Identification of a troponin-I like protein in platelet preparations as histone H2B

D.I.H. Stewart, K. Golosinska and L.B. Smillie

Medical Research Council Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton T6G 2H7, Canada

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A tropomyosin-binding protein (app. M_r 17000) was detected in equine platelet preparations by a gel overlay technique. Its isolation, amino acid and partial sequence analyses have shown it to be histone H2B. As with a similar protein from pig platelet preparations [der Terrossian et al. (1983) FEBS Lett. 152, 202–206], it inhibits Mg^{2+}-dependent actomyosin S1 ATPase. This inhibition is partially reversed in the presence of calmodulin and Ca^{2+} but is not potentiated, unlike troponin-I, by tropomyosin. This protein, along with the other histones, is almost certainly derived from a low level of contaminating nucleated cells in most platelet preparations.

Tropomyosin binding *Calmodulin* *Actin binding* *Actomyosin ATPase* *Ca^{2+} regulation*

1. INTRODUCTION

The study of contractile mechanisms in non-muscle cells has led to the identification of a large number of actin-binding proteins [1] which fulfill a wide variety of functions including cross-linking, length regulation and capping of the actin filaments. The actin-binding protein tropomyosin (TM) has been identified in the platelet [2], and although many of its properties have been documented [3–5], a specific role in vivo for the protein in the non-muscle system has not, as yet, been described. Although skeletal TMs give stability to actin filaments [6–8], this may not be the only role of the platelet protein which binds only weakly to F-actin under conditions considered physiological [9].

A recent receptor [10] describes the purification of a basic Tn-I like protein from pig platelets, which binds to calmodulin and inhibits the actin-activated Mg^{2+}-dependent ATPase activity of skeletal muscle myosin. Here, we have isolated a basic TM-binding protein of similar molecular mass which also inhibits the actin-activated Mg^{2+}-dependent ATPase of myosin S1, but not in a specific manner. We identify this protein, which has an app. M_r of 17000 on SDS gels, as the core histone, H2B, which has previously been shown to bind to calmodulin in a Ca^{2+}-dependent manner [11].

Abbreviations: SDS, sodium dodecyl sulfate; EGTA, ethylene glycolbis(β-amino-ethyl ether)-*N,N,N',N'*-tetraacetic acid; TM, tropomyosin; Tn-T, troponin-T; Tn-I, troponin-I; Tn-C, troponin-C; kDa, kilo dalton; DTT, dithiothreitol

2. METHODS

2.1. *Protein preparation*

Calmodulin was prepared from bovine brain [12] and platelet TM as in [3]. Histones were isolated from bovine thymus [13].

2.2. *Analytical methods*

Amino terminal sequence data were obtained by automatic Edman degradation as in [14]. ATPase assays were performed by the pH stat method described in [4]. SDS–polyacrylamide gel electrophoresis was performed as in [15]. DNA was

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determined as in [16]. The method in [17] was modified for the identification of TM-binding proteins by gel overlay, using a 'binding buffer' consisting of 10 mM $MgCl_2$, 100 mM KCl, 1 mM EGTA, 2 mM DTT, 2 mM ATP and 10 mM Tris–HCl, pH 7.5.

2.3. *Purification of 17 kDa TM-binding protein*

A 10-g sample of lyophilized equine platelets, prepared as in [3], was suspended in 300 ml of 1 M KCl, 1 mM DTT, 10 mM Tris–HCl, 1 mM EGTA (pH 7.4) containing 0.2 μg/ml pepstatin, 0.2 μg/ml leupeptin and 0.1 mM phenylmethane sulfonyl fluoride. The suspension was homogenized briefly and then further extracted by stirring for 30 min. The combined extracts were centrifuged at 50000 × *g* for 60 min and the supernatant dialyzed against 3 changes of 0.1 M NaCl, 1 mM DTT, 1 mM EGTA, 10 mM Tris (pH 7.4) overnight. This fraction was adjusted to pH 4.5 with 5 M HCl and centrifuged at 10000 × *g* for 10 min. The supernatant was dialyzed against 3 changes of water overnight and concentrated by lyophilization. All procedures were performed at 4°C.

