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Dissecting the free energy of formation of the 1:1 actomyosin complex

Enrico Grazi*, Raffaella Adami, Orietta Cintio, Paola Cuneo, Ermes Magri, Giorgio Trombetta

Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Via Borsari 46, Ferrara 44100, Italy

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Abstract

The behaviour of solutions of pure myosin, of pure F-actin and of the equimolar mixture of myosin and of F-actin is studied. It is found that the chemical potential of the two proteins, in separate solutions, increases monotonically with the increase of protein osmotic pressure. A method is presented to determine the chemical potential of the 1:1 actin-myosin complex formed from equimolar solutions of myosin and of F-actin (as monomer).This is the first evaluation of the chemical potential of actomyosin under conditions similar to those of skeletal muscle. It is found that the filament suspensions of myosin and of the 1:1 actin-myosin complex display a high non-ideal behavior as well as distinctly different energy profiles as a function of protein osmotic pressure. This supports the hypothesis that, in muscle: (a) detached cross-bridge change significantly their free energy when sarcomere is shifting from the relaxed to the active or to the rigor state; and (b) the cross-bridge attachment-detachment process is accompanied by changes of muscle protein osmotic pressure.

Keywords: Free energy; Myosin; Actomyosin; Attached-detached cross-bridges

* Corresponding author. Tel.: +39-532-291-421; fax: +39-532-202-723.
E-mail address: gre@ifeuniv.unife.it (E. Grazi).

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1. Introduction

Important studies were performed by Reiss et al. [1,2], Ogston [3], Lebowitz et al. [4], Gibbons [5], Ross and Minton [6], Minton [7] on the non-ideality of macromolecular solutions. Parsegian et al. [8] investigated the relationship between macromolecular osmotic pressure and the energetics of macromolecules and of complex biological structures.

We employed these techniques to study the behaviour of the 'contractile' proteins of skeletal muscle, with the aim to contribute to the unraveling of the mechanism of muscle contraction.

We took advantage of the geometrical constraint induced by hexagonal packing of actin filaments under an osmotic stress, to show that interfilament distance decreases with the increase of protein osmotic pressure [9,10]. On this ground, we proposed a new model of the actin filament, the 'fluttering wing' model, which predicts the diameter, the orientation of the monomers and the intermonomer contacts of the actin filament to vary as a function of protein osmotic pressure [11].

We compared the effect of osmotic stress on the pure and on the myosin sub-fragment-1 decorated actin filament and showed that the decorated actin filament displays a larger rigidity than the pure actin filament. On this ground, we made the hypothesis that the stiffness of the cross-bridge components and the contractile force of skeletal muscle are a function of the intrinsic osmotic pressure generated by the cross-bridge itself [12–14].

We estimated the free energy of interaction of myosin filaments with pure and with regulated F-actin under the conditions occurring in the A-band in vivo [15]. In that occasion we made no attempt to partition the free energy change between free myosin and actomyosin. We tackle now this goal by providing a method that, on the sole basis of osmotic pressure measurements, allows to partition the free energy change between free myosin and actomyosin. The result is achieved by analyzing three systems:

1. a solution of myosin;
2. a solution of F-actin; and
3. a solution of myosin and of F-actin,

the systems being at the macromolecular osmotic equilibrium, through a semipermeable membrane, with an external reservoir of very large volume and of given macromolecular osmotic presssure, obtained by the suitable addition of poly(ethyleneglycol).

2. Materials and methods

Globular(G)-actin [16] and myosin [17] were prepared from rabbit muscle. Molar concentrations were calculated on the basis of molecular masses of 42 kDa for actin [18] and of 470 kDa for myosin [17]. The absorption coefficients used were as follows: actin, $A^{1\%}_{290}$, of 6.2 [19]; myosin, $A^{1\%}_{280}$ of 5.3 [17]. Bipolar myosin filaments were prepared according to Honda and Asakura [20]. In our hands, this procedure gave rise to filaments of the average length of 0.7, 0.1 μm.

Buffer solutions for osmotic stress experiments contained in 1000 g of water: KCl, 0.1 mol; triethanolamine, 0.01 mol; $MgCl_2$, NaN_3, 2-mercaptoethanol, 2 mmol each. pH was taken to 7.5 with 6 M HCl.

