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Melting of myosin rod as revealed by electron microscopy. I. Effects of glycerol and anions on length and stability of myosin rod

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Myosin rod — helix-coil transition — length — stability

The length of the rods of intact myosin molecules and of isolated myosin rods were determined under a variety of conditions by electron microscopy. In all experiments, except for freeze-drying, the temperature and *p*H were kept at 20 °C and 7.0.

Glycerol was found to have a marked effect on the stability of myosin especially for air-dried molecules. In the presence of 0.3 M of volatile buffer salts, e.g., ammonium acetate, -formate, -benzoate, -bicarbonate, -carbamate, 30 to 50% of glycerol were needed to get average lengths of myosin rods comparable to published values and to the values of freeze-dried molecules (145–149 nm). Below 10% glycerol the average length of rods was shorter by about 10 and 20 nm in intact myosin and isolated rods, respectively. Chloride caused a significant concentration-dependent shortening of myosin rods due to destabilization of the α-helical double coiled rod structure. Similar or higher concentrations of volatile salts, not containing chloride as an anion, had no shortening effect.

Thus, subtle influences depending on the composition of the dispersion solution on the final appearance and lengths of myosin rods have to be considered, before studying temperature- and *p*H-dependent changes of myosin rod structure [15].

Introduction

The myosin molecule, consisting of 2 heavy chains and two pairs of different light chains [16] displays an α-helical coiled-coil rod, 140 to 150 nm long [1, 5, 9] and 2 condensed globular heads of 20 nm in length and 3.5 to 6.5 nm in width [1, 2, 14]. Cyclic attachment and detachment of myosin cross-bridges in conjunction with structural changes within the myosin molecule, e.g., rotation of myosin heads accompanied by retraction of a spring-like elastic component in the myosin S-2 segment [4] or active shortening (melting) of a defined region within S-2 caused by a temperature-dependent helix-coil transition [3] are thought to produce the force necessary to slide the interdigitating thick and thin filaments during contraction. In order to see whether a shortening of myosin rods as a function of temperature and *p*H [3] may be directly visualized by electron microscopy, we found it necessary to test first several parameters involved in the preparation of myosin specimens for electron microscopy for their effect on the length, stability and/or flexibility of myosin molecules and myosin rods. Our results show that the amount of glycerol, the composition of dispersion buffers and especially the type of anions used for the preparation of myosin molecules for electron microscopy influence the apparent length and stability of the myosin rod. Only after controlling these parameters the effect of temperature and *p*H on the length of myosin rods and rod fragments can be studied [15].

Materials and methods

Isolation and preparation of myosin and myosin rod

Myosin was isolated from chicken pectoralis muscle by high ionic strength extraction, low salt precipitation and ammonium sulfate fractionation followed by chromatography on DEAE-Sephadex A-50 [8] and further processed and stored as described [2, 13]. Myosin rod was prepared by papain digestion of intact chicken myosin at low ionic strength in the presence of divalent cations [11], and the ethanol resistant fraction was processed as described [11] and stored frozen at −20 °C. Before use, myosin or myosin rod was dialyzed overnight against 40 mM NaCl, 5 mM P_i, *p*H 6.8, centrifuged and the pellet resuspended in 0.7 M ammonium acetate, *p*H 7.0. Immediately before spraying, myosin or rod was diluted to 10 µg/ml into the solutions indicated in Tables I to III after adjusting the *p*H to 7.0 with 0.3 M ammonium carbamate.

Air-drying experiments in vacuo

Myosin and myosin rod were diluted into the different buffers containing glycerol and then sprayed onto freshly cleaved mica or carbon-coated grids, air dried, in vacuo shadowed and further processed for electron microscopy as described [12, 14].

Freeze-drying experiments

Myosin and myosin rod were diluted into the same buffers as for air-drying experiments but omitting glycerol. The molecules were directly sprayed onto carbon-coated grids, frozen by immmersion into supercooled liquid nitrogen, freeze-dried, shadowed and further processed for electron microscopy as described [12, 14].

