Human Erythrocyte Dematin and Protein 4.2 (Pallidin) Are ATP Binding Proteins[†]

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Received July 27, 1995; Revised Manuscript Received December 5, 1995[⊗]

ABSTRACT: Dematin and protein 4.2 are peripheral membrane proteins associated with the cytoplasmic surface of the human erythrocyte plasma membrane. Isoforms of dematin and protein 4.2 exist in many nonerythroid cells. In solution, dematin is a trimeric protein containing two subunits of 48 kDa and one subunit of 52 kDa. Recent determination of the primary structure of the 52 kDa subunit of dematin showed that it contains an additional 22-amino acid sequence in the headpiece domain. An alignment of the 22-amino acid insertion sequence revealed that the 52 kDa subunit of dematin shares a novel 11-amino acid motif with protein 4.2. In this communication, we report that the conserved 11-amino acid motif in dematin[52] and protein 4.2 contains a nucleotide binding P-loop. Direct binding of ATP is demonstrated to the glutathione *S*-transferase fusion proteins containing corresponding segments of dematin[52] and protein 4.2 as well as to purified protein 4.2. The binding of ATP to the recombinant domains of dematin[52] and protein 4.2 is specific, saturable, and of high affinity. The nucleotide specificity of the P-loop is restricted to ATP since no detectable binding was observed with GTP. These results show that the 11-amino acid motif provides an ATP binding site in dematin[52] and protein 4.2. Although the functional significance of ATP binding is not yet clear, our findings open new perspectives for the function of dematin and protein 4.2 *in vivo*.

Dematin is an actin binding and bundling phosphoprotein of the erythrocyte membrane skeleton (Chishti *et al*., 1988; Siegel & Branton, 1985). Human erythrocyte dematin consists of two subunits of 48 and 52 kDa (Chishti *et al*., 1988). Previously, we have shown that the primary structure of the 48 kDa subunit contains a headpiece domain which was first identified in villin, an actin binding and bundling protein of the brush border cytoskeleton (Rana *et al*., 1993). The headpiece domain of dematin and villin contains an actin binding site and is essential for the morphogenic function of villin (Friederich *et al*., 1992; Rana *et al*., 1993). Recently, a similar headpiece domain was identified in the *Drosophila quail* gene product (Mahajan-Miklos & Cooley, 1994). The *Drosophila quail* gene encodes a villin-like protein, and null mutations in the *quail* gene cause female sterility due to the defects in the actin bundling activity of the villin-like protein (Mahajan-Miklos & Cooley, 1994).

More recently, we have reported the primary structure of the 52 kDa subunit of dematin which is identical to that of the 48 kDa subunit except that it contains an additional 22-amino acid sequence within its headpiece domain (Azim *et al*., 1995). Sequence alignment of the 52 kDa subunit of dematin showed that it shares an 11-amino acid motif with protein 4.2, a major protein of the human erythrocyte membrane (Azim *et al*., 1995). The presence of a shared motif between dematin and protein 4.2 is surprising since the two proteins do not appear to share any known structural and functional characteristics. In fact, the topographical location of dematin and protein 4.2 at the distal ends of a spectrin heterodimer in principle minimizes the possible interaction between these proteins at the plasma membrane [see Bennett (1989) for review]. Dematin binds to actin filaments which are associated with the tail ends of spectrin heterodimers, whereas protein 4.2 binds to band 3 proteins which associate with the head ends of spectrin heterodimer via ankyrin [see Bennett (1989) for review]. In mature red blood cells, dematin is believed to function by anchoring actin−spectrin junctional complexes to the plasma membrane (Derick *et al.*, 1992; Rana *et al.*, 1993). The functional significance of the actin bundling activity of dematin is not yet clear, but it may play a role in tissues such as brain, skeletal muscle, heart, kidney, and lung where the dematin transcripts are abundantly expressed (Rana *et al.*, 1993). Alternatively, the actin bundling activity of dematin may be of functional importance during erythroid development where the reorganization of actin bundles precedes enucleation of the erythroblasts (Koury *et al.*, 1989).