Solutions of F-actin, of myosin filaments and of their mixtures at 1:1 molar ratio were prepared and dialysed for 17 h at 2°C against the same buffer. Immediately before mixing, the solutions containg F-actin were gently homogenized by hand in a Potter-Elvhejm device to fragment the filaments of actin and allow a more homogeneous interaction with the filaments of myosin. Since we are dealing with equilibrium studies, the presence of ATP, which would affect the assembly of myosin with actin, had to be avoided. To achieve this, F-actin, which usually is kept in 0.2 mM ATP, was sedimented and the pellet was suspended in the buffer, which does not contain ATP and dialysed as described above. The osmotic pressure of the protein system was measured using a secondary osmometer, protein solutios (1 ml) were equilibrated by dialysis against buffer solutions (100 ml) supplemented with weighed amounts of poly(ethyleneglycol) 40 000. The osmotic pressure

associated with the poly(ethyleneglycol) solutions was measured directly with a pressure gauge [10]. Equilibration of protein solutions was carried out for 48–96 h, at 22°C, in stopped bottles, immersed in a shaking water bath thermostatically controlled to within ± 0.1°C. At the end of the equilibration, protein concentration was measured as previously described [10,15].

For electron microscope observation, bipolar myosin filaments were diluted with the same suspension buffer to a protein content of 0.1 mg/ml and immediately applied to carbon-coated 400 mesh grids washed once with a drop of water and stained with five drops of 1% uranyl acetate, pH 4.25.

Embedding in Araldite. To observe the packing of actomyosin, in some cases, at the end of the equilibation against poly(ethyleneglycol), glutaraldehyde (final concentration 1%) was added to the poly(ethylenglycol) solution and dialysis was continued for 17 h at 22°C. The dialysis bag was then opened and the small protein clot was immersed in liquid nitrogen. The frozen sample was thus treated essentially, as described by Craig et al. [21], in their procedure 1 and embedded in Araldite. Thirthy-five-nano meter sections were collected on naked grids and stained with 4% aqueous uranyl acetate, followed by lead citrate [22].

3. Theory

3.1. Premise

Changing the volume fraction or concentration of the macromolecular species in its phase by applying osmotic stress is physical work done on that species. This work is most naturally expressed in terms of the chemical potential of the macromolecules subject to stress at the fixed values of the intensive thermodynamic variable pertaining to the particular preparation:

$$\Delta\mu_w(T,P,a_i) = -\pi\Delta V$$

If the exchangeable medium is considered as a single multicomponent system, one can speak of chemical potentials of all species, the multicomponent system s, the stressing polymer p and the macromolecule of interest m by the Gibbs–Duhem relation for each phase:

$$n_s d\mu_s = -n_m d\mu_m$$

$$n_s d\mu_s = -n_p d\mu_p.$$

The chemical potential of each is related in an obvious way to the osmotic pressure of water in the two solutions [8]. The macromolecules being charged does not impair the validity of the measurements since, the overall free energy change is referred to the system composed by the macromolecules plus the surrounding cloud of the neutralysing ions. This means that 'the component is electrically neutral even when the species is charged' as it was pregnantly stated by Scatchard [23]. We are well aware that both myosin and actin are present as filaments and that osmotic pressure ought to be a direct function of their number density. We have preferred, however, to relate the free energy change of actin and myosin, under osmotic stress to the concentration of the monomeric species of actin and of myosin. The choice is justified by the fact that the system is highly non-ideal and that, in any case, its behaviour ought to be described phenomenologically by making use of an activity coefficient. It would thus appear an unnecessary complication to try to relate the free energy change to the number density of actin and myosin filaments.

3.2. Experimental design

Let us consider four compartments. Compartment (A) contains pure water and compartment (B) contains a solution of small electrolytes. The two compartments are separated by a membrane permeable only to water. Water chemical potential is lower in compartment (B) and an osmotic pressure P_e generates. Compartment (C) contains the solution of small electrolytes plus a macromolecule, poly(ethyleneglycol) and is separated from compartment (B) by a membrane permeable to water and to the small electrolytes but not to

the macromolecule. After equilibration of the diffusible species, water chemical potential in compartment (C) turns out to be lower than in compartment (B) and the osmotic pressure P_i, larger than P_e. (P_i–P_e) represents the macromolecular osmotic pressure (P). Compartment (D) contains the solution of small electrolytes plus the proteins under study and is separated from compartment (C) by a membrane permeable to water and to the small electrolytes but not to the macromolecules. After equilibration, the water chemical potential as well as the macromolecular osmotic pressure are the same in compartments (C) and (D). The chemical potentials of all the diffusible species, except water, are the same in all the compartments. In practice the system is simpler, the solution of compartment (D), is equilibrated against a much larger volume of the solution of compartment (C), which is at the desired macromolecular osmotic pressure (P), so that at the equilibrium macromolecular osmotic pressure is the same in compartments (C) and (D).

