Spatial relationship between SH_1 and the actin binding site on myosin subfragment-1 surface

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Received 31 July 1984

To examine the spatial relationship between SH_1 thiol and actin binding site on subfragment-1 surface, we studied the interaction with actin of subfragment-1 whose SH_1 was labeled with an iodoacetate derivative of biotin and covered with avidin. Subfragment-1–avidin complex bound F-actin and its Mg^{2+} ATPase activity was activated by actin. Considering the size and the location of biotin binding site on avidin, our results suggest that SH_1 is separated from the actin binding site on subfragment-1 surface by at least 17–20 Å.

Muscle myosin *SH_1 thiol* *Actin binding site* *Biotin–avidin interaction*

1. INTRODUCTION

Rabbit skeletal muscle myosin has one very reactive SH group called SH_1 [1]. The cysteinyl residue having SH_1 is the 103rd residue from the COOH end of subfragment-1 (S-1) on the primary structure [2] and is located at about 130 Å from the head–rod junction of myosin on the tertiary structure [3]. Since chemical modification of SH_1 and cross-linking to another SH group called SH_2 alter the affinity of S-1 to actin [4–6], the spatial relationship between the actin binding site and SH_1 is of interest. In this study, we treated SH_1 with iodoacetate derivative of biotin (IAA-biotin) [3] and attached avidin to the biotinylated SH_1 in order to examine whether this modification interferes with the interaction between myosin head and actin.

2. EXPERIMENTAL PROCEDURE AND RESULTS

Myosin prepared from rabbit back muscle was treated with 4-fold molar excess of IAA-biotin in 0.05 M KCl, 20 mM Tris–HCl, pH 8.5, at 0°C for 2.5 h. In this way nearly half of SH_1 was biotinylated [3]. pH of the reaction mixture was lowered to 6.7 and biotinylated myosin was collected by centrifugation. The precipitate was dialyzed against 0.25 M KCl, 10 mM sodium phosphate, pH 7.0, 1 mM EDTA, and 0.2 mM dithioerythritol. S-1 was prepared from this myosin (20 mg/ml) by the digestion with chymotrypsin (0.05 mg/ml) for 20 min at 25°C. The solution was diluted with an equal volume of 6 mM $MgCl_2$, and undigested myosin and rod were removed by centrifugation. S-1 was collected by ammonium sulfate fractionation (67% saturation). Precipitate was dissolved in a small volume of 50 mM Tris–HCl, pH 8.0, and an equal amount of avidin by weight was mixed in the presence of 2 mM Mg-ADP. The mixture was left for 15 h at 4°C and then applied to a Sephacryl S-200 column (1.4 × 50 cm) equilibrated with 0.3 M KCl, 20 mM Tris–HCl, pH 8.0, and 0.5 mM 2-mercaptoethanol. The elution profile is shown in fig.1. The first peak was a complex of biotinylated S-1 and avidin. The shoulder of the first peak and the second peak were S-1 and avidin, respectively, as judged from the elution profile of the mixture of unlabeled S-1 and avidin (broken line in fig.1). The densitometry of SDS-polyacrylamide gel elec-

Abbreviations: S-1, subfragment-1; IAA-biotin, iodoacetate derivative of biotin

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00145793/84/$3.00

trophoreogram of the first peak showed that it contained S-1 and avidin at an equimolar amount (fig.2a).

ATPase activities of the S-1–avidin complex was compared with those of unlabeled S-1, 1 : 1 mixture of unlabeled S-1 and avidin, and S-1 obtained from IAA-biotin treated myosin (table 1). The presence of avidin did not seem to interfere with any of the ATPase activities of unlabeled S-1. S-1 prepared from IAA-biotin treated myosin showed high Ca^{2+} ATPase and low EDTA-K^+ ATPase activities as expected for the partial modification of SH_1. EDTA-K^+ ATPase activity of S-1–avidin complex was very low as expected for the SH_1 blocked S-1 but its Ca^{2+} ATPase activity was not so high as expected. Although the affinity constant for actin in the presence of ATP was not determined, Mg^{2+} ATPase activity of S-1–avidin complex was activated by actin in a similar extent to that of unlabeled S-1 with avidin.