Human erythrocyte protein 4.2 is a 72 kDa protein which is tightly bound to the inner membrane surface via the band 3 (anion channel) protein [reviewed in Cohen *et al.* (1993)]. While the exact function of the protein 4.2 is not known, numerous examples of hemolytic anemia associated with protein 4.2 deficiency demonstrate that the protein is essential for normal erythrocyte survival and function [see Cohen *et al.* (1993) and Yawata (1994a,b) for reviews]. Erythrocytes lacking or deficient in protein 4.2 have a shortened life span and have shape abnormalities ranging from mild to pronounced spherocytosis or ovalocytosis (Yawata, 1994a,b). While the detrimental changes in shape and survival of protein 4.2 deficient erythrocytes suggest that protein 4.2

[†] This work is supported by National Institutes of Health grants (HL51445 and HL37462). A.H.C. is an established investigator of the American Heart Association.

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[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1996.

0006-2960/96/0435-3001$12.00/0

may play some role in membrane skeletal function, protein 4.2 does not behave like a typical cytoskeletal protein. Unlike cytoskeletal proteins, protein 4.2 is readily extracted from erythrocyte membranes by the nonionic detergent Triton X-100 and is retained on membranes from which all membrane skeletal proteins have been eluted (Korsgren & Cohen, 1986; Steck & Yu, 1973; Yu *et al.*, 1973). It has been suggested that protein 4.2 may be involved in the stabilization of ankyrin−membrane binding (Rybicki *et al.*, 1988), but this has not been confirmed in other studies (Ideguchi *et al.*, 1990; Korsgren & Cohen, 1988). Nevertheless, since protein 4.2 binds to the cytoplasmic domain of band 3 which serves as a crucial anchorage site for several cytoskeletal−membrane links, it is possible that protein 4.2 plays some role in the promotion or stabilization of membrane skeletal linkages.

Protein 4.2 has strong sequence similarity to the transglutaminase family of proteins [reviewed in Cohen *et al.* (1993)] but does not exhibit transglutaminase activity. This similarity is present also at the genomic level (Korsgren & Cohen, 1991), suggesting that the genes for protein 4.2 and the transglutaminase proteins share a common ancestor. Nonerythroid forms of protein 4.2 have been detected in many cell types (Cohen *et al.*, 1993; Friedrichs *et al.*, 1989). The murine protein 4.2 gene has been identified as being the likely locus for the pallid mutation, which affects the formation or function of storage granules in melanocytes, platelets, and kidney (White *et al.*, 1992). The pallid mouse is considered a model for human platelet storage pool diseases which are characterized by decreased or absent platelet dense granules resulting in prolonged bleeding times and by defective melanosomes resulting in albinism. These observations suggest that nonerythroid protein 4.2 may play an important role in the formation or function of intracellular vesicles.

In this communication, we show that ATP binds to the P-loop consensus sequence present in the 52 kDa subunit of dematin and protein 4.2. The ATP binding to dematin[52] and protein 4.2 is specific, saturable, and of high affinity. Implications of this finding are discussed in terms of the functions of dematin and protein 4.2 *in vivo*.

EXPERIMENTAL PROCEDURES

Materials. Glutathione−Sepharose and the TA cloning vector were purchased from Pharmacia and Invitrogen, respectively. The DH5α competent cells were obtained from GIBCO BRL. Radiolabeled [α-^{32}P]- and [γ-^{32}P]-ATP and [γ-^{32}P]-GTP were purchased from ICN. ATP binding measurements were carried out using the Millipore-1225 sampling manifold apparatus. The HA-type nitrocellulose filters (0.45 μm) were purchased from Millipore Inc. Purified guinea pig liver transglutaminase was obtained from Sigma.