3.3. Solution of a single macromolecular solute

The sample solution is equilibrated with the external reservoir at the macromolecular osmotic pressure, P, the small electrolytes solution is considered here as a single multicomponent system. μ_1, a_1, n_1 and m_1, respectively, are the chemical potential, the activity, the number of moles and the molality of water, μ_2, a_2, n_2 and m_2, are the chemical potential, the activity, the number of moles and the molality of protein. V is the partial molar volume of water (18 cm^3) and ϕ is the molal osmotic coefficient.

From the followig relationships:

$$m_1 VP/RT = -m_1 \ln[a_1] = \phi m_2 \quad (1)$$

and

$$(n_1 d\mu_1 + n_2 d\mu_2 = 0)_{T,P,n_2}, \quad (2)$$

the chemical potential of the protein is calculated:

$$RT\, d\ln[a_1] + n_2/n_1\, RT\, d\ln[a_2] = 0,$$

$$RT\, d\ln[a_1] + m_2/m_1\, RT\, d\ln[a_2] = 0,$$

$$m_2\, RT\, d\ln[a_2] = -m_1\, RT\, d\ln[a_1] = RT\, d(\phi\, m_2),$$

$$m_2 RT\, d\ln[a_2] = RT(m_2 + d\phi + \phi + dm_2). \quad (3)$$

$$d\mu_2 = RT(d\phi + \phi/m_2 \cdot dm_2) \quad (3\text{ bis})$$

$$\Delta\mu_2 = RT \times \int_{m_2}^{m_2'} \frac{\phi}{m_2} dm_2 + RT \times (\phi' - \phi). \quad (4)$$

From Eq. (3) the activity coefficient of the protein is also calculated [24]:

$$m_2 d\ln[a_2] = m_2 d\ln[m_2] + m_2 d\ln[\gamma_2] = \phi dm_2 + m_2 d\phi$$

$$d\ln[\gamma_2] = (\phi - 1) d\ln[m_2] + d\phi = (\phi - 1)/m_2 dm_2 + d\phi. \quad (5)$$

Integration of Eq. (5) since $\phi \to 1$ as $m_2 \to 0$, gives

$$\ln[\gamma_2] = \int_0^{m_2} \frac{(\phi - 1)}{m_2} dm_2 + (\phi - 1). \quad (6)$$

3.4. Solution of two macromplecular solutes undergoing association, the reaction being at chemical equilibrium

A solution of F-actin plus myosin and a solution of myosin alone are equilibrated at the same osmotic pressure:

$$n_1 d\mu_1 + n_m d\mu_m + n_a d\mu_a + n_{ma} d\mu_{ma} + n_{ma_2} d\mu_{ma_2} = 0 \quad (7)$$

$$d\mu_1 = -(n_m^*/n_1^*) d\mu_m^* \quad (8)$$

where, in the actomyosin solution, n_1, n_m, n_a, n_{ma}, n_{ma_2} indicate the number of moles of water, of free myosin, of free F-actin monomer, of the 1:1 actin-myosin complex and of the 2:1 actin-myosin compex, respectively, while, in the pure myosin solution, n_1^* and n_m^* indicate the number of moles of water and of myosin. In the actomyosin solution the total number of moles of myosin equals the total number of moles of F-actin (as monomer), futhermore, the two solutions contain the same amount of total myosin (n_m^*). It thus follows that:

$$n_m^* = n_m + n_{ma} + n_{ma_2} = n_a + n_{ma} + 2n_{ma_2}$$

and Eq. (7) becomes:

$$\begin{aligned} &n_1 d\mu_1 + (n_m^* - n_{ma} - n_{ma_2}) d\mu_m \\ &\quad + (n_m^* - n_{ma} - 2n_{ma_2}) d\mu_a + n_{ma} d\mu_{ma} \\ &\quad + n_{ma_2} d\mu_{ma_2} = 0 \end{aligned}$$

$$\begin{aligned} &n_1 d\mu_1 + n_m^*(d\mu_a + d\mu_m) - n_{ma}(d\mu_a + d\mu_m) \\ &\quad - n_{ma_2}(d\mu_a + d\mu_m) - n_{ma_2} d\mu_a + n_{ma} d\mu_{ma} \\ &\quad + n_{ma_2} d\mu_{ma_2} = 0. \end{aligned} \tag{9}$$

