S100A1 modulates skeletal muscle contraction by desensitizing calcium activation of isometric tension, stiffness and ATPase

Bishow B. Adhikari, Kuan Wang*

Laboratory of Physical Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Building 6, Room 408, Bethesda, MD 20892, USA

Received 19 March 2001; revised 27 April 2001; accepted 29 April 2001

First published online 9 May 2001

Edited by Amy M. McGough

Abstract S100, a subfamily of the EF-hand type calcium sensing proteins, is implicated in many cellular functions including muscle contractility. Two isoforms, S100A1 and S100B, at 2–10 μM significantly inhibit active tension, stiffness and ATPase of skinned single rabbit psoas muscle fibers at submaximal (pCa ~6.1-5.6), but not at maximal levels of activation (pCa 4.0). S100A1 is a more potent inhibitor than S100B. Hill analysis of the ATPase–pCa and tension–pCa curves indicates that these proteins reduce calcium sensitivity and enhance the cooperativity toward calcium. We propose S100A1, and perhaps S100B, are viable candidates as physiological modulators of muscle contraction. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Thin filament regulation; Actin; Myosin; Nebulin; Calcium sensor

1. Introduction

The S100s are a subfamily of the EF-hand type of calcium sensing proteins that have been implicated in a multitude of cellular functions and diseases, including muscle contractility (reviewed in [1,2]). Two isoforms, S100A1 (20.8 kDa) and S100B (21 kDa), which occur as dimers of identical subunits with two EF-hand calcium-binding motifs per subunit, are particularly strong candidates for physiological modulation of muscle function. These proteins interact with the proteins in the sarcomere and are present in significant amounts in skeletal and cardiac muscles [3–7]. They are also capable of modulating smooth muscle actin–myosin interaction in the presence of the regulatory proteins caldesmon or calponin [8–10]. Furthermore, both isoforms are implicated in the normal development [11,12] and functioning of the heart – the expression of S100A1 is reduced in human cardiomyopathy [13] and increased in hypertrophy-induced right ventricles [14], and S100B is capable of regulating cardiac hypertrophy [15].

In order to determine potential roles of the S100s in contractility of skeletal muscle, we have examined the effect of S100A1 and S100B on the calcium activation of isometric tension, stiffness and ATPase in chemically and mechanically skinned single rabbit psoas muscle fibers. At physiological levels of concentration (2–10 μM), both proteins desensitized the activation of tension, stiffness, and ATPase in parallel without significantly altering the maximum values. Interestingly, the inhibition by S100A1 was much greater than by S100B, indicating an isoform-specific function of S100A1.

2. Materials and methods

2.1. Doubly skinned fiber preparation

Chemically skinned psoas fiber bundles were prepared from adult, white New Zealand rabbits (~2 kg) as described [16]. Single fibers were dissected from bundles in rigor solution (10 mM imidazole, 12 mM potassium ethylene glycol-bis(β-amino-ethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (K-EGTA), 1.3 mM Mg-acetate, 138 mM potassium propionate (KPr), 1 mM dithiothreitol (DTT), 20 μg/ml leupeptin, pH 7.0), and the sarcolemma of a ~5 mm segment was peeled off with tweezers (Dumont #5) under a dissecting microscope (SZ60, Olympus). All experiments were carried out at or near the slack sarcomere length (SL) = 2.1–2.2 μm by allowing the fiber to buckle first (~30 min), followed by slow stretching until the force began increasing just above the baseline level.

S100A1 and S100B were procured in lyophilized form (bovine brain source, Sigma). Prior to use, the identity and the purity (>95%) were routinely verified by matrix-assisted laser desorption ionization–time of flight mass spectroscopy and sodium dodecyl sulfate–polyacrylamide gel electrophoresis, respectively. The protein concentrations were determined from absorbance at 280 nm using extinction coefficients (mg/ml) of 0.91 and 0.20 for S100A1 and S100B [17,18].

2.2. Muscle mechanics

Two mechanical setups were used. Tension and stiffness were measured in a previously described setup with modifications [19]. The force transducer (Ackers, model AE 801E, Horten, Norway) and the length transducer (Cambridge Technology, Inc., model 300S, Cambridge, MA, USA) were mounted over a sample chamber on top of an inverted microscope. Stainless steel pin-hooks were attached to the transducers for fiber mounting via aluminum 'T-clips'. The SL was monitored continuously by focusing the first order diffraction spots from the fiber on a linear photo-diode array that was calibrated with gratings of known spacing. In-house developed software was used to acquire data and control the instrument. Stiffness (the ratio of amplitudes of tension and percent fiber length) was acquired by the sinusoidal method at 2 kHz with a 0.1% of fiber length amplitude and 16 sine waves. The fiber cross-sectional area was calculated from the average of 4–8 equidistant measurements along fiber axis at a magnification of 400×.