C*onstruction of Recombinant Proteins.* To produce recombinant proteins, the cDNA sequence corresponding to the defined domains of dematin and protein 4.2 was amplified from the human reticulocyte cDNA pool using the polymerase chain reaction. The primers for the headpiece domain of dematin with and without the insert sequence were 5′-TCAGGGAGTGAGACTGGAAG CCCA (sense) and 5′-GAAGAGAGAGGCCTTC TTCTTGA (antisense). The primers for the protein 4.2 constructs were as follows: protein 4.2 (amino acids 294−347) sense primer, 5′-GCTGGATCCTCA GCACAGGGCACC, and antisense primer, 5′-CGCGAATTCCTGCCATCCATCATA; protein 4.2 (amino acids 175−352) sense primer, 5′-CGCGGATC-CGCGGATGCTGTTTTCCTGAAGAAT, and antisense primer, 5′-CGGGAATTCGATTCTGCCTCTCTGGCCTTC. The PCR[1] amplified products were subcloned into a TA cloning vector and then transferred to the pGEX-2T vector using an asymmetric BamH1 and EcoR1 adaptor present in the PCR products. Alternatively, the PCR products were directly cloned into pGEX-2T with compatible primer restriction sites [protein 4.2 (175−352)]. Recombinant fusion proteins were expressed in the DH5α cells in the presence of IPTG and purified from bacterial lysates by affinity chromatography on the glutathione−Sepharose column (Frangioni & Neel, 1993). The protein concentration was determined either by the Micro BCA Protein Assay Kit (Pierce) or elution of the bound dye by pyridine (Fenner *et al.*, 1975). All cDNA constructs were sequenced to confirm the frame of the cDNA fragments and the fidelity of the PCR.

Purification of Erythrocyte Protein 4.2. Human erythrocyte protein 4.2 was purified by the method of Dotimas *et al.* (1993).

Filter Binding Assay for ATP. We used a nitrocellulose filter binding assay to measure the binding of ATP to the fusion proteins in solution. The assay is rapid, sensitive, and allows processing of 12 samples in one cycle. The ATP binding buffer contains 10 mM HEPES (pH 7.6), 50 mM KCl, 75 mM NaCl, 1.0 mM $MgCl_2$, 0.5 mM 1,2-bis(2-aminoethoxy)ethane-N,N,N,N-tetraacetic acid (EGTA), 0.5 mM dithiothreitol (DTT), 500 nCi of [γ-^{32}P]-ATP, 100 nM or 50 μM (as indicated) of cold ATP, and the indicated amounts of the respective GST fusion proteins. For the GTP binding measurements, ATP was substituted with 500 nCi of [γ-^{32}P]GTP and 100 nM of cold GTP in the binding buffer as described above. The fusion proteins were incubated with the binding buffer for 3 h on ice. After the nucleotide binding was complete in solution, the assay mixture (150 μL) was applied onto prewetted HA-type filters using a Millipore-1225 sampling manifold apparatus. The protein-bound nucleotides were retained on the nitrocellulose. For the protein 4.2 construct shown in Figure 4C, the volume of the incubation mixture was increased to 300 μL. The binding buffer was the same except that the ATP concentration was 50 μM and [γ-^{32}P]ATP was present at a concentration of 2 $\times$ 10^5 cpm/nmol. Competition with either cold ATP or GTP was done in the presence of 1.0 mM ATP or GTP. The nitrocellulose filters were washed extensively in the binding buffer without ATP or GTP, and the protein-bound radioactivity was measured in a scintillation counter. The concentration of ATP was determined by measuring the absorbance at 259 nm.

ATP binding to purified human erythrocyte protein 4.2 was done by incubation of 20 μg of protein 4.2 in 20 mM Tris-HCl (pH 7.5), 1.0 mM ethylenediaminetetraacetic acid (EDTA), 1.2 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 1.0 mM DTT, and 20 μM ATP plus either 1.0 μCi of [α-^{32}P]-ATP or 0.3 μCi of [γ-^{32}P]ATP in a final volume of 100 μL. The mixure was incubated at 30 °C for 2 h, and the reaction

[1] Abbreviations: SDS, sodium dodecyl sulfate; IPTG, isopropyl thio-β-D-galactoside; PCR, polymerase chain reaction; GST, glutathione *S*-transferase.