Since the system is at the equilibrium the following relationships hold:

$$d\mu_m + d\mu_a = d\mu_{ma}; \quad d\mu_{ma} + d\mu_a = d\mu_{ma2}$$

and equation (9) becomes:

$$d\mu_1 = -(n_m^*/n_1) d\mu_{ma}. \tag{10}$$

Since osmotic pressure is the same in both systems, Eq. (8) and Eq. (10) can be compared. If now, as it is experimentally feasible, the two systems undergo the same infinitesimal change of μ_1, the right terms of Eq. (8) and Eq. (10) can be equated:

$$(n_m^*/n_1) d\mu_{ma} = (n_m^*/n_1^*) d\mu_m^*$$

and

$$d\mu_{ma} = n_1/n_1^* d\mu_m^* = m_m^*/m_m d\mu_m^*. \tag{11}$$

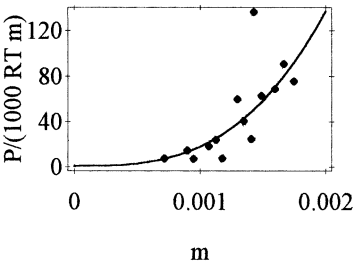

Fig. 1. Protein osmotic pressure as a function of myosin concentration. Myosin concentration (m) is expressed in molality, protein osmotic pressure (P) is expressed in Pa. Data are fitted by the equation:

$$P = 2.45 \times 10^6 \ (m_m + 100 \ m_m^2 + 1.7 \times 10^{10} \ m_m^4).$$

where, m_m^*, is the molality of myosin in the solution containing myosin alone and, m_m, is the concentration of total myosin in the solution containing both myosin and F-actin.

4. Results

4.1. Myosin

Myosin solutions were equilibrated at increasing protein osmotic pressure. It was found that, with the increase of the pressure from 13.5 to 479 kPa, the molality of myosin increased from 0.72 to 1.75 mmolal. In Fig. 1 the observed values of protein osmotic pressure are plotted as a function of myosin molality. Data are fitted by the equation:

$$P = 2.45 \times 10^6 (m_m + 100 m_m^2 + 1.7 \times 10^{10} m_m^4) \quad \text{Pa}. \tag{12}$$

By making use of Eq. (12) the osmotic molal coefficient ($\phi = m_1 VP/RT/m_2$), the $\Delta\mu$ of myosin and the activity coefficient of myosin were calculated.

The $\Delta\mu$ of myosin was calculated by analytic integration of Eq. (4). 3.68×10^{-4} molal myosin was taken as a reference, corresponding to the protein osmotic pressure of 1.7 kPa, the lowest pressure attained in our experiments.

The logarithm of the activity coefficient of

Table 1
Protein osmotic pressure, molal osmotic coefficient, ln γ and $\Delta\mu$ of myosin as a function of myosin molality[a]

Myosin (mmolal)	Osmotic pressure (kPa)	Molal osmotic coefficient	ln (activity coefficient)	$\Delta\mu$ (J mol^{-1})
0.7	11.8	6.90	7.91	1.80×10^4
0.8	19.1	9.78	11.76	2.77×10^4
0.9	29.7	13.48	16.70	4.01×10^4
1.0	44.3	18.10	22.86	5.55×10^4
1.1	63.9	23.73	30.38	7.42×10^4
1.2	99.6	30.49	39.41	9.65×10^4
1.3	122.5	38.48	50.06	12.28×10^4
1.4	163.9	47.79	62.47	15.34×10^4
1.5	215.0	58.52	76.80	18.86×10^4
1.6	277.5	70.79	93.16	22.89×10^4
1.7	352.7	84.69	111.70	27.44×10^4
1.8	442.4	100.32	132.55	32.57×10^4

[a]Osmotic pressure was calculated from the equation: Osmotic pressure = 2.45×10^6 ($m_m + 100\ m_m^2 + 1.7\times10^{10}\ m_m^4$), PaMolal osmotic coefficient = $m_1\ VP/(RT\ m_m)$. $\Delta\mu_m$ was calculated as described in the text, with reference to 3.68×10^{-4} molal myosin, corresponding to the protein osmotic pressure of 1.7 kPa, the lowest pressure attained in our experiments. ln [γ_m] was calculated as described in the text.

myosin was calculated by analytic integration of Eq. (6). The values are reported in Table 1.