The free calcium concentrations in the relaxing solution (pCa > 8, 10 mM imidazole, 12 mM K-EGTA, 3 mM Mg-acetate, 117 mM KPr, 1.8 mM sodium adenosine 5′-triphosphate, 5 mM phosphocreatine (CP) and 200 U/ml of creatine phosphokinase (CPK), 1 mM DTT, 20 μg/ml leupeptin, pH 7.0) and the activating solution (pCa 4, same as relaxing solution except with 2.5 mM Mg-acetate and with K-EGTA replaced with Ca-EGTA) were routinely measured. In the

*Corresponding author. Fax: (1)-301-402 0009.
E-mail: wangk@exchange.nih.gov

Abbreviations: CP, phosphocreatine; CPK, creatine phosphokinase; KPr, potassium propionate; DTT, dithiothreitol; NADH, nicotinamide adenine dinucleotide; K- or Ca-EGTA, potassium or calcium ethylene glycol-bis(β-amino-ethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TnC, troponin C

PII: S0014-5793(01)02444-9

activating solution of pCa 5.7, obtained by mixing appropriate amounts of the relaxing and activating solutions, the pCa electrode measurement (see below) showed no change in pCa upon the addition of 80 μM S100A1 or S100B, thus ruling out changes in free calcium by the S100s.

Simultaneous measurements of tension and ATPase over the entire calcium activation range were done with a commercial instrument (K. Guth, Scientific Instruments, Heidelberg, Germany). The relaxing and activating solutions in this setup contained 0.4 mM nicotinamide adenine dinucleotide (NADH), 140 U/ml L-lactic dehydrogenase, 100 U/ml pyruvate kinase, and 5 mM phosphoenolpyruvate for the enzyme-coupled assay [20] in lieu of the CP and CPK, with ionic strength adjusted by KPr. pCa of solution at the fiber was varied by mixing these solutions with a gradient mixer. The actual pCa at the fiber was monitored using a fluorescent calcium indicator, Calcium Green II (Molecular Probes) and verified with a calcium selective electrode (Orion Research Inc., Beverly, MA, USA) previously calibrated with calcium standards (WPI, Sarasota, FL, USA). The ATPase was determined using the fluorescence decay of NADH in an enzyme-linked assay system [20].

To account for variability between fibers and conditions, each set of experiments were carried out on the same day with the same fiber and the same solutions. The tension/pCa and ATPase/pCa curves were fitted with the Hill equation using the non-linear fitting routine (Levenberg–Marquardt) in MathCAD, version 8.0 (MathSoft, Inc.): $y = y_{max}/(1+10^{n(pCa-pK)})$, where y_{max} is the maximum value, n is the Hill coefficient and pK is the calcium concentration in pCa units where the measured quantity (y) is at 50% of y_{max}.

3. Results

3.1. Inhibition of isometric tension and stiffness by S100

Both S100A1 and S100B exhibit a concentration-dependent and reversible inhibition of tension and stiffness at pCa 5.7, a sub-maximal calcium activation level. As shown for S100A1 in Fig. 1A, incremental increase of S100A1 from 1 to 4 μM (downward arrows) resulted in a significant inhibition of both tension and stiffness, up to 60% at 4 μM, and appeared to plateau at 60–70% beyond 10 μM (data not shown). The effect was rapid, observed within 20 s of the addition, and reversible, as shown by the rapid recovery of tension and stiffness upon treating the fiber with activating solution. Stiffness varied in parallel with tension over the entire trace in Fig. 1A. Repeated cycles of application and removal of S100 proteins resulted in qualitatively similar inhibition (data not shown). However, neither S100A1 nor S100B produced a significant change in the tension and stiffness measurements under maximum calcium activation (pCa 4.0) and under either rigor or relaxing conditions (data not shown).