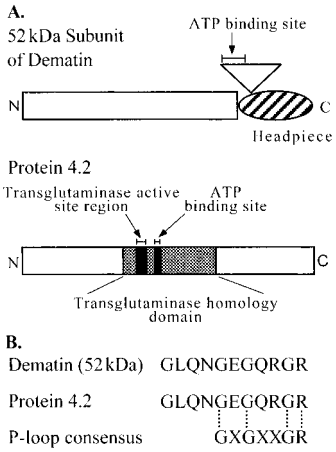

FIGURE 1: (A) Location of the ATP binding motif in dematin and protein 4.2. In dematin, the ATP binding site is located within the 22-amino acid insertion present in the headpiece domain. Only the 52 kDa subunit of dematin contains the binding site for ATP. The headpiece domain in dematin also contains an actin binding site at the carboxyl terminus. In protein 4.2, the ATP binding site lies within the transglutaminase homology domain. It should be noted that the ATP binding site is not found in transglutaminases and is unique to protein 4.2. (B) Consensus sequence for the ATP binding P-loop.

was stopped by the addition of 2.0 mL of ice cold 20 mM Tris-HCl (pH 8.0) plus 100 mM NaCl (stop buffer). Aliquots of the samples were applied to the nitrocellulose filters and washed five times with 2.0 mL aliquots of stop buffer. Filters were counted for ^{32}P in a scintillation counter. All measurements were made on duplicate samples which agreed to within 10%.

Gel Filtration Assay. The separation of free and protein-bound [γ-^{32}P]ATP was carried out on a Sephadex G-25 gel filtration column (0.5 × 7.5 cm). The assay conditions were identical to those described in the filter binding assay except that the assay volume was reduced to 60 μL.

RESULTS

The 52 kDa subunit of dematin (dematin^{52}) and protein 4.2 contain an identical 11-amino acid motif (Azim *et al.*, 1995). This shared motif originated from the 22-amino acid insertion found in the headpiece domain of the 52 kDa subunit of dematin (Azim *et al.*, 1995). Of the 11-amino acids present in the shared motif, 9 residues are contributed by the 22-amino acid insertion whereas the remaining 3 amino acids are derived from the original headpiece domain of the 48 kDa subunit of dematin (Figure 1). Numerically, the dematin homology motif spans from glycine-317 to arginine-327 in dematin^{52} and from glycine-312 to arginine-322 in protein 4.2 (Figure 1). The dematin homology motif is also highly conserved in murine protein 4.2. Only arginine-322 is substituted for histidine in the murine protein 4.2 (Korsgren & Cohen, 1994).

While investigating the functional significance of the dematin homology motif, we observed a nucleotide binding consensus sequence within the shared 11-amino acid motif (Figure 1). A typical phosphate binding sequence, often termed P-loop, contains the consensus GXGXXGK (Saraste *et al.*, 1990). The dematin homology motif shows a conservative substitution of lysine with arginine resulting in the following consensus, GXGXXGR (Figure 1). Similar substitution of arginine in the P-loop has been previously

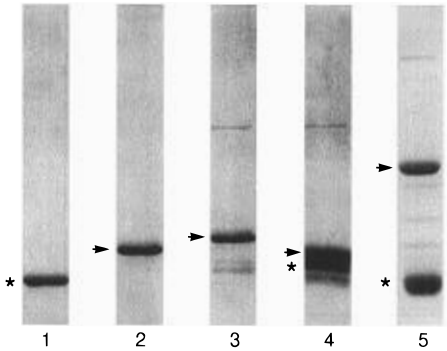

FIGURE 2: Expression of recombinant proteins in *Escherichia coli*. Coomassie-stained SDS−polyacrylamide gel electrophoresis. The details of the GST fusion protein constructs are described in Experimental Procedures. (1) Purified GST protein alone. (2) The headpiece domain of dematin^{48} without the insertion sequence. (3) The headpiece domain of dematin^{52} containing the 22-amino acid insertion. (4) A 54-amino acid segment of the human protein 4.2 containing the dematin homology motif. (5) A 178-amino acid domain of protein 4.2 containing the dematin homology motif. The asterisks (*) indicate the position of the GST protein. Note that the purified proteins in lanes 4 and 5 are partially degraded to produce free GST protein. The amino acid boundaries of the these constructs are described in Results. The dematin constructs, shown in lanes 2 and 3, are the same as published previously (Azim *et al.*, 1995). The amino acid numbers for protein 4.2 constructs are based on the published sequence of Korsgren and Cohen (1991).