4.2. F-actin

F-actin solutions were equilibrated at increasing protein osmotic pressure. It was found that, with the increase of the pressure from 1.7 to 223.8 kPa, the molality of F-actin (as monomer) increased from 5.67 to 22.2 mmolal. In Fig. 2 the observed values of protein osmotic pressure are plotted as a function of F-actin molality (as monomer). Data are fitted by the equation:

$$P = 2.45\times10^6(m_a - m_a^{1.04} + 1.28\times10^7 m_a^{5.09}),\quad Pa. \tag{13}$$

By making use of Eq. (13) the osmotic molal coefficient ($\phi = m_1VP/RT/m_a$)and the $\Delta\mu$ of actin were calculated.

The $\Delta\mu$ of F-actin was calculated by analytic integration of Eq. (4), 5 mmolal F-actin (as monomer) was taken as a reference, corresponding to the protein osmotic presssure of 2.4 kPa.

The logarithm of the activity coefficient of actin was calculated by analytical integration of Eq. (6). The values are reported in Table 2.

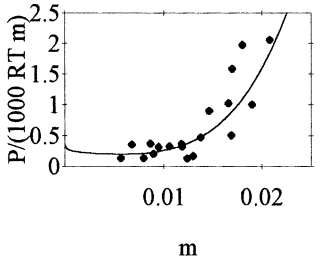

Fig. 2. Protein osmotic pressure as function of F-actin concentration. F-actin concentration (as monomer) (m) is expressed in molality; protein osmotic pressure (P) is expressed in Pa. Data are fitted by the equation:

$P = 2.45\times10^6\ (m_a - m_a^{1.04} + 1.28\times10^7\ m_a^{5.09})$.

4.3. Actomyosin

Myosin and F-actin (equimolar as monomers) were equilibrated at increasing protein osmotic pressure. It was found that, with the increase of the pressure from 1.7 to 229 kPa, the molality of total myosin increased from 0.45 to 1.8 mmolal. In Fig. 3 the observed values of protein osmotic pressure are plotted as a function of total myosin

Table 2
Calculated protein osmotic pressure, molal osmotic coefficient, ln γ and $\Delta\mu$ of F-actin as a function of F-actin molality (as monomer)[a]

Actin (nmolal)	Osmotic pressure (kPa)	Molal osmotic coefficient	ln (activity coefficient)	$\Delta\mu$ (J mol^{-1})
5	2.4	0.196	−21.028	0
6	2.9	0.195	−21.175	72.7
7	3.4	0.199	−21.295	135
8	4.1	0.209	−21.391	191
9	5.0	0.226	−21.466	244
10	6.2	0.253	−21.520	297
11	7.8	0.290	−21.553	352
12	10.0	0.340	−21.562	412
13	12.9	0.407	−21.546	478
14	16.9	0.492	−21.502	553
15	22.0	0.599	−21.427	639
16	28.6	0.731	−21.317	739
17	37.1	0.891	−21.168	854
18	47.8	1.084	−20.975	986
19	61.2	1.314	−20.735	1140
20	77.7	1.585	−20.441	1320
21	97.8	1.901	−20.089	1520
22	122.2	2.268	−19.672	1750

[a] Pressure was calculated from the equation: Osmotic pressure = 2.45×10^6 ($m_a - m_a^{1.04} + 1.28 \times 10^7\ m_{ma}^{5.09}$), PaMolal osmotic coefficient = $m_1\ VP/(RT\ m_a)$. $\Delta\mu$ was calculated as described in the text, with reference to 5 mmolal F-actin (as monomer), corresponding to the calculated protein osmotic pressure of 2.4 kPa. ln γ was calculated as described in the text.