Measurements at pCa 5.7 and 4.0 from 23 separate fibers were normalized, averaged and summarized in Fig. 1B,C. Compared to values in the absence of the proteins, the relative tension and stiffness at pCa 5.7 under the saturating concentrations of S100 (≥10 μM) were, respectively, 0.41 ± 0.07 ($n = 9$) and 0.52 ± 0.07 ($n = 20$) for S100A1 and 0.76 ± 0.03 ($n = 5$) and 0.75 ± 0.04 ($n = 5$) for S100B. Based on Student's t-test, the inhibitions for both S100s became significant only at the sub-maximal activation at pCa 5.7 ($P \ll 0.05$) but not at pCa 4.0. Thus, the S100A1 isoform appears to be a potent inhibitor of fiber mechanics at sub-maximal activation levels, while S100B appears to have a lesser effect.

3.2. Desensitization of calcium activation of tension and ATPase

The calcium dependence of the inhibition was explored further by simultaneously measuring the tension–pCa and ATPase–pCa curves over the entire range of activation. Figs. 2 and 3 show the representative data sets of tension- and ATPase–pCa curves that are analyzed with the Hill equations for the sensitivity and the degree of cooperativity of activation. S100A1 (at 6 and 10 μM) caused a significant shift of the curves towards lower pCa (therefore lowered calcium sensitivity): pK reduced from 5.95 to 5.88 (6 μM) and to 5.78 (10 μM) for the ATPase curves and 5.88 to 5.83 (6 μM) and to 5.71 (10 μM) for the tension curves (Fig. 2). A somewhat increased cooperativity, as reflected by the larger Hill coefficients, was also observed. n Increased from 6.8 to 7.9 (6 μM) but was unchanged at 10 μM for the ATPase curves and it increased from 9.3 to 10.4 (6 μM) and to 10.6 (10 μM) for the tension curves. The changes for S100B (at 10 μM) were much smaller, yet reproducible, as demonstrated by the highly precise measurements (Fig. 3). The pKs changed from 5.84 to 5.81 for the ATPase curve and from 5.79 to 5.75, for the tension curve. Hill coefficients in the presence of S100B also increased slightly: an increase from 8.7 to 9.1 for the ATPase curve and 8.8 to 9.4 for the tension curve (Fig. 3).

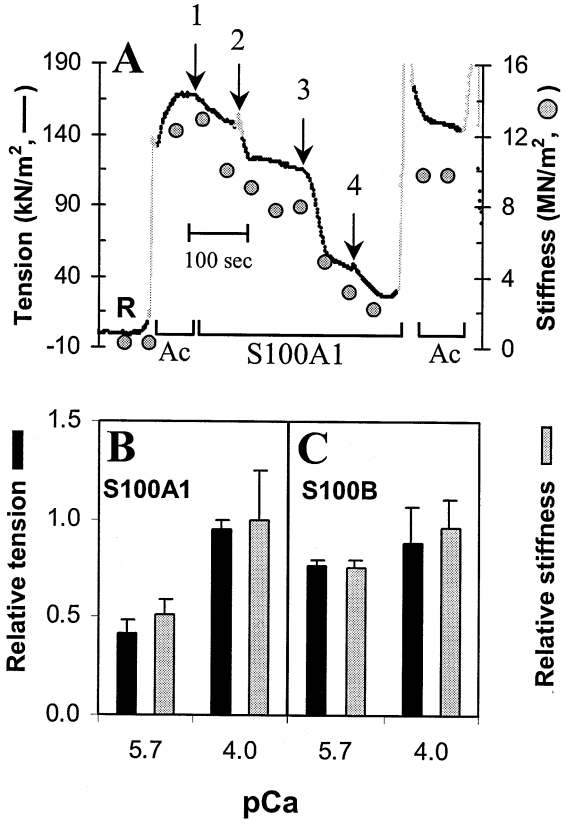

Fig. 1. Inhibition of isometric tension and stiffness by S100A1 and S100B. A: A doubly skinned single fiber (2.0×95 μm, SL = 2.1 μm) in relaxing solution (R) was initially activated in activating solution at pCa 5.7 (Ac) and S100A1 was added in 1 μM increments at the arrows, followed by treatment with the activating solution (Ac). The spikes in tension, shown in lighter shade, are artifacts due to the solution exchanges. B and C: Relative tension (dark bars) and stiffness (lighter bars) at pCa 5.7 and 4.0 in the presence of S100A1 and S100B with error bars (S.E.M., $n = 2$–19). Fiber diameter = 64–83 μm, SL = 2.1 μm, S100 = 5–20 μM. All comparisons were made in the same fiber and then averaged among the different fibers.

