (f = the frictional coefficient of the protein, and f_0 = the frictional coefficient of the equivalent sphere, with a volume equal to that of the solvated protein). From $s_{m,20,w}^{0} = 5.05$ S, we deduce $f = 9.2 \times 10^{-8}$ g/s (see eq 7) and $f_0 = 7.8 \times 10^{-8}$ g/s. On the other hand, f_{min} (frictional coefficient of the equivalent unsolvated sphere) is given by (Tinoco et al., 1978; eq 6.35)

$$f_{min} = 6\pi\eta[3M\bar{V}/(4\pi N_A)]^{1/3} \qquad (21)$$

η = viscosity of water at 20 °C. For the monomer, we obtain $f_{min} = 6.0 \times 10^{-8}$ g/s. According to Tinoco et al. (1978; eq 6.40), we also have

$$f_0/f_{min} = (1 + \delta V_1{}^0/\bar{V})^{1/3} \qquad (22)$$

where $V_1{}^0$ is the specific volume of the bulk solvent (1.002 mL/g for pure water at 20 °C) and δ is the degree of hydration of the protein. Here, we obtain $\delta = 0.9$ g/g. This number is in good agreement with the recently proposed value (0.65 g/g; Garcia de la Torre & Bloomfield, 1980). For a 1:4 prolate ellipsoid, we have $\nu = 4.66$ (Yang, 1961). Now, the intrinsic viscosity is given by (Tinoco et al., 1978; eq 6.59)

$$[\eta] = \nu(\bar{V} + \delta V_1{}^0) \qquad (23)$$

We get, for the monomer, $[\eta] = 7.6$ cm^3/g, which is in good agreement with the measured value (6.5 cm^3/g; Yang & Wu, 1977).

It is well-known that δ steeply depends on the choice of the ellipsoid (see eq 23, for instance), and for this reason, we shall now present an independent reasoning which confirms that the S-1 monomer is highly hydrated. Elliott & Offer (1978) have shown that the volume of the entirely dehydrated myosin head is 142000 Å^3 (their Figure 6). Now, Margossian et al. (1981) have shown that, in solution, complete S-1 (Mg·S-1) has a molecular weight of 130000. From these two results, it is easy to calculate the specific volume V_{sp} of the dehydrated protein, and we get $V_{sp} = 0.658$ mL/g. We shall consider, as a reasonable assumption, that chymotryptic S-1 has the same V_{sp}. The apparent specific volume $\bar{V}$ of a protein is given by (Tanford, 1967; eq 20.5)

$$\bar{V} = V_{sp} + \delta(V_1 - V_1^0) \qquad (24)$$

where V_1 is the specific volume of bound water. Since V_1 and V_1^0 are not very different and since $\bar{V}$ and V_{sp} are quite different, eq 24 leads to the conclusion that δ is unusually high for S-1, as proposed above. By the way, it is interesting to calculate V_1 if δ is assumed to be 0.9 g/g (see above). Equation 24 gives a specific gravity of bound water of $1/V_1 = 0.915$ g/cm^3. It is highly surprising to find a value extremely close to that of ice (0.917 g/cm^3). Since a 1:3 prolate (Kretzschmar et al., 1978) or a 1:6 oblate ellipsoid (Yang & Wu, 1977) gives the same qualitative results ($\delta = 1.2$ g/g and $1/V_1 = 0.934$ g/cm^3 for a 1:3 prolate; $\delta = 0.55$ g/g and $1/V_1 = 0.869$ g/cm^3 for a 1:6 oblate), one may wonder whether the old hypothesis of an icelike structure for bound water is not confirmed here. However, more work should be done in this field in order to definitely confirm this exciting conclusion.