Table 3
Observed and calculated concentrations of total myosin in the F-actin-myosin solutions. Activities ratios and $\Delta\mu$ of the 1:1 actin-myosin complex as a function of protein osmotic pressure[a]

Pressure (kPa)	Total myosin observed (mmolal)	Total myosin calculated (mmolal)	Activity ratios of the 1:1 actomyosin complex	$\Delta\mu$ (J mol^{-1})
1.7	0.450	0.468	1.0	0
3.6	0.600	0.574	4.8	0.38×10^4
6.9	0.635	0.687	39.4	0.90×10^4
8.5	0.830	0.729	97	1.12×10^4
10.0	0.830	0.763	219	1.32×10^4
13.2	0.770	0.824	1166	1.73×10^4
14.0	0.800	0.838	1683	1.82×10^4
16.0	0.860	0.870	4483	2.06×10^4
18.0	0.890	0.90	11 005	2.28×10^4
20.9	0.860	0.938	40 629	2.60×10^4
32.3	1.000	1.06	4.26×10^6	3.74×10^4
56.2	1.325	1.24	1.90×10^{10}	5.80×10^4
85.1	1.56	1.40	1.51×10^{14}	8.00×10^4
229.0	1.80	1.86	4.00×10^{29}	16.70×10^4

[a] Total myosin concentration was calculated by Eq. (6). The ratios of the activities of the 1:1 actomyosin complex was calculated by Eq. (19). The $\Delta\mu$ for the 1:1 actin-myosin complex was calculated by Eq. (18). The pressure of reference was 1.7 kPa, the lowest experimental pressure attained in our experiments.

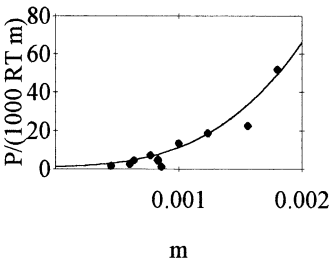

Fig. 3. Protein osmotic pressure of an equimolar mixture of myosin and of F-actin as a function of total myosin concentration. The mixtures contain equimolar concentrations of myosin and of F-actin (as monomer). Concentration (m) is expressed in molality of total myosin (free myosm + actomyosin). Protein osmotic pressure (P) is expressed in Pa. Data are fitted by the equation:

$P = 2.45 \times 10^6\ (m_{\mathrm{m}} + 2600\ m_m^2 \times 7 \times 10^9\ m_m^{3.99})$.

molality. Data are fitted by the equation (where, m_{m}, indicates total myosin molality) (Table 3):

$$P = 2.45 \times 10^6 (m_{\mathrm{m}} + 2600 m_{\mathrm{m}}^2 + 7 \times 10^9 m_{\mathrm{m}}^{3.99}),$$

$$\text{Pa}. \qquad (14)$$

To calculate the free energy of formation of actomyosin from myosin and from F-actin the behaviours of the actomyosin solution and of a solution containing only myosin were compared.

At the time of mixing the actomyosin solutions contained, n_{m}^*, moles of myosin and an identical number of moles of F-actin (as monomer). At the equilibrium the solutions contained, $(n_{\mathrm{m}}^* - n_{\mathrm{ma}} - n_{\mathrm{ma_2}})$, moles of myosin, $(n_{\mathrm{m}}^* - n_{\mathrm{ma}} - 2n_{\mathrm{ma_2}})$, moles of F-actin monomer, n_{ma} moles of the 1:1 actin-myosin complex and $n_{\mathrm{ma_2}}$ moles of the 2:1 actin-myosm complex.

The pure myosin solutions (the same solutions as in Fig. 1) contained, n_{m}^* moles of myosin and were equilibrated at the same osmotic pressures as the actomyosin solutions.

In order to compare the two sets of solutions, the molality of myosin was expressed as a function of osmotic pressure. The data from the solutions containing myosin alone were fitted by the equation:

$$\text{Myosin molality} = 0.000349777 + 1.16659 \times 10^{-5} P^{0.4} - 2.00647 \times 10^{-9} P, \quad (15)$$

which is equivalent to Eq. (12). The data from the actomyosin solutions were fitted by the equation:

$$\text{Total myosin molality} = 0.0000171694(1 + 1.51356 P^{0.18} + 2.04174\ \mathrm{P}^{0.31}), \quad (16)$$

which is equivalent to Eq. (14).