noted in many ATP binding proteins such as cAMP-dependent protein kinases, a cGMP-dependent protein kinase, a lymphoid cell tyrosine kinase LCK, and the *Drosophila* gene product related to the EGF receptor tyrosine kinase (Hanks *et al.*, 1988). These observations suggested that the P-loop consensus sequence present in dematin^{52} and protein 4.2 may confer nucleotide binding activity to these proteins. In order to test this hypothesis, we measured the binding of ATP and GTP to portions of these proteins containing the putative ATP binding domains expressed as glutathione *S*-transferase (GST) fusion proteins in bacteria (Figure 2). The following cDNA constructs expressing recombinant fusion proteins were produced. (1) The headpiece domain of dematin^{48} without the insertion sequence was one. This construct does not contain the dematin homology motif and includes residues serine-309 to phenylalanine-383 of the human erythroid dematin^{48}. (2) The headpiece domain of dematin^{52} containing the 22-amino acid insertion was another. This construct includes the dematin homology motif and spans from serine-309 to phenylalanine-405 of the dematin^{52}. (3) A truncated segment of the human protein 4.2 containing the dematin homology motif was a third. This construct includes the amino acids from serine-294 to glutamine-347. (4) A relatively longer segment of the N-terminal domain of human protein 4.2, which includes amino acids from aspartic acid-175 to arginine-352 was another. Purified human erythrocyte protein 4.2 was also used in binding experiments.

Using a qualitative gel filtration assay, we first examined the binding of ATP to the GST−dematin fusion proteins. As shown in Figure 3, the radiolabeled ATP bound only to the GST−dematin^{52} protein. No binding of ATP occurred with either GST−dematin^{48} or GST alone (Figure 3). These results indicated that ATP specifically binds to the GST−dematin^{52} fusion protein which contains a P-loop consensus sequence. In order to quantify the binding of ATP to the fusion proteins, a nitrocellulose-based ATP binding assay

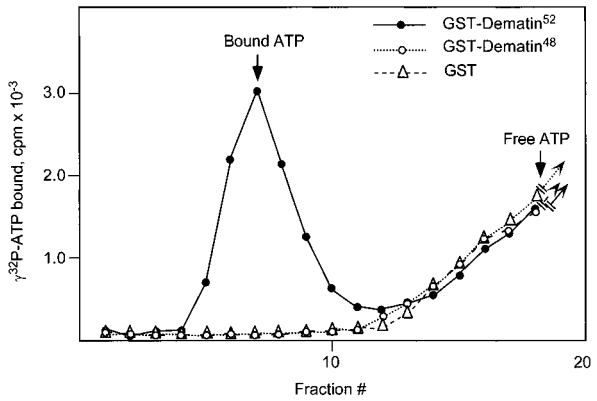

FIGURE 3: Binding of radiolabeled ATP to dematin. Protein-bound and free [γ-^{32}P]ATP were separated by chromatography on a gel filtration column of Sephadex G-25. The details are described in Experimental Procedures. Note that the ATP binding occurred only to the dematin construct containing the ATP binding P-loop.

was used. It should be noted that the filter binding assay measures nucleotide binding to proteins in solution. After the binding is complete, the nitrocellulose filter is used to separate free and protein-bound nucleotides. Using this filter binding assay, the binding of ATP to the fusion proteins containing the P-loop consensus sequence was detected. As shown in Figure 4, ATP binds to GST−dematin^{52}, GST−protein 4.2 (294−347), and GST−protein 4.2 (175−352). Again, no binding of ATP was detected with either GST alone or GST−dematin^{48} (Figure 4). The binding of radiolabeled ATP to the respective fusion proteins of dematin^{52} and protein 4.2 was completely reversed in the presence of a molar excess of unlabeled ATP (Figure 4). The nucleotide binding of the P-loop consensus sequence is specific for ATP since a molar excess of cold GTP did not inhibit ATP binding to GST−dematin^{52} (Figure 4A). Similarly, no direct binding of radiolabeled GTP was detected with either GST−dematin^{52} or the GST−protein 4.2 fusion proteins (data not shown). The radiolabeled ATP also bound to the native protein 4.2 purified from human red cell membranes, and ATP binding to the native protein 4.2 was independent of the position of radiolabeled phosphate in ATP (Figure 5). The native protein 4.2 bound 0.12 mol of [γ-^{32}P]-ATP per mole of protein and 0.2 mol of [α-^{32}P]ATP per mole of protein. Native protein 4.2 bound no detectable [γ-^{32}P]GTP under the conditions of these experiments (data not shown). Similarly, little or no binding of [^{32}P]ATP was detected with either the protein 4.2-related enzyme transglutaminase or bovine serum albumin (Figure 5). These results show that the headpiece domain of dematin^{52} and a motif present within the transglutaminase homology domain of protein 4.2 contain an ATP binding site.