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Electron microscopy

Micrographs were recorded at 50000 ×, using a Jeol JEM 100 C electron microscope operated at 100 kV. The magnification was calibrated using the unit cell dimension of catalase crystals (8.75 nm) as a reference. For length measurements, the negatives were projected onto a screen [14], using the 8.75 nm repeat of catalase and the diameter of co-prepared polystyrene spheres (diameter 91 ± 3 nm, Balzers Union/Liechtenstein) as internal calibration references. The contour lengths of extended particles were recorded by tracing with a "map-liner".

Most of the length measurements were done on rotary shadowed particles dried on mica in vacuo since this mode of shadowing routinely yielded most satisfactory pictures.

Results and discussion

Morphology

The overall morphology of myosin molecules (not shown) and myosin rods (Fig. 1) after air-drying or freeze-drying

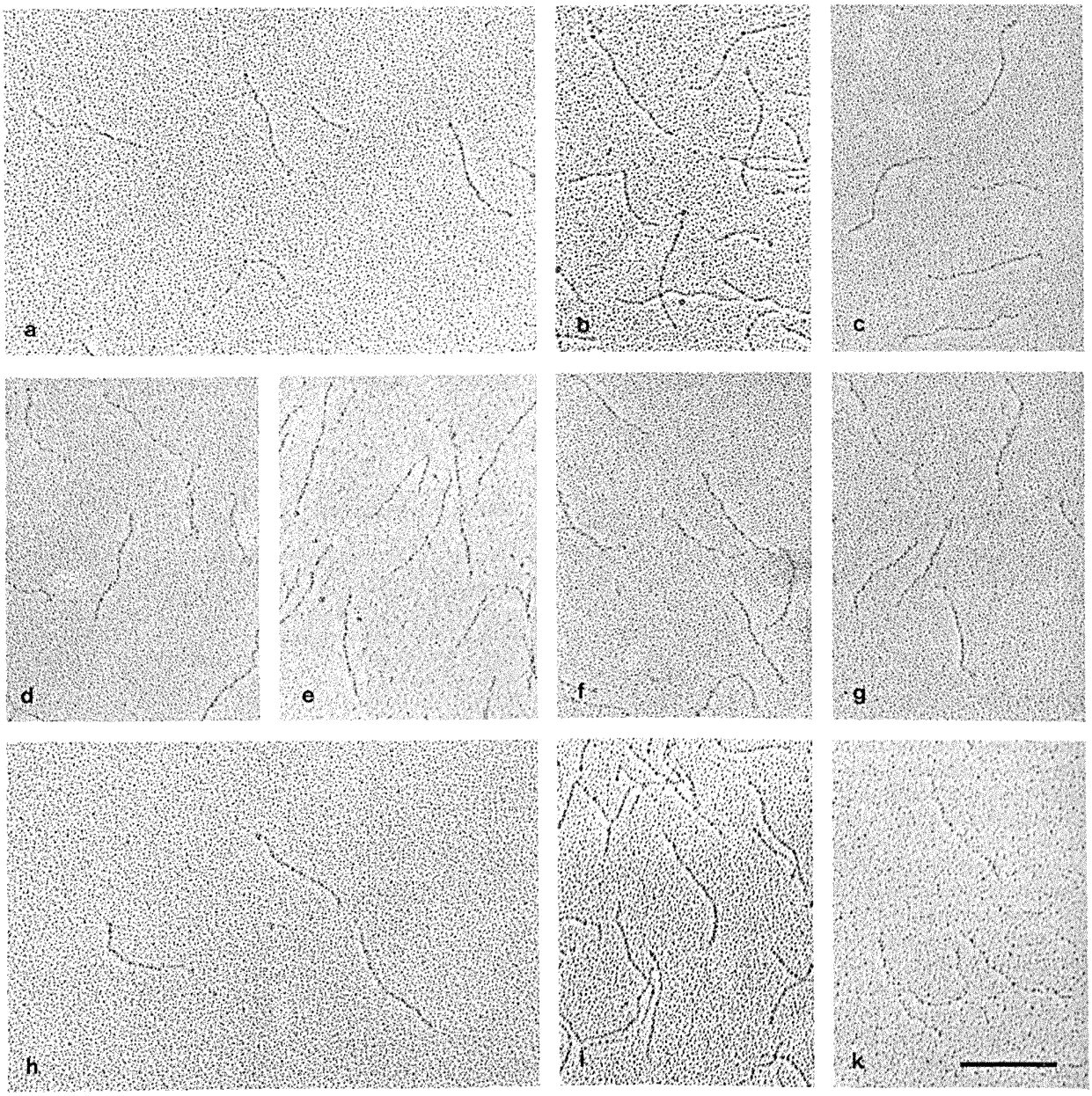

Fig. 1. Isolated chicken pectoralis myosin rod (papain) prepared for electron microscopy after air-drying in vacuo from 0.3 M ammonium acetate containig 50% (**a**), 30% (**b**), 20% (**c**), 10% (**d**), 5% (**e**), 1% (**f**), and 0% glycerol (**g**), 0.3 M NaCl (**h**), 0.3 M Na-acetate (**i**), and 0.3 M ammonium chloride (**k**), each containing 50% glycerol, rotary shadowed with Pt/C. — Bar 100 nm.