Since the (3 *bis*) is: $d\mu_{\mathrm{m}}^* = RT(d\phi^* + \phi^*/m_{\mathrm{m}}^* dm_{\mathrm{m}^*})$, where ϕ^* is the molal osmotic coefficient of myosin in the solution containing myosin alone, Eq. (11) becomes:

$$d\mu_{\mathrm{ma}} = \frac{m_{\mathrm{m}}^*}{m_{\mathrm{m}}} \times RT \times \left(d\phi^* + \frac{\phi^*}{m_{\mathrm{m}}^*} \times dm_{\mathrm{m}}^* \right), \quad (17)$$

where, m_{m}^*, is the molality of myosin in the solution containing myosin alone and m_{m} is the molality of total myosin in the solution containing both myosin and F-actin. The expressions for the molalities as a function of osmotic pressure, Eq. (15) and Eq. (16), are then introduced into Eq. (17), which by integration, becomes:

$$\Delta\mu_{\mathrm{ma}} = RT \times \int_P^{P'} \frac{\phi^*}{m_{\mathrm{m}}} \times \frac{dm_{\mathrm{m}}^*}{dP} dP + RT \times \int_P^{P'} \frac{m_{\mathrm{m}}^*}{m_{\mathrm{m}}} \times \frac{d\phi^*}{dP} dP. \quad (18)$$

The free energy of formation of actomyosin is then calculated by numerical integration of Eq. (18), after analytical differentiation of the terms, dm_{m}^*/dP and $d\phi^*/dP$. In the equation the terms marked by the asterisk belong to the system containing myosin alone.

The activity coefficient cannot be calculated, since the actual concentration of the 1:1 actomyosin complex is unknown, yet the ratios of the activities were calculated from the equation:

$$\Delta\mu_{ma}/RT = \ln\left[a'_{ma}/a_{ma}\right]. \quad (19)$$

5. Discussion

The aim of this work is to provide a picture of the behaviour of F-actin, of myosin filaments and of their interacting mixtures under conditions approaching the in vivo conditions, i.e. under conditions reproducing closely the in vivo protein osmotic pressure. Our observations will be discussed from a purely thermodynamic point of view as well as a contribution to the unraveling of the connections between work delivery and free energy changes in the contractile structures.

5.1. Thermodygamic significance

In pure F-actin and in pure myosin filaments solutions, the chemical potential of the two proteins increases monotonically with protein osmotic pressure. Even though we are dealing with complex filamentous structures, we have related the free energy change of actin and myosin under osmotic stress to the concentrations of the monomeric species of actin and of myosin. The choice is justified by the fact that the system is highly non-ideal and that, in any case, its behaviour ought to be described phenomenologically by making use of an activity coefficient. It would thus appear an unnecessary complication to try to relate the free energy change to the number density of actin and myosin filaments.

Due to the high polymerization degree of F-actin, molal osmotic coefficients lower than 1 are found. Their values range from 2.268 to 0.196, between 22 and 5 mmolal F-actin and are expected to tend to 1 at infinite dilution of actin and accompanying solutes.

Ln $[\gamma_a]$ is -21.028 at 5 mmolal F-actin and reaches a minimum of -21.562 at 12 mmolal F-actin, then increases to -19.672 at 22 mmolal F-actin. Interestingly Ln $[\gamma_a]$ is negative even at F-actin concentrations larger than 18 mmolal, where the molal osmotic coefficient is larger than 1. This is because Ln $[\gamma_a]$ is obtained by integration starting from zero F-actin concentration [Eq. (6)]. The high negative value of Ln $[\gamma_a]$ indicates the much higher stability (lower energy) of the F-monomer as compared to the G-monomer in the ideal solution.

In the study of the chemical potential of the 1:1 actin-myosin complex, formed from equimolar (as monomers) suspensions of myosin filaments and of F-actin, particular care was employed to insure complete and homogeneous formation of the actomyosin complex. To allow the two species of filaments to interact properly, dilute suspensions (~ 10 μM as monomers) of the two proteins were mixed, moreover, immediately before mixing, F-actin was fragmented mechanically to favour recognition of actin monomers by the myosin heads. The actomyosin clots did not certainly attain the sarcomere organization, nevertheless, we could observe that the decorated myosin filaments were organized quite regularly after equilibration against poly(ethyleneglycol) (data not shown).

