Removal of the Amino-terminal Acidic Residues of Yeast Actin

STUDIES IN VITRO AND IN VIVO*

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We have examined the role of the acidic residues Asp1 and Glu1 at the NH2 terminus of Saccharomyces cerevisiae actin through site-directed mutagenesis. In DNEQ actin, these residues have been changed to Asn1 and Glu4, whereas in ΔDSE actin, the Asp1-Ser-Glu tripeptide has been deleted. Both mutant actins can replace wild type yeast actin. Peptide mapping studies reveal that DNEQ, like wild type actin, retains the initiator Met and is NH2 terminally acetylated, whereas ΔDSE has a free NH2 terminus and has lost the initiator Met. Interestingly, microscopic examination of filaments of these two actins reveal the appearance of bundled filaments. The DNEQ bundles are smaller and more ordered, whereas the ΔDSE bundles are larger and more loosely organized. Additionally, both mutant actins activate the ATPase activity of rabbit muscle myosin S1 fragment to a lesser extent than wild type. We have also developed a sensitive assay for actin function in vitro that enabled us to detect a slight defect in the ability of these mutant actins to support secretion, an important function in yeast. Thus, although the mutant actins resulted in no gross phenotypic changes, we were able to detect a defect in actin function through this assay. From these studies we can conclude that: 1) although NH2-terminal negative charges are not essential to yeast life, the loss of such charges does result in a slight defect in the actins’ ability to support secretion, 2) removal of the NH2-terminal negative charges promotes the bundling of actin filaments, and 3) mutants lacking NH2-terminal negative charges are unable to activate the myosin S1 ATPase activity as well as wild type actin.

Acts sequenced to date contain at least 2 and as many as 4 acidic residues at the NH2 terminus (1-4). These acidic residues have been implicated in the binding of several actin-binding proteins to actin as a result of cross-linking studies with the zero-length cross-linker 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (6-10). One of these, myosin, can be cross-linked to some or all of these acidic residues (11), and the binding of myosin to actin in the presence of ATP can be inhibited by the binding of an antibody directed against the NH2 terminus of actin (12).

Although control experiments indicate that nonspecific cross-linking is unlikely to occur (13), it has been demonstrated that these amino-terminal acidic residues are the acidic residues most likely to react with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (14). Site-directed mutagenesis of these acidic residues is a way in which to directly test their role in actin function without concern for their general reactivity to cross-linking agents. Sutoh et al. (15) examined the effect of changing 1 or 2 of the 3 acidic residues of Dictyostelium discoideum actin to His. This resulted in an impaired ability of these actins to activate the ATPase activity of rabbit heavy meromyosin. This effect was caused by a decrease in the Vmax of the heavy meromyosin rather than a change in the K1/2 of heavy meromyosin to actin. A study by Aspensstrom and Karlsson (16) examined a series of mutant chicken β-actins expressed in yeast. In these experiments, 2 of the 3 NH2-terminal Asp residues were either deleted or changed to Ala or Lys. These actins exhibited normal polymerization, but the Lys mutation was not decorated with myosin S1, indicating an interference with binding.

Although these two studies provide evidence that the NH2-terminal acidic residues of actin are important at least for the proper interaction of actin with myosin, a number of important questions about this region remain unanswered. In both these studies, at least 1 acidic residue remained at the actin NH2 terminus, and in one case a bulky positively charged imidazole was substituted for the acidic residues. This substitution might possibly cause steric hindrance of the protein-protein interaction, leading to the observed effect. In both these studies, the mutant actin was co-expressed with the endogenous wild type actin, which made it difficult to assess what, if any, effect these mutations had in vivo.

We have been using site-directed mutagenesis of yeast actin to study structure-function relationships of the actin NH2-terminal region in vivo and in vitro. Earlier (17), we demonstrated that the actin NH2 terminus is not post-translationally modified in yeast as it is in other cells. We further showed that addition of a Cys at the NH2 terminus had no effect on yeast viability, even though one-third of this actin had an unacylated NH2 terminus.