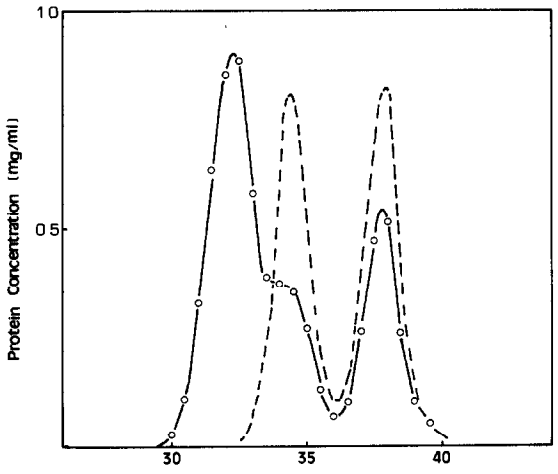

Fig. 1. The elution profile of a mixture of partially biotinylated S-1 and avidin. Protein concentration was determined by the Bio-Rad Protein Assay. The broken line is the elution profile of a mixture of unlabeled S-1 and avidin.

Binding of S-1–avidin complex with actin was studied by centrifugation. S-1–avidin complex (2.5 μM) or a mixture of unlabeled S-1 (2.5 μM) with avidin (2.5 μM) were added to F-actin (10 μM), in 0.13 M KCl, 2 mM $MgCl_2$, 20 mM Tris–HCl, pH 8.0, incubated for 1 h at 20°C, and centrifuged at 200 000 × g for 1 h. As shown in fig.2, almost all S-1–avidin complex coprecipitated with actin (lanes e and g). Since a small amount of avidin stuck to F-actin under our experimental conditions (lanes c and d), we tested whether the binding of S-1–avidin complex really occurs through the actin binding site of S-1. An important characteristic of the actin binding site is that the affinity to actin is weakened by the binding of ATP or its analogue to the active site of S-1. When the centrifugation experiment was performed in the presence of 2 mM Mg–AMPPNP, most of the S-1–avidin complex was dissociated from actin (lanes i and k). The amount of S-1–avidin complex coprecipitated with actin was the same as that of unlabeled S-1 in the presence of avidin (lanes h and j) suggesting that they were entangled in the precipitate by F-actin. When the ionic strength was raised to 0.35 M KCl to reduce the nonspecific interaction of avidin with actin, we observed that a considerable amount of

Table 1
ATPase activity

S-1 species	ATPase activity (μmol P_i/min per mg)			
	Ca^{2+} ATPase	EDTA-K^+ ATPase	Mg^{2+} ATPase	Actin activated Mg^{2+} ATP ase
S-1–avidin	1.45	0.897	0.0047	0.212
Unlabeled S-1	0.916	10.1	0.0089	0.293
Unlabeled S-1 + avidin	1.08	10.6	0.0103	0.303
Partially biotinylated S-1	6.26	3.23	0.0398	0.412

Ca^{2+} ATPase activity was measured at 37°C in 0.5 M KCl, 5 mM $CaCl_2$, 2 mM ATP and 20 mM histidine buffer, pH 7.6. EDTA-K^+ ATPase activity was measured at 37°C in 0.5 M KCl, 1 mM EDTA, 2 mM ATP, and 20 mM histidine, pH 7.6. Mg^{2+} ATPase activity was measured at 20°C in 0.05 M KCl, 3 mM $MgCl_2$, 2 mM ATP, and 20 mM Tris–HCl buffer, pH 8.0. Actin-activated Mg^{2+} ATPase activity was measured at 20°C in 0.05 M KCl, 3 mM $MgCl_2$, 2 mM ATP, and 20 mM Tris–HCl buffer, pH 8.0, in the presence of 1.05 mg/ml actin