For the S-1 dimer, we only know the sedimentation coefficient and the molecular weight. However, we can have an idea of its shape. From $s^0_{d,20,w} = 6.05$ S, we deduce $f = 16.3 \times 10^{-8}$ g/s. From eq 21, we deduce $f_{min} = 7.5 \times 10^{-8}$ g/s. By assuming the same value of δ for the dimer and the monomer (0.9 g/g), eq 22 leads to $f_0 = 9.8 \times 10^{-8}$ g/s. Thus, we have $f/f_0 = 1.66$, and according to Tinoco et al. (1978; Table 6.2), this value corresponds to a 1:12 prolate ellipsoid: the dimer is necessarily end to end. Note that a 1:8 prolate ellipsoid would have been expected. In this case, eq 19 gives $[\eta] = 25.5$ cm^3/g ($\bar{V}_d = 0.725$ mL/g; $M_d = 215000$; $(f_0/f)\nu^{1/3} = 1.51$; Tinoco et al., 1978; Table 6.3). From eq 23 we deduce $\delta = 1.8$ g/g ($\nu = 10.10$; Yang, 1961). This value is considerable and well above the upper limit of 1.1 g/g proposed by Yang (1961). Thus, it is possible that the two protomers are more elongated than the monomer, when they are included in the dimer. We shall not go deeper into this question, since many physical properties of the dimer are not yet known. The only firm conclusion is that the dimer is end to end (this result is intuitive, in view of the rather low difference between $s^0_{m,20,w}$ and $s^0_{d,20,w}$).

Conclusion

We conclude, from the present study, that chymotryptic S-1 can form a dimer, in rapid reversible equilibrium with the monomer. The sedimentation coefficient of the monomer is 5.05 ± 0.05 S and that of the dimer 6.05 ± 0.05 S. The two protomers making up the dimer are stuck together in an end-to-end arrangement, and both the monomer and the dimer are highly hydrated. It is important to point out that these two conclusions are independent of the choice for the exact shape of S-1. Incidentally, we have found that the water bound to S-1 has a specific gravity close to that of ice.

The monomer–dimer constant of equilibrium depends on the temperature, the hydrostatic pressure, the pH, the ionic strength, the presence or absence of a Mg–(phosphate compound), the presence or absence of Mg^{2+}, and the presence or absence of Ca^{2+} (Margossian & Lowey, 1977). We can rule out the possibility that the formation of the dimer might be related to the absence of the LC-2 light chain in chymotryptic S-1 (Figure 2B), since Margossian et al. (1975) have found that, at 60000 rpm, pH 7.0, 100 mM KCl, and 0.2 mM MgP_i, complete S-1 and LC-2 free S-1 sediment at about equal rates, 5.80 S (Lowey et al., 1969); this value corresponds to a monomer–dimer mixture (see also legend to Figure 4).

In this study, we did not intend to present an exhaustive approach of the monomer–dimer equilibrium and the individual properties of the monomer and the dimer. However, it appears that the question of the myosin subfragments is reopened. For instance, the usual enzymatic studies have probably been done on monomer–dimer mixtures, with the possibility of cooperative effects in the dimer. It should now be fundamental to study the true enzymatic properties of the monomer and the dimer, as well as their other physical and chemical properties.

Another problem is very likely related to the existence of an S-1 dimer, i.e., the problem of the refractory state described by Fraser et al. (1975). The site of dimerization is not known, but if it were close to the actin-binding site, the dimer should be unable to interact with F-actin, and it would be identified with the refractory state. With this hypothesis in mind, it is easy to understand that the amount of S-1 in the refractory state should depend on the composition of the buffer, on the hydrostatic pressure, on the temperature, and on the S-1 concentration. This conclusion agrees with observation and gives an attractive answer to the puzzling results concerning the existence and the properties of the refractory state. In a more general way, it is important to take into account the dimerization of S-1 in the experiments concerned with the binding of these subfragments to F-actin. Moreover, Fraser et al. (1975) have also shown that HMM can be in a refractory state, and this result suggests that HMM also can form a dimer. Therefore, the problems evoked here may be the same for HMM as those for S-1.

Finally, we mention the possible importance of the existence of an S-1 dimer as concerns the structure of the myosin filaments. Indeed, Morel & Gingold (1979) have suggested that, in the presence of a Mg–(phosphate compound), one head of a myosin molecule might be inserted in the backbone and might interact with a head of the opposite myosin molecule. The second head would lie outside to interact with actin. Now, we have shown here that the S-1's making up the dimer are strongly bound in the presence of MgADP. Thus the present results might support such an assumption. However, more work should be done in this direction, and it is, in particular, necessary to reconcile this model with that of Offer & Elliott (1978). We are now working on this problem.