The 1:1 actin to myosin (as monomers) stoicheiometry was selected to allow the analysis of the data. This stoicheiometry differs from that of 2.6 calculated for the dense region of the A-band in muscle [15]. Nevertheless, our data, which are obtained directly and without any limiting assumption from osmotic pressure measurements, provide the first evaluation of the chemical potential of the 1:1 actin-myosin complex under conditions similar to those of skeletal muscle.

5.2. Work performance and free energy change in the contractile structure

The performance of work by muscle is determined with great precision. Problems arise, on the contrary, when identifying the contractile structures of highest energy and the free energy change accompanying their decay. To overcome this difficulty the reconstruction of muscle energetics was attemped from studies on actomyosin kinetics in diluted solution, i.e. in a quasi ideal solution. Unfortunately, muscle is a highly non-ideal 'solution'. This is not at all unexpected but is documented beyond any reasonable doubt in the present report. A concentrated myosin filaments suspensions (detached cross-bridges in the

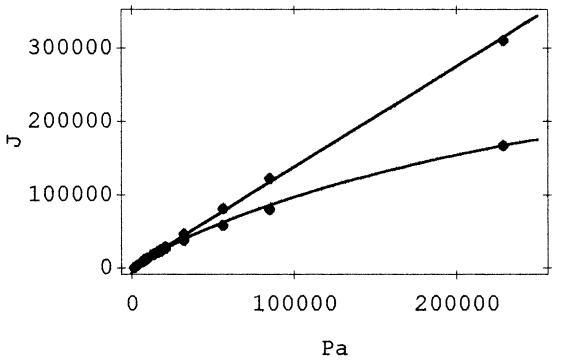

Fig. 4. Energy profile of myosin and of the 1:1 actin-myosin complex as a function of protein osmotic pressure. Myosin, upper line. Data are taken from Table 1 and are fitted by the equation:

$$\Delta\mu = 3.41389\,(P - 0.17\times10^{4})^{0.91} + 2.3988\times10^{-3} \times (P - 0.17\times10^{5})^{1.38}, \text{J}$$

1:1 actin-myosin complex, lower line. Data are taken from Table 3 and are fitted by the equation:

$$\Delta\mu = 4.31613\,(P - 0.17\times10^{4})^{0.91} - 0.0245796 \times (P - 0.17\times10^{4})^{1.27}, \text{J}$$

sarcomere) behaves non-ideally (Fig. 1). Detached cross-bridges are thus, expected to change significantly their free energy when sarcomere is shifting from the relaxed to the active or to the rigor state. The 1:1 actin-myosin complex (attached cross-bridges in rigor) also behaves non-ideally (Fig. 3). The free energy profiles of myosin and of the 1:1 actin-myosin complex, as a function of protein osmotic pressure, are distinctly different (Fig. 4). Again this is not unexpected but it is worth while stressing, the conversion of myosin into the 1:1 actin-myosin complex is controlled by protein osmotic pressure. So far in our studies, we have taken 18 kPa as the physiological protein osmotic pressure of skeletal muscle [25]. However, if mixing myosin and F-actin (the formation of attached cross-bridges in rigor) alters protein osmotic pressure, the question arises whether such a thing really does exist as the physiological protein osmotic pressure of skeletal muscle. Let us take actomyosin as a reasonable model of muscle in rigor and myosin as a poorer model of relaxed muscle. Now, if 18 kPa is the protein osmotic pressure of relaxed muscle, the corresponding myosin molality in the model is 7.87×10^{-4} molal [Eq. (12)]. Once actomyosin is formed the same total myosin molality generates [Eq. (14)] the protein osmotic pressure of 12.9 kPa. If, on the contrary, 18 kPa is the protein osmotic pressure of muscle in rigor, the corresponding total myosin molality in the model is 8.79×10^{-4} molal [Eq. (14)]. The same concentration of myosin, after relaxation [Eq. (12)] generates the protein osmotic pressure of 27.2 kPa. Thus, actomyosin formation has a clearly detectable influence on muscle protein osmotic pressure, i.e. on the water chemical potential of the highly non-ideal solution that is the contractile apparatus. Alteration of the water chemical potential necessarily influences the energetics of all the contractile structures, including those complexed with ATP, which cannot be studied by means of an equilibrium method. We are aware that our observations are not exhaustive. Our aim is to provide a borderline for an educated guess on muscle energetics.

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