4. Discussion

The present data demonstrate for the first time that the

S100 proteins at 2–10 μM reversibly inhibit isometric tension and stiffness in skinned rabbit skeletal muscle. These proteins lower the sensitivity and enhance the cooperativity of both tension and ATPase toward calcium (i.e. a shift of p*K*s toward lower pCa and an increase of Hill coefficient) without significant changes at maximal levels of activation. The significantly higher potency of S100A1 inhibition compared to S100B, combined with the higher abundance of S100A1, suggests a strong physiological relevance for S100A1 in skeletal muscle function.

Although the S100 content in rabbit psoas fibers is not currently available, the estimated content by immunoassay

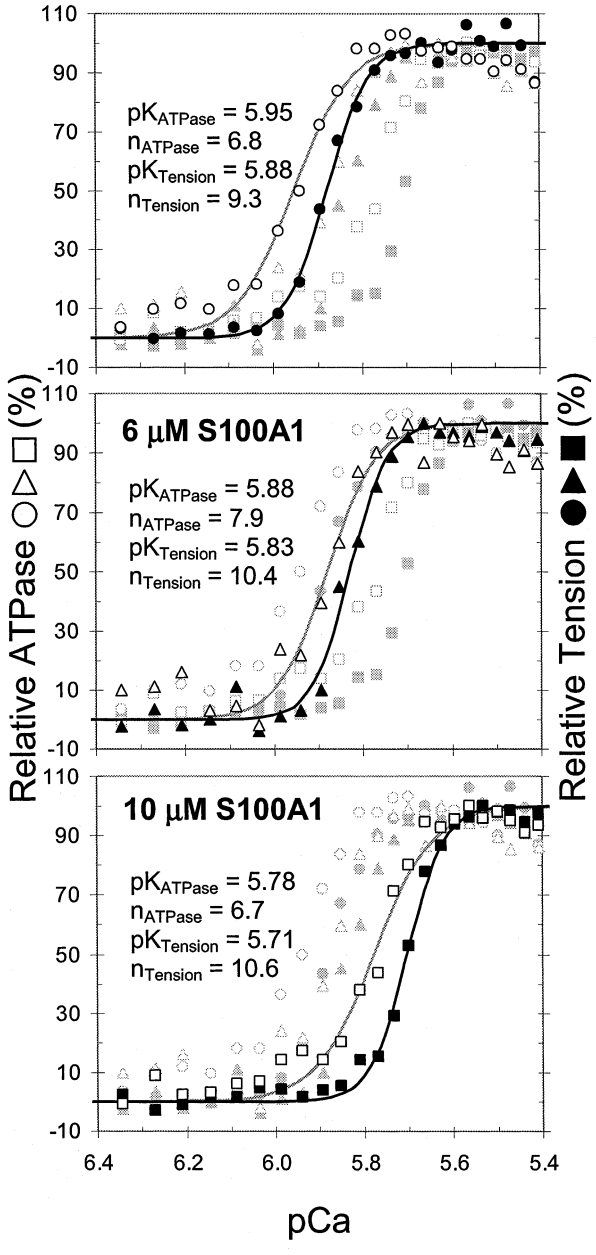

Fig. 2. Desensitization of calcium activation of tension and ATPase by S100A1. The relative ATPase (open symbols) and tension (closed symbols) from doubly skinned single fibers are plotted as a function of solution pCa. The lines are fit to the data using the Hill equation with the parameters shown next to the figures. Top: without S100A1; middle: 6 μM S100A1; bottom: 10 μM S100A1, $T = 23°C$, SL = 2.1 μm.