Tab. I. Dependence of length of chicken intact myosin rod and isolated rod on the concentration of glycerol at constant ammonium acetate concentration (0.3 M) at *p*H 7.0 and at 20 °C.

	Mean length (nm) ±S.D. of	
	Intact myosin rod	Isolated myosin rod
Glycerol air dried in vacuo		
0%	137.1±9.4 (19)	117.3±6.0 (28)
1%	138.9±3.9 (22)	122.9±6.0 (14)
5%	141.6±5.6 (21)	127.6±8.4 (29)
10%	147.9±4.8 (39)	125.9±6.0 (26)
20%	143.8±4.1 (26)	135.7±6.9 (33)
30%	149.5±8.4 (34)	142.7±7.1 (25)
50%	149.4±6.5 (62)	139.0±6.8 (38)
Freeze dried without glycerol	146.7±7.5 (24)	137.5±6.6 (8)

S.D. Standard deviation. — Values in brackets: n.

from different solutions in the presence or absence of glycerol was similar to that reported earlier [12, 13, 14]. Myosin molecules, although of significantly lower contrast when air dried in the presence of only 5% glycerol, were still visible in their usual appearance consisting of a long rod and two heads (not shown). Surprisingly, myosin rods were clearly visible without any glycerol (Fig. 1g).