The protein powder was suspended in 20 ml of 5% formic acid, and centrifuged at 5000 × *g* for 10 min. The supernatant, which had an $A_{280}^{1\,cm}$ of 6–7, was applied to a 2.5 × 200 cm G50 Sephadex column with a flow rate of 20 ml/h in 5% formic acid. Fractions were monitored for protein at 280 nm. The 17 kDa protein was eluted in the first peak following the void volume peak. This fraction (50–60 mg protein) was lyophilized and then dissolved in 20 ml of 8 M urea, 50 mM cacodylate buffer (pH 6.5) and applied to a 1.2 × 25 cm CM52 cellulose column. Proteins were eluted with a 0.0–0.3 M KCl linear gradient in the sample buffer. The column was run at 10 ml/h. The 17 kDa protein was eluted at 0.16–0.18 M KCl. This peak represented 10–20 mg of protein purified from 10 g platelets.

3. RESULTS AND DISCUSSION

3.1. *Identification and characterization of a 17 kDa TM-binding protein*

Using ^{125}I-labelled platelet TM as a probe in the gel overlay technique, a band with an app. M_r of 17000 was identified on autoradiograms of 13% SDS–polyacrylamide gels of whole platelets. In control experiments it was demonstrated that [^{125}I]TM bound to standard actin, Tn-I and Tn-T.

The 17 kDa protein was isolated as described above. The protein, which is basic, runs as a close doublet on SDS–polyacrylamide gels in the 16–17 kDa region, and was judged to be greater than 95% pure. Its amino acid composition (table 1) is very similar to the bovine core histone H2B and the first 20 residues of its N-terminal sequence were found to be identical to this protein.

The binding of the protein to TM was confirmed by an affinity chromatographic procedure using platelet TM immobilized on Sepharose 4B. It was eluted with a salt gradient from this column at the same position as calf thymus histone H2B. An equimolar mixture of the core histones, H2A, H2B, H3 and H4 did not bind to this column. The 17 kDa protein was also shown to bind to F-actin by pelleting experiments [5]. The 17 kDa protein comigrated with H2B on SDS–polyacrylamide gels

Table 1

Amino acid analysis of equine platelet 17 kDa protein

	Amino acid composition (mol/mol of peptide)		
	Platelet 17 kDa protein[a]	Bovine uterus histone H2B [11]	Bovine thymus histone H2B[b]
ASP	6.1	7.8	6
THR	8.3	7.1	8
SER	10.1	10.6	14
GLU	11.4	11.6	10
PRO	5.8	6.6	6
GLY	8.5	10.0	7
ALA	13.9	12.6	13
VAL	8.2	8.2	9
MET	1.6	1.7	2
ILE	6.3	5.9	6
LEU	8.0	8.9	6
TYR	4.7	4.1	5
PHE	2.6	2.6	2
HIS	2.8	3.4	3
LYS	15.9	15.9	20
ARG	11.4	8.9	8
TRP	0.0	0.0	0
CYS	0.0	n.d.	0

[a] Calculated from an M_r of 13700

[b] From the sequence of [21] and [22]

and in view of these findings we conclude that the 17 kDa TM-binding protein is histone H2B. This protein, of M_r 13700, runs with an anomalous high molecular mass on SDS gels.

3.2. *Comparison of 17 kDa protein with Tn-I*

A comparison of the effects of Tn-I, 17 kDa protein and salmine on the actin-activated Mg^{2+} ATPase of skeletal muscle myosin S1 in the absence and presence of platelet TM is shown in fig.1. Although the inhibitory activity of Tn-I is markedly potentiated by TM, that of the 17 kDa protein shows no such effect, and higher molar ratios of 17 kDa protein to actin are required to obtain the same level of inhibition in the presence of TM. Salmine, a 6 kDa basic protein, also inhibits the ATPase in the presence or absence of TM. This suggests that the inhibitory effect of the 17 kDa protein is of a non-specific nature attributable to its small size and basic properties. We have also observed that the inhibition produced by the 17 kDa protein can be reversed by about 50% with the inclusion of calmodulin and Ca^{2+} in the assay. This observation is consistent with a previous demonstration that the interactions of both myelin basic protein and histone H2B with calmodulin are sensitive to the presence or absence of Ca^{2+} [11].

In the indirect non-competitive ELISA assay, the 17 kDa protein showed no specific interaction with a polyclonal antiserum raised to SDS-denatured rabbit skeletal Tn-I. Lack of apparent sequence homology was also demonstrated by tryptic peptide mapping. We therefore suggest that the inhibition of the ATPase is a non-specific effect due to the binding of the basic 17 kDa protein to F-actin.