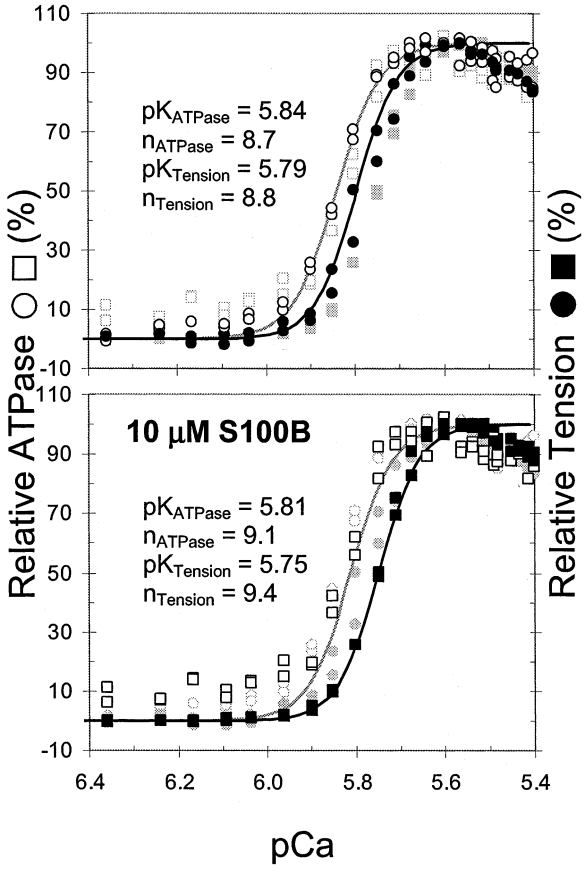

Fig. 3. Desensitization of calcium activation of tension and ATPase by S100B. The relative ATPase (open symbols) and tension (closed symbols) from doubly skinned single fibers are plotted as a function of solution pCa. Two sets of activation data are shown for each condition to show repeatability. The lines are fit to the one set of data using the Hill equation with the parameters shown next to the figures. Top: without S100B; bottom: 10 μM S100B, $T = 23°C$, SL = 2.1 μm.

methods is ~1 μg for S100A1 per mg of wet tissue in human skeletal muscle [21,22]. S100A1 content varies with the fiber type, and is 3-fold lower (~0.3 μg/mg of wet tissue) in the fast-twitch than in the slow-twitch in mice [5]. Assuming 1 g of wet muscle occupies 1 ml volume, these values convert to ~50 μM and ~15 μM, respectively, in the two fiber types. Thus, the S100A1-induced modulation of activation demonstrated here is physiologically significant. S100B is reportedly lower (yet unspecified) in content than S100A in human skeletal muscle [22]. Its lower potency of inhibition and lower content obscure the physiological relevance of this isoform in skeletal muscle activation.

Our observation that inhibition of tension is accompanied by parallel inhibitions of stiffness and ATPase in skinned fibers indicates a direct inhibition of the interaction between actin and myosin by the S100s. Several potential mechanisms are plausible. The trivial possibility that the observed effects are due to an exchange of S100 with other EF-hand proteins in the fiber, such as troponin C (TnC) and myosin light chains, is excluded by the following observations. (a) the S100 effects are much more rapid than the lengthy and drastic procedures required for the exchange of analogous proteins with TnC or light chains [23,24], and (b) the inhibition was rapidly reversed upon removal of the added protein by buffer,

indicating no loss of endogenous proteins. In principle, a direct blocking of the molecular interface between actin and myosin by the S100s could produce the inhibitions. Indeed, both S100s are known to bind to CapZα [25,26] and the PEVK fragment of titin [27]. S100A1 also binds to myosin heavy chain [7] and F-actin [28]. However, S100A1 does not directly alter the actin-activated ATPase activities of either the skeletal muscle myosin or the thiophosphorylated smooth muscle myosin in solution [10,29]. Therefore, it is necessary to invoke protein interactions that involve additional regulator(s) that control actin and myosin interactions to explain these inhibitions. In this connection, two observations are intriguing: (a) the calcium-regulated binding of S100A1 to F-actin [28], and (b) the capability of the S100s to modulate actin–myosin interactions in the presence of secondary regulatory proteins, such as smooth muscle caldesmon and calponin [8–10]. Since these regulatory proteins are thought to function analogously to nebulin in skeletal muscle [30], we speculate that the S100s may interact simultaneously with nebulin and actin in a calcium-dependent manner and regulate the interaction of myosin with the thin filament. This is a potentially significant role for the S100s in muscle, in addition to the multitude of other functions implicated from previous studies [1,2].

In summary, S100A1 and perhaps S100B are capable of modulating contractility at physiological concentrations by desensitizing the calcium activation of skeletal muscle. Such a modulation represents a distinct and heretofore unappreciated branch of the calcium signaling pathways in skeletal muscle.

Acknowledgements: We are grateful to Alan Brady and the late Victor Chen for advice and guidance. We are grateful to Dr. Glenn Kerrick (University of Miami) for consulting on the Guth ATPase-tension equipment.