In this paper, we have further examined the importance of the NH2-terminal acidic residues through the expression of two yeast actin mutants lacking NH2-terminal negative charges in the yeast Saccharomyces cerevisiae. The 2 acidic residues Asp1 and Glu1 of yeast actin have either been changed to Asn and Glu, respectively, or deleted entirely. Coding sequences of these mutant actins were then used to replace the wild type actin sequence in yeast. We report here the effects of these actin mutations in vivo using a new sensitive

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† The abbreviations used are: S1, myosin subfragment 1; DNEQ, a mutant actin in which Asp1 has been replaced with Asn and Glu1 with Gin; ΔDSE, a mutant yeast actin in which the Asp1-Ser-Glu tripeptide has been deleted.
assay for actin function based on the secretion of invertase. Additionally, we report that these two actins behave very differently from wild type actin in their interactions with myosin S1 and in the bundling tendencies of filaments made from these actins.

MATERIALS AND METHODS

[35S]H2SO4, 1500 Ci/mmol, was purchased from ICN. The site-directed mutagenesis kit and [3P]ATP were purchased from Amer- sham Corp. Proteases were obtained from Boehringer Mannheim and Worthington. The Sequenase DNA sequencing kit was purchased from US Biochemical Corp. Protease inhibitors, glucose oxidase, peroxidase, and c-diaminodisulfone were obtained from Sigma. The S1 fragment of rabbit skeletal muscle myosin was prepared by the method of Weeds and Taylor (18). The oligodeoxynucleotides used in the mutagenesis procedure were synthesized by the University of Iowa DNA Core facility.

Yeast Strains and Manipulations—S. cerevisiae strain TdYDD was a gift of D. Shortle (Johns Hopkins University). HMSF 176 was a gift of S. Simon (Rockefeller University). Strain KC26 was con- structed by standard genetic techniques (19). See Table I for a list of strains used in this study. Yeast were grown as described (17). Yeast cells were grown on 2% peptone, 1% yeast extract, 2% glucose, or synthetic defined medium (19). For labeling of proteins, cells at OD660 = 0.1 were labeled in medium lacking sulfate with 20 μCi/ml [35S]H2SO4 for 6–8 h (17). Labeled cells were harvested by centrifugation and washed in unlabeled medium, and the cell pellet was frozen at −20 °C. Transformation of yeast was by a modification of the method of Ito et al. (20).

Haploid yeast containing a deleted chromosomal actin gene and a wild type or mutant plasmid-borne actin sequence were isolated through tetrad dissection of transformed diploids that had been induced to undergo sporulation (19). The desired Ura+ (plasmid), Leu+ (deleted chromosomal actin) cells were identified and used in these studies. Plasmid Manipulations and Mutagenesis—Plasmid manipulations were as described in Ausubel et al. (21). The yeast actin gene (1, 2) containing all necessary transcriptional regulatory elements but lacking the 309-base pair intron (a gift of D. Shortle) was cloned into the replicative form of the bacteriophage M13 mp18, and the resulting DNA was used to transform competent E. coli. Single-stranded viral recombinant DNA was then isolated and utilized as a template for oligodeoxynucleotide-dependent site-directed mutagenesis using the Amersham mutagenesis kit. For one mutant actin, an oligodeoxynucleotide of the sequence 5′-CTCAATGTTACAATGACGTTCAAA-3′ was used to change Asp1 = Glu4 = Asp′-Ser-Glu. After sequencing to verify the mutation, the mutant DNA was cloned into the centromeric plasmid yCp50 (22) (a gift of D. Shortle), which carries the yeast URA3+ gene. Plasmid pCEN-ADSE was generated by cloning the mutant actin-coding sequence plus the entire yeast actin fragment will only be labeled if the initiator Met remains. Chemical Acetylation of Peptides—The isolated peptides were treated with acetic anhydride and pyridine as previously described (24) and the products were analyzed by thin-layer electrophoresis at pH 6.3 and visualized by autoradiography.

RESULTS

Can Yeast Acts Lacking an Acidic NH2 Terminus Support Yeast Life?—We first used site-directed mutagenesis to change the codon for Asp1 to Asn and that for Glu4 to Gln. The NH2-terminal sequence of this actin, called DNEQ, is shown in Fig. 1. These substitutions remove the negative charges from the actin NH2 terminus. We subcloned this mutant actin-coding sequence plus the entire yeast actin promoter into yCp50, a yeast centromeric plasmid carrying a URA3+-selectable marker, forming the plasmid pCEN-DNEQ. Yeast strain TdYDD was transformed with this plasmid. This diploid strain carries one functional actin allele and one that has been deleted, and the deletion is marked with LEU2+ (Table I). Sporulation of these transformed cells allowed isolation of a URA1, LEU2+ haploid strain designated TdYDD. These nutritional markers indicate that the mutant actin-coding sequence is the only functional actin sequence in these cells. Since functional actin is essential for yeast life (29), these cells live because this mutant actin with no NH2-terminal concentration of negative charge supports all vital actin functions. Although the negative charges associated with Asp1 and Glu4 are no longer present, the Asn2 and Gln4 residues may