3.3. *Identification of histones in platelet preparations*

The above observations suggest that the effects of the 17 kDa protein, identified as histone H2B, are non-specific, and that the presence of this histone in platelet preparations is attributable to contamination with low levels of nucleated white blood cells. All 4 core histones were shown to be present in our platelet preparations by their coelectrophoresis with the components of a core bovine histone preparation on SDS–polyacrylamide gels and 'spiking' of the platelet preparation with individual core histones. The ratios of the proteins to each other were the same in the platelet as in the core histone preparation (table 2). The individual histones in the latter are known to be present in a 1:1:1:1 molar ratio [18,19]. Histone H1, which is less well conserved between species [20], is present

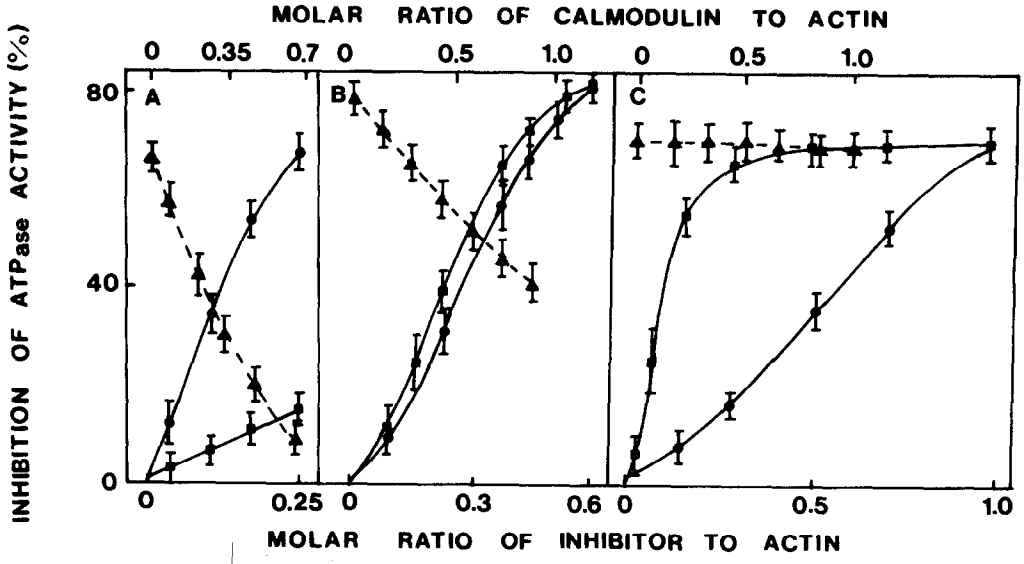

Fig.1. Inhibition of actin-activated Mg^{2+} ATPase of S1 by: (A) Tn-I; (B) 17 kDa protein; (C) salmine in the presence (●—●) and absence (■—■) of platelet TM in a ratio to actin of 0.3:1. The effect of calmodulin with Ca^{2+} on the inhibition in the presence of TM is shown (▲—▲).

Table 2

Relative peak size of Coomassie-stained core histone protein bands on SDS–polyacrylamide gels determined by densitometric scanning

	% Peak size of Coomassie-stained bands[a]			
	H2A	H2B	H3	H4
Platelet preparation	21	26	30	24
Platelet preparation spiked with histone complex	20	28	31	23
Histone complex	18	25	31	26

[a] Average of scans of 7 gels

Each protein was present in the histone mixture in a 1:1:1:1 molar ratio. The results are expressed as a percentage of the total peak size of the 4 histone bands

as a novel band in the platelet preparation. Cell counting and DNA analyses showed that white blood cells were present at a level of 0.14% of the platelet count. Calculation of the protein content contributed by this level of cell contamination strongly suggests that the histones observed in our preparation were derived from this source.

3.4. *Conclusions*

A 17 kDa basic protein in equine platelet preparations which binds to TM and F-actin has been identified as histone H2B. Although it mimics Tn-I in its inhibition of the Mg^{2+}-dependent actomyosin S1 ATPase activity and in the partial reversal of this inhibition by calmodulin and Ca^{2+}, its inhibitory action, unlike Tn-I, is not potentiated by platelet TM. We suggest that a similar protein recently reported in pig platelet preparations [10] is also histone H2B and that its presence along with the other core histones in a 1:1:1:1 molar ratio is due to a low level of contamination with nucleated cells. Although this histone could conceivably play a role in the control of thin filament assembly in nuclei, we ascribe no function to it in the contractile phenomena of platelet activation.

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