The binding of ATP to the GST−dematin^{52} and GST−protein 4.2 fusion proteins (294−347) was saturable and of high affinity (Figure 6). At saturation, 1.0 nmol of ATP is bound per 7.0 nmol of GST−dematin^{52} with an estimated K_d value of 1.82 μM (Figure 6A). Similarly, the ATP binding to GST−protein 4.2 (294−347) saturates at 1.0 nmol of ATP per 29 nmol of the fusion protein (Figure 6B). The estimated K_d value for the interaction between ATP and GST−protein 4.2 (294−347) is 0.83 μM (Figure 6B). To test whether the stoichiometry of the ATP binding is influenced by the method of protein purification, we isolated GST−dematin^{52} by solubilizing bacteria in 1% Triton X-100 (Marfatia *et al*., 1994). Although the protein yield was low, the stoichiometry of ATP binding was improved to 1.0 nmol of ATP per 3.0 nmol of the fusion protein. On the basis of this result, we suggest that the apparently low binding stoichiometry of ATP to the fusion proteins may be a reflection of partially folded fusion proteins. Similarly, the affinity of these interactions may be modulated by post-translational modifications as well as by the elevated concentrations of dematin and protein 4.2 at the plasma membrane.

DISCUSSION

The evidence presented in this paper demonstrates that the human erythrocyte dematin^{52} and protein 4.2 contain an ATP binding site within a shared 11-amino acid motif (Azim *et al.*, 1995). The shared 11-amino acid motif constitutes the amino terminal half of the 22-amino acid insertion found in the 52 kDa subunit of dematin (Azim *et al.*, 1995). In

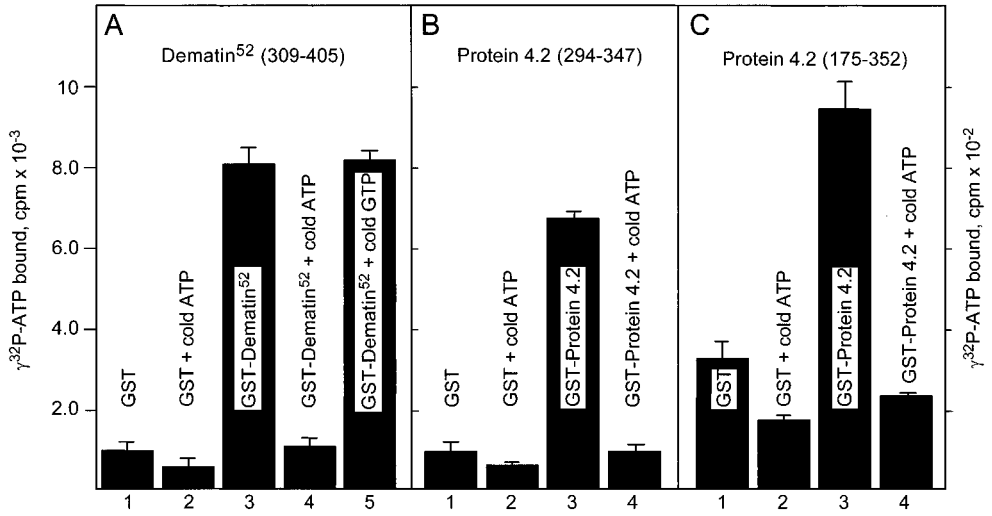

FIGURE 4: ATP binding to the recombinant domains of dematin and protein 4.2. The details of the GST fusion proteins are described in the legend of Figure 2. The ATP binding was measured using a filter binding assay as described in Experimental Procedures. (A) Binding of ATP to the headpiece domain of dematin^{52} containing the 22-amino acid insertion; 2.5 μg of GST fusion protein was used. (B) Binding of ATP to the truncated segment of the human protein 4.2 containing the dematin homology motif; 2.5 μg of GST fusion protein was used. (C) Binding of ATP to the longer segment of the amino terminal domain of protein 4.2; 12 μg of protein 4.2 domain (amino acids 175−352) fusion protein was used. Panel A and panel B share the same ordinate.