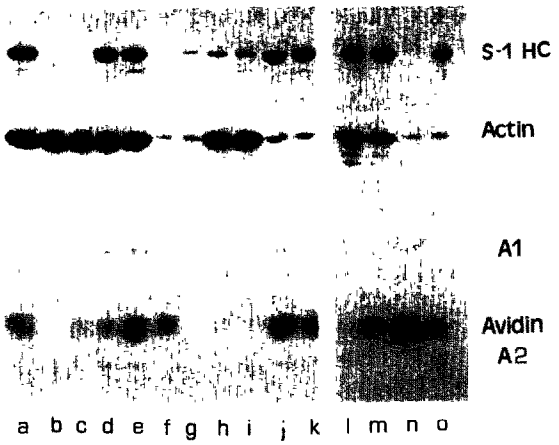

Fig. 2. Binding of S-1–avidin complex to actin studied by centrifugation. SDS-PAGE of (a) S-1–avidin complex with actin before centrifugation, (b) precipitate of F-actin alone, (c) precipitate of F-actin with avidin, (d) precipitate of F-actin and unlabeled S-1 with avidin, (e) precipitate of F-actin and S-1–avidin complex, (f) supernatant of (d), (g) supernatant of (e), (h) precipitate of F-actin and unlabeled S-1 with avidin in the presence of AMPPNP, (i) precipitate of F-actin and s-1–avidin complex in the presence of AMPPNP, (j) supernatant of (h), (k) supernatant of (i), (l) precipitate of F-actin and unlabeled S-1 with avidin at 0.35 M KCl, (m) precipitate of F-actin and s-1–avidin complex at 0.35 M KCl, (n) supernatant of (l), (o) supernatant of (m). Unless otherwise stated centrifugation experiments were performed in 0.13 M KCl, 2 mM $MgCl_2$, and 20 mM Tris-HCl, pH 8.0, at 20°C and 200 000 × g for 1 h. Concentration of F-actin, s-1–avidin complex, unlabeled S-1, and avidin were 10 μM, 2.5 μM, and 2.5 μM, respectively. After centrifugation, supernatant was removed and precipitate suspended in the same volume of water as original solution. Exactly the same volume of the supernatant and the suspension of precipitate were applied to SDS-PAGE.

S-1–avidin complex was dissociated from actin (lanes m and o) while almost all unlabeled S-1 precipitated with actin (lanes l and n).

3. DISCUSSION

The depth of the biotin binding site of avidin, using bis-biotin derivatives with different spacer arm length, was studied by authors in [7]. According to their results, the biotin binding site is quite deep because the spacer of 1,12-diaminododecane is required for the stable cross-linking of two

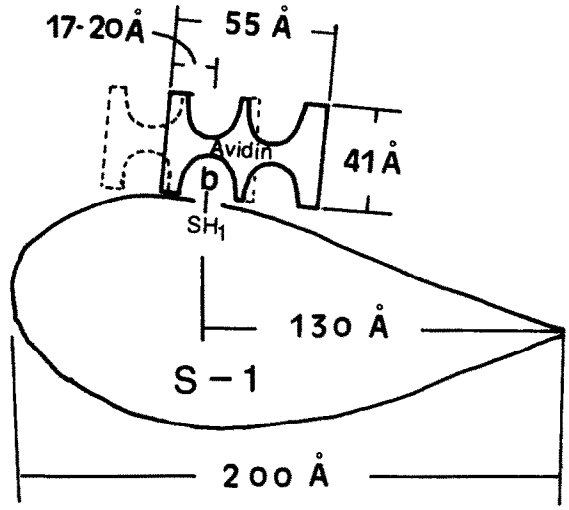

Fig. 3. Schematic illustration of the binding of avidin to SH_1.

avidin molecules with bis-biotin. Although our IAA biotin has 1,6-diaminohexane between biotin and iodoacetate [3], the binding of avidin to the myosin biotinylated at SH_1 is very slow and the binding rate was enhanced only in the presence of a high concentration of Mg-ADP [3]. This observation agrees well with the mobility of spin compound attached to SH_1 [8]. SH_1 is probably buried inside the S-1 in the absence of nucleotide. Since Mg- ADP was removed from S-1–avidin complex after the chromatography on Sephacryl S-200 column and the ATP brought by actin solution during the binding experiment is very low (~10 μM), avidin on biotinylated SH_1 seems to adhere tightly on the surface of S-1. Our results – that such a S-1–avidin complex can bind F-actin and the Mg^{2+} ATPase activity of the complex is activated by F-actin – suggest that the actin binding site on the surface of S-1 is not so close to SH_1. According to authors in [7], 4 biotin binding sites of avidin are located about 17–20 Å from the nearest edge of avidin molecule. Therefore, our results suggest that SH_1 is separated from the actin binding site by at least 17–20 Å (fig.3). After removal of Mg-ADP, S-1–avidin complex may still take a conformation similar to that of S-1–ADP complex because SH_1 is pulled by avidin. This may be the reason for the low affinity of S-1–avidin complex to F-actin at a high KCl concentration. Oxidation of other SH groups such as SH_2 is improbable

because the S-1–avidin complex, prepared very carefully with regard to the SH protection (adding dithioerythritol and bubbling N_2 gas in every solution), gave the same results.

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