![Amino acid sequences of the NH2-terminal tryptic peptides of wild type yeast actin and the two actin mutants generated in this study (1, 2). Arrows indicate the site of cleavage by thermolysin, which produces two fragments, Nt and Ct. Labeling with [35S]H2SO4 will always yield a labeled Ct fragment. However, the Nt fragment will only be labeled if the initiator Met remains.](Fig. 1)
still form hydrogen bonds with other residues. It is thus possible that Asn\(^2\) and Gln\(^1\) could participate in a hydrogen bond with an actin-binding protein that usually interacts with actin through an ionic bond. To test this possibility, we constructed a second yeast actin mutant in which the tripeptide Asp\(^2\)-Ser-Glu was deleted. This actin sequence, which codes for Met-Val-Ala-actin (Fig. 1), was cloned into \(\gamma\)Cp50 under the control of the yeast actin promoter, forming the plasmid pCEN-\(\Delta\)DSE.

When pCEN-\(\Delta\)DSE was used to transform yeast strain Td\(y\)DD, URA\(^+\) transformants were again obtained. Following sporulation, URA\(^+\) LEU\(^+\) haploid cells were isolated and designated Td\(y\)\(\Delta\)DSE. The only functional actin in this strain is the mutant actin in which the tripeptide Asp\(^2\)-Ser-Glu has been deleted. This mutant actin can therefore carry out all vital yeast actin functions as well.

We then determined the growth rates of the wild type and mutant strains at 30°C in liquid YPD medium. All strains had doubling times of approximately 120 min. We also examined the ability of these cells to grow on YPD plates at 20, 30, and 37°C. All strains grew equally well at all temperatures. Additionally, all strains are capable of growth on a nonfermentable carbon source. Microscopic examination of the cells revealed no overt morphological defects in the mutants (data not shown). It appears from these results that there are no gross deleterious effects of these NH\(_2\)-terminal mutant actins in yeast under a number of different growth conditions.

**NH\(_2\)-terminal Analysis of the Mutant Actins—** Yeast actin does not undergo the unique actin-processing reaction seen in other organisms in which the initiator Met is removed post-translationally as acetyl-Met by an actin-processing enzyme (17). However, wild type yeast actin, like other actins, is NH\(_2\) terminally acetylated and therefore begins acetyl-Met-Asp-actin. The Met-Asn sequence of DNEQ actin and the Met-Pro sequence of ADSE actin is approximately one-half that of the wild type peptide; this result is predicted for a peptide that has approximately the same mass as the wild type oxidized actin NH\(_2\) terminus but only one-half the negatively charged residues. Since two of the original four negative charges on this peptide were removed by the mutagenesis, we predicted that this actin is NH\(_2\) terminally acetylated.

The mobility of the NH\(_2\)-terminal fragment of the \(\Delta\)DSE actin is approximately one-half that of the DNEQ fragment, despite the fact that this peptide has the same number of negatively charged residues as the DNEQ NH\(_2\)-terminal tryptic peptide and is 3 amino acids smaller. If this actin were acetylated, the peptide should migrate faster than that from DNEQ. This result is probably explained by the presence of one additional positive charge in the \(\Delta\)DSE actin from an unacetylated NH\(_2\) terminus.

To further examine the NH\(_2\) termini of these actins, we eluted these labeled tryptic peptides from the electrophoretogram and digested them with thermolysin. This enzyme cleaves the peptide into two fragments, Nt and Ct, which are separable by electrophoresis at pH 6.3 (Fig. 1). The Ct fragment, which contains both a Met and Cys, will always be labeled. The Nt fragment, however, will only be labeled if the initiator Met has been retained. The results of this digestion and electrophoresis are shown in Fig. 3. The NH\(_2\)-terminal tryptic peptides from all three types of actin give rise to identical Ct fragments upon thermolysin digestion. However, only the wild type and DNEQ NH\(_2\)-terminal peptides give rise to labeled Nt fragments. This result indicates that the DNEQ actin, like wild type actin, retains the initiator Met, whereas the Met has been removed from the \(\Delta\)DSE actin, presumably by the normal ribosome-associated Met-amino peptidase (31). The mobility of the Nt fragment of the NH\(_2\)-