Measurements

Length measurements on the rods of intact myosin and on isolated myosin rods after preparation under a variety of conditions, e.g., varying the concentration of glycerol, ammonium acetate, Na-acetate, NaCl, and of the different volatile buffer salts, are summarized in Tables I to III. Except for the freeze-drying experiments in which the temperature was at −35 °C, the temperature and *p*H were kept constant at 20° and *p*H 7.0, respectively, in all other experiments. The average length of the rods of intact chicken pectoralis myosin sprayed according to standard methods, e.g., from 0.3 to 0.6 M ammonium acetate in the presence of 50% glycerol was 149.4±6.5 nm (n=62) (Tab. I) and that of freeze-dried molecules in the absence of glycerol was 146.7±7.5 nm (n=24) which is in agreement with earlier values for chicken myosin [12], but shorter by about 6 to 9 nm compared to rabbit myosin with values of 155.9±4.8 nm (n=33) (Tabs. I, II in [15]). In addition, our data showed that the length of myosin rods measured from the C-terminal end to the S-1 neck region was consistently longer by about 10 nm than the total length of isolated rod fragments (139.0±6.8 nm, n=38) (Tab. I). A similar difference was also found with myosin and isolated papain rod from rabbit (Tabs. I, II in [15]). The value for rod length of papain digested rabbit myosin (145.8±5.8 nm, n=31) ([15], Tab. I) correspond well to published data. Our experiments indicated that the length of isolated papain rods, was somewhat shorter than that of chymotryptic rod [9] even though the enzymes are thought to cleave myosin at similar sites near the head-tail junction [6]. Some rod fragments showed at one end a blob that could have resulted from crumpling of the digestion site (Fig. 1b). A similar phenomenon was observed by Stewart and Edwards [9] who found that the sum of the length of isolated long S-2 and light meromyosin (LMM) was also shorter by approximately 10 nm than total chymotryptic myosin rod. This was explained by the possibility that a C-terminal fragment of LMM could have been missing [9]. The exact nature of the small difference between chymotryptic and papain rod cannot be explained at the moment, but it is not relevant for the sort of studies performed with intact myosin, myosin rod and rod fragments [15] since relative effects of glycerol, salts, *p*H and temperature are all measured on the very same preparations of myosin, rod and rod fragments and thus represent relative measurements.

Tab. II. Influence of different buffer solutions at constant glycerol (50%) and salt concentration (0.3 M) on the length of intact myosin rod and isolated rod at *p*H 7.0 (adjusted by 0.3 M ammonium carbamate) and 20 °C.

Buffer solution	Mean length (nm) ±S.D. of	
	Intact myosin rod	Isolated myosin rod
Ammonium acetate +0.1 mM IAA	144.2± 7.9 (24)	137.0± 6.4 (17)
Ammonium acetate −iodoacetic acid	144.7± 7.5 (24)	139.0± 6.8 (24)
Ammonium acetate +BME, EDTA	149.3±11.7 (22)	138.4± 5.4 (16)
Ammonium carbamate	151.0± 5.9 (66)	142.6± 5.9 (37)
Ammonium bicarbonate	150.1± 5.0 (52)	143.7± 7.6 (38)
Ammonium formate	150.6± 7.1 (62)	143.6± 4.7 (63)
Ammonium benzoate	151.9± 7.6 (32)	142.9± 7.2 (54)
NaCl	139.0± 5.4 (22)	131.1±10.4 (25)
Na acetate	141.6± 5.6 (21)	133.0± 5.5 (22)
Ammonium chloride	137.9± 5.7 (35)	132.5± 4.6 (32)

S.D. Standard deviation. — Values in brackets: n.

Effects of glycerol on myosin rod

In Table I the different lengths of the rods of intact chicken myosin and isolated myosin rod after air-drying in vacuo at different concentrations of glycerol in the spraying buffer (0.3 M ammonium acetate, *p*H 7.0) are summarized and compared to freeze-dried molecules. Rods of intact myosin and isolated myosin rods (papain) sprayed in the presence of only 1 to 5% glycerol were significantly shorter by about 10 to 12 nm and 20 to 22 nm, respectively, than similar molecules sprayed at 30 to 50% glycerol indi-

Tab. III. Influence of chloride concentration (including Cl^-) on the length of isolated myosin rod at 50% glycerol, *p*H 7.0 and 20 °C.

Salt concentration	Mean length (nm) ±S.D. of isolated rod			
	Ammonium acetate	NaCl	Na-acetate	Ammonium chloride
0.3 M	139.0±6.8 (24)	131.1±10.4 (25)	133.0±5.5 (22)	132.5±4.6
0.4 M	139.6±5.8 (29)	126.0± 6.7 (25)	136.2±5.9 (24)	135.9±7.4
0.5 M	139.6±5.1 (25)	119.3± 7.5 (26)	135.8±8.2 (22)	132.7±6.5
0.6 M	139.2±3.6 (29)	122.0± 9.3 (18)	139.2±5.5 (7)	133.8±6.8