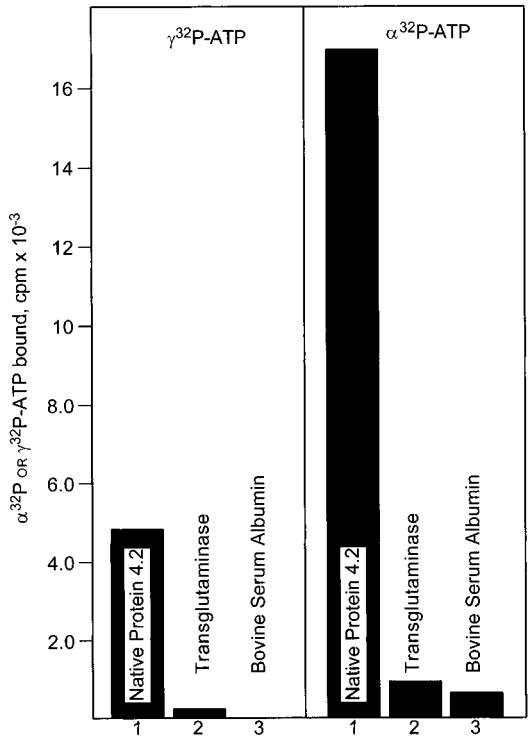

FIGURE 5: ATP binding to purified human erythrocyte protein 4.2. Binding of [α-^{32}P]ATP or of [γ-^{32}P]ATP to 10 μg of native protein 4.2, bovine serum albumin, and guinea pig liver transglutaminse was done as described in Experimental Procedures. The values shown are the means of two independent measurements which were within 10% of each other. Similar results were obtained with two other independent preparations of protein 4.2 (not shown).

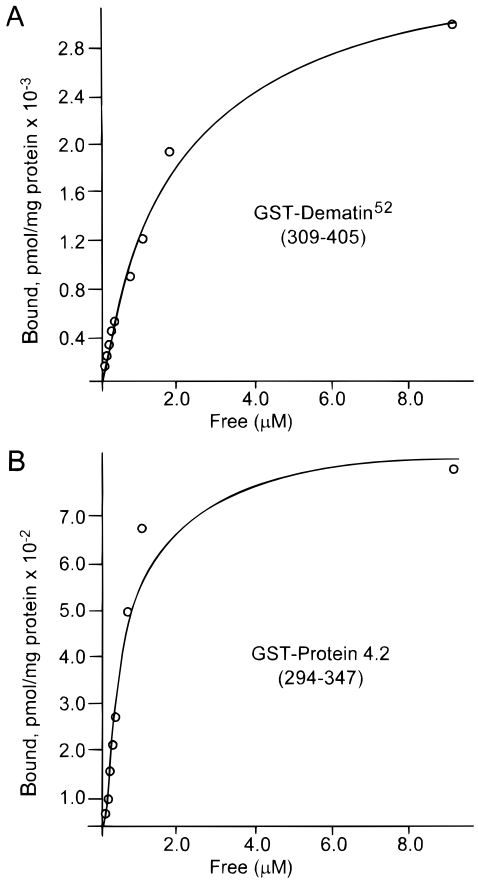

FIGURE 6: Quantification of ATP binding to the recombinant GST fusion proteins of dematin (A) and protein 4.2 (B). ATP binding to the GST fusion proteins was measured using a filter binding assay as described in Experimental Procedures. The extent of ATP binding was calculated in the presence of increasing amounts of cold ATP. The binding parameters were calculated using the ENZFITTER (Elsevier-BIOSOFT) computer program.