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### Table I

**Yeast strains used in this study**

| Strain       | Genotype                                      | Plasmid          | Source       |
|--------------|-----------------------------------------------|------------------|--------------|
| Td\(y\)DD    | \(\alpha\) leu2/\(\alpha\) leu3-52/ura3-52,lys2/+\(+,ade2-101/+\),act1::LEU2/+ | None             | D. Shortle   |
| Td\(y\)WN    | \(\alpha\) leu2,ura3-52,lys2,act1::LEU2       | pCEN-WN          | Ref. 17      |
| Td\(y\)DNEQ  | \(\alpha\) leu2,ura3-52,lys2,act1::LEU2       | pCEN-DNEQ        | This study   |
| Td\(y\)\(\Delta\)DSE | \(\alpha\) leu2,ura3-52,lys2,act1::LEU2       | pCEN-\(\Delta\)DSE | This study   |
| HMSF173      | \(\alpha\) sec18-Itg,gal2                     | None             | S. Simon     |
| KC26-WN      | \(\alpha\) leu2,ura3-52,sec18-Itg,act1::LEU2 | pCEN-WN          | This study   |
| KC26-DNEQ    | \(\alpha\) leu2,ura3-52,sec18-Itg,act1::LEU2 | pCEN-DNEQ        | This study   |
| KC26-\(\Delta\)DSE | \(\alpha\) leu2,ura3-52,sec18-Itg,act1::LEU2 | pCEN-\(\Delta\)DSE | This study   |

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**Fig. 2.** NH\(_2\)-terminal tryptic peptides of wild type, DNEQ, and \(\Delta\)DSE actins isolated from yeast fed \([35S]H_2SO_4\). The actins were oxidized with performic acid and digested with trypsin, and the resulting peptides were subjected to electrophoresis at pH 6.3 and visualized by autoradiography. Oxidation with performic acid converts Met to methionine sulfoxide and Cys to cysteic acid. The band representing the NH\(_2\)-terminal tryptic peptide of each actin is labeled with an arrow. A, wild type actin; B, DNEQ actin; C, \(\Delta\)DSE actin.
that the fragment is NH₂ terminally acetylated. If it had a free NH₂-terminal tryptic peptides from the mutant
conditions of partial acetylation
result reflects the acetylation of the Lys residue at position 18. Since there is no evidence of a second more negatively charged species, the NH₂ terminus of this actin must already be blocked. The structure of the NH₂ terminus of DNEQ actin is therefore acetyl-Met-Asn-Ser-actin. Chemical acetylation of the ΔDSE peptide, in contrast, produces two new species, marked with one and two stars, denoting the presence of two groups that can become acetylated. One of the acetylations occurs at the Lys residue. The only other possible free amino group in this peptide is the NH₂ terminus. Therefore, ΔDSE actin has an unacetylated NH₂ terminus.

Amino Acid Sequencing of the ΔDSE Actin NH₂ Terminus—Since ΔDSE actin is not NH₂ terminally acetylated, we sequenced the NH₂ terminus to directly verify the identity of the NH₂-terminal amino acid as Val. Automated sequencing showed that the first 4 residues had the sequence Val-Ala-Ala-Leu. Therefore, the NH₂-terminal sequence of ΔDSE actin is +H₃N-Val-Ala-Ala-actin. Thus, not only are negative charges unnecessary at the NH₂ terminus of yeast actin, but a full positive charge can apparently be accommodated there in vivo as well.

Electron Microscopic Studies of Wild Type and Mutant Actin Filaments—Since these mutant actins did not result in any apparent growth defects, we were able to use these cells as a source of pure mutant actins for in vivo analysis of the effects of altering the NH₂ terminus of actin. Purified wild type, DNEQ, and ΔDSE actins were polymerized and examined by electron microscopy. All three actins had essentially the same critical concentrations for polymerization, as all three had essentially the same concentration of G-actin in the supernatant following polymerization and centrifugation to pellet the F-actin (data not shown). As seen in Fig. 5A, wild type yeast actin exhibits normal F-actin morphology. Individual actin filaments can be observed in the negatively stained sample. However, the majority of the filaments in the DNEQ (Fig. 5, B and C) and ΔDSE (Fig. 5, D–F) samples are bundled. Bundles of both types of actin are clearly composed of individual filaments (arrows), which can be seen leaving and joining the bundles. However, although both mutant F-actins form bundles, the morphology of each type