References

[1] Zimmer, D.B., Cornwall, E.H., Landar, A. and Song, W. (1995) Brain Res. Bull. 37, 417–429.
[2] Donato, R. (1999) Biochim. Biophys. Acta 1450, 191–231.
[3] Maco, B., Brezova, A., Schafer, B.W., Uhrik, B. and Heizmann, C.W. (1997) Gen. Physiol. Biophys. 16, 373–377.
[4] Maco, B., Uhrik, B. and Heizmann, C.W. (2000) Gen. Physiol. Biophys. 19, 237–244.
[5] Haimoto, H. and Kato, K. (1987) J. Neurochem. 48, 917–923.
[6] Haimoto, H. and Kato, K. (1988) Eur. J. Biochem. 171, 409–415.
[7] Zimmer, D.B. (1991) Cell Motil. Cytoskeleton 20, 325–337.
[8] Pritchard, K. and Marston, S.B. (1991) Biochem. J. 277, 819–824.
[9] Polyakov, A.A., Huber, P.A., Marston, S.B. and Gusev, N.B. (1998) FEBS Lett. 422, 235–239.
[10] Fujii, T., Machino, K., Andoh, H., Satoh, T. and Kondo, Y. (1990) J. Biochem. Tokyo 107, 133–137.
[11] Kiewitz, R., Acklin, C., Minder, E., Huber, P.R., Schafer, B.W. and Heizmann, C.W. (2000) Biochem. Biophys. Res. Commun. 274, 865–871.
[12] Kiewitz, R., Lyons, G.E., Schafer, B.W. and Heizmann, C.W. (2000) Biochim. Biophys. Acta 1498, 207–219.
[13] Remppis, A., Greten, T., Schafer, B.W., Hunziker, P., Erne, P., Katus, H.A. and Heizmann, C.W. (1996) Biochim. Biophys. Acta 1313, 253–257.
[14] Ehlermann, P., Remppis, A., Guddat, O., Weimann, J., Schnabel, P.A., Motsch, J., Heizmann, C.W. and Katus, H.A. (2000) Biochim. Biophys. Acta 1500, 249–255.
[15] Tsoporis, J.N., Marks, A., Kahn, H.J., Butany, J.W., Liu, P.P., O'Hanlon, D. and Parker, T.G. (1998) J. Clin. Invest. 102, 1609–1616.
[16] Granzier, H.L. and Wang, K. (1993) Biophys. J. 65, 2141–2159.
[17] Isobe, T., Nakajima, T. and Okuyama, T. (1977) Biochim. Biophys. Acta 494, 222–232.
[18] Isobe, T., Ishioka, N., Masuda, T., Takahashi, Y., Ganno, S. and Okuyama, T. (1983) Biochem. Int. 6, 419–426.
[19] Granzier, H.L.M. and Wang, K. (1993) J. Gen. Physiol. 101, 235–270.
[20] Guth, K. and Wojciechowski, R. (1986) Pflug. Arch. 407, 552–557.
[21] Hidaka, H.E.T. and Kato, K. (1983) Methods Enzymol. 102, 256–261.
[22] Kato, K. and Kimura, S. (1985) Biochim. Biophys. Acta 842, 146–150.
[23] Moss, R.L. (1992) Circ. Res. 70, 865–884.
[24] Wagner, P.D. (1982) Methods Enzymol. 85, 72–81.
[25] Ivanenkov, V.V., Jamieson, G.A., Gruenstein, E. and Dimlich, R.V. (1995) J. Biol. Chem. 270, 14651–14658.
[26] Ivanenkov, V.V., Dimlich, R.V. and Jamieson Jr., G.A. (1996) Biochem. Biophys. Res. Commun. 221, 46–50.
[27] Gutierrez-Cruz, G., Van Heerden, A.H. and Wang, K. (2000) J. Biol. Chem. 17, 17.
[28] Mandinova, A., Atar, D., Schafer, B.W., Spiess, M., Aebi, U. and Heizmann, C.W. (1998) J. Cell Sci. 111, 2043–2054.
[29] Zhao, X.Q., Naka, M., Muneyuki, M. and Tanaka, T. (2000) Biochem. Biophys. Res. Commun. 267, 77–79.
[30] Root, D.D. and Wang, K. (1994) Biochemistry 33, 12581–12591.