Added in Proof

After our paper had been submitted for publication, an article by Flamig & Cusanovich (1981) appeared. These authors confirm that cardiac S-1 can form reversible polymers. Incidentally, they claim that, under their conditions, skeletal S-1 (A1) is in the form of a monomer. In the following, we discuss the case of skeletal S-1 only. The conditions of their Figure 8 are 20 °C, 1 atm, 0 mM KCl, 10 mM $MgCl_2$, 0.1 mM DTT, and 50 mM BTP, pH 7.0. In light of our results, a monomer–dimer mixture would have been expected. There are two possible explanations for this apparent contradiction. (i) S-1 (A1) alone cannot form dimers. Figure 3 in Margossian et al. (1981) shows that, for S-1 (A1) in a benign buffer, at 5 °C, we have $K = 0.03$ mL/mg ($r = 0.632$; $r_{min} = 0.440$). The value of r is significant and the value of K is comparable to that for total chymotryptic S-1 under the same conditions (our Figure 4): in imidazole, S-1 (A1) can form dimers. (ii) BTP can disrupt the S-1 dimers. In order to check this possibility, we performed two sedimentation–diffusion equilibrium experiments on total S-1, at 4 °C. The first one was done in the buffer of Flamig & Cusanovich (1981) and the second one in the same buffer, except the BTP was replaced by imidazole. In both buffers, we found a monomer–dimer mixture. However, the proportion of dimer was higher in imidazole (~60% at 3.6 mg/mL S-1) than in BTP (~40% at 3.6 mg/mL S-1). In both buffers, total chymotryptic S-1 can form dimers, but it is possible that S-1 (A1) alone behaves quite differently in BTP and in imidazole.

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Appendix

Mathematical Treatment of the Experimental Points Obtained in Figure 3. The hydrostatic pressure P at a radial position r is defined by $P = \rho\omega^2(r^2 - r_0^2)/2$ (Harrington & Kegeles, 1973). It is possible to define a mean pressure $\bar{P}$ in the working zone by $\bar{P} = \rho\omega^2(\bar{r}_{1/2}^{\,2} - r_0^2)/2$, where $\bar{r}_{1/2}$ is defined under Theory. ρ was taken as 1 g/cm^3 for both buffers. According to eq 13, we get for the mean value of the equilibrium constant $K = K_0 \exp(-5.57\Delta\bar{V}X)$ ($\Delta\bar{V}$ in mL/g). The choice of a mean value for K is justified, since the variations of K with $r_{1/2}$ induce only small variations in s^{app} (see Discussion). The expression of K was introduced in eq 5. In this equation, there are four unknown parameters: s_{m}^0, s_{d}^0, K_0, and $\Delta\bar{V}$. For estimations of these parameters, the two sets of experimental points were each fitted by single exponentials of the form $s^{\mathrm{app}} = \alpha - \beta \exp(-\gamma X)$. When $X \rightarrow \infty$, the asymptote is α and $s_{\mathrm{d}}^0/(1 + k_s c)$ in eq 5 ($\Delta\bar{V} < 0$). Since k_s is known (0.010 mL/mg), s_{d}^0 was estimated from $s_{\mathrm{d}}^0 = \alpha(1 + k_s c)$. When $X \rightarrow 0$, the exponential equation gives $s^{\mathrm{app}} = \alpha - \beta$, and eq 5 is unchanged, except that K is replaced by K_0. s_{m}^0 was taken as 5.00 S (Figure 1) and K_0 could be estimated by equating eq 5 with $\alpha - \beta$. The estimated values of s_{m}^0, s_{d}^0, and K_0 were introduced in eq 5, and an estimation of $\Delta\bar{V}$ was obtained by the least-squares method. Finally, the estimated values of s_{m}^0, s_{d}^0, and K_0 were varied by steps of 0.01 unit, and the least-squares method was repeated, leading to new values of $\Delta\bar{V}$. It was observed that the SD reaches a minimum value, corresponding to curves a and b and to the different values given in the legend to Figure 3.

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