S.D. Standard deviation. — Values in brackets: n.

cating a strong stabilizing effect of glycerol on the rod structure. This fact was also discussed by other investigators [1, 10] although they could not visualize myosin molecules at lower than 10% glycerol. The length of air-dried myosin rod from chicken in the presence of 30 to 50% glycerol was identical to that of freeze-dried molecules in the absence of glycerol (146.7 ± 7.5, $n = 24$). Glycerol used in the standard procedure (30–50% glycerol) seemed to prevent destabilization of the myosin rod during dehydration in vacuo either by substituting for water around the molecule or by preventing the loss of structurally bound water and thus preventing collapse and shrinkage of the molecules. Therefore, for studying the effects of temperature and *p*H on myosin rod and rod fragments [15], 50% glycerol was included to give consistent and reproducible length values very similar to those of freeze-dried molecules.

Effects of volatile buffers

With 50% glycerol in the spraying solution all volatile buffers tested at concentrations of 0.3 M and *p*H 7.0, e.g., ammonium acetate, -bicarbonate, -formate, -carbamate and -benzoate gave identical results as far as myosin rod length was concerned (Tab. II). The lengths compared favorably to or were slightly longer than those obtained with freeze-dried molecules (Tab. I). The inclusion of 1 mM EDTA, 1 mM β-mercaptoethanol (BME) or the alkylation of exposed SH-groups by 0.1 mM iodoacetic acid (IAA) had no significant effect on myosin rod length (Tab. II). Thus, all volatile buffers in combination with glycerol are well suited to give consistently reproducible values of myosin rod lengths after air-drying in vacuo.

Effects of chloride

In Table III, the values of myosin rod obtained after drying from different buffer solutions, ammonium acetate, NaCl, Na-acetate, and ammonium chloride with increasing molarity of 0.3 to 0.6 M, are summarized. A concentration-dependent destabilizing effect of chloride anions on isolated myosin rod dried from NaCl was noticed. The average length of papain rod from chicken myosin at 0.3 M NaCl, 131.1 ± 10.4 nm ($n = 25$) dropped to 122.0 ± 9.3 nm ($n = 18$) at 0.6 M NaCl (Tab. III) being shorter by about 15 nm compared to freeze-dried rod (Tab. I) or rod after air-drying in vacuo in volatile buffers containing no chloride (Tab. III). Since a shortening effect was also seen with 0.3 M to 0.6 M ammonium chloride, but not with 0.6 M Na-acetate, it was concluded that this shortening effect was mainly due to the chloride anion (Tab. III). This confirmed the results obtained by Stafford [7] who found that increasing concentrations of chloride had a marked shortening effect on the length of myosin rods in solution whereas acetate as an anion had none [7]. The cation Na^+ seemed to have a slight shortening effect at 0.3 M, but no effect at 0.6 M concentrations (Tab. III). Possible changes of *p*H during evaporation of volatile salts in vacuo cannot be excluded, although all buffers were adjusted with ammonium-carbamate to *p*H 7.0 immediately before use to maintain the *p*H at neutrality during evaporation. However, since the length values obtained with different volatile buffers were all very similar, shortening due to possible *p*H effects [15] were likely to be negligible. In conclusion, evidence is provided that glycerol had a concentration-dependent stabilizing effect on myosin rods. Reducing the glycerol concentration to lower than 10% in air-drying experiments led to a significant shortening of the myosin rods. By contrast, chloride had a destabilizing effect resulting in shortening of the rod structure by about 10 to 15 nm. Volatile ammonium buffers at concentrations up to 0.6 M did not seem to affect myosin rod length if 30 to 50% glycerol was present. After establishing these parameters shown here to critically influence the stability and length of myosin rods, the stage was set to study the effects of temperature and *p*H on the length of myosin rods and rod fragments under controlled and optimized conditions [15].

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