the primary structure of protein 4.2, the dematin homology motif is adjacent to the active site region of the transglutaminase homology domain (Figure 1). Although the dematin homology motif is located within the transglutaminase-like domain in protein 4.2, it does not share any sequence similarity with transglutaminases. The nucleotide binding appears to be mediated by the P-loop consensus sequence present in these proteins. The binding is specific for ATP since no detectable binding could be observed with GTP. The binding of ATP to dematin^{52} and protein 4.2 may suggest new approaches for the purification of these proteins from the red blood cell membranes. The high-affinity ATP binding property might be exploited to purify dematin and protein 4.2 using an affinity chromatography approach on a Cibacron blue coupled to agarose (Miller & Brenchley, 1981). Similar purification strategies have been used previously to purify enzymes containing a "dinucleotide fold" which specifically bind to Cibacron blue (Thompson *et al.*, 1975). The new purification alternatives may be of significance for protein 4.2 since it is rather difficult to purify protein 4.2 in the homogeneous form (Dotimas *et al.*, 1993).

The binding of ATP to dematin^{52} and protein 4.2 raises the possibility that the bound ATP may modulate protein−protein interactions mediated by dematin and protein 4.2 *in vivo*. Such a paradigm has been well-established in several cytoskeletal proteins which show striking parallels with dematin^{52} and protein 4.2 (Alberts & Miake-Lye, 1992; Gilbert *et al.*, 1995). The prototypical example is myosin whose ATPase activity drives muscle contraction and movement of the vesicles along actin filaments [see Alberts and Miake-Lye (1992) for review]. The ATP binding site is located in the myosin head domain. Similarly, the hydrolysis of bound nucleotides plays a critical role in several cytoplasmic proteins such as kinesins, dyneins, ras, hsp70, and related chaperonins (Alberts & Miake-Lye, 1992; Gilbert *et al.*, 1995). Whether ATP bound to dematin^{52} and protein 4.2 plays any such role(s) remains to be determined.

Interestingly, the ATP binding site in dematin^{52} is located in the headpiece domain which binds to actin filaments. The actin bundling activity of dematin is completely inhibited by phosphorylation and is restored upon dephosphorylation (Chishti *et al.*, 1988). However, the actin binding measurements indicated that phosphorylation had no effect on the actin binding properties of dematin (Azim *et al.*, 1995). The hydrolysis of ATP in the headpiece domain of dematin^{52} may generate a conformational change necessary for the phosphorylation-induced inhibition of actin bundling activity. The existence of similar mechanisms has been documented in other cytoskeletal proteins (Alberts & Miake-Lye, 1992; Gilbert *et al.*, 1995). Alternatively, the 52 kDa subunit of dematin may function as a membrane-associated ATPase, thus modulating the dematin-mediated cytoskeletal interactions at the plasma membrane.

Protein 4.2 binds to the cytoplasmic domain of band 3 and may play a role in the organization of the cytoskeleton or in modulation of cytoskeletal membrane interactions (Cohen *et al.*, 1993; Yawata, 1994a,b). It is possible that ATP binding or hydrolysis serves some regulatory role in these interactions. Moreover, ATP binding to nonerythroid

forms of protein 4.2 may have some influence on the proposed role of this protein in the formation or function of intracellular storage granules (White *et al.*, 1992). ATP binding by protein 4.2 and dematin may also serve other functions. Hoffman and colleagues have identified a membrane-bound pool of ATP, estimated at approximately 160 000 molecules per cell, which is utilized by the erythrocyte Na pump as well as by the Ca pump (Proverbio & Hoffman, 1977; Proverbio *et al.*, 1989). The locus of this membrane-associated pool of ATP is unknown. There are approximately 200 000 copies of protein 4.2 on the erythrocyte membrane and 40 000 copies of trimeric dematin. Thus, these proteins are present in sufficient quantity to account for the membrane-associated ATP pool. This suggests the possibility that the nucleotides associated with these cytoskeletal proteins may serve as a reservoir for membrane transport molecules. Further study will reveal whether ATP associated with protein 4.2 or dematin serves such functions.

ACKNOWLEDGMENT

We thank Donna-Marie Mironchuk and Andrew Geffken for assistance during the preparation of the manuscript.

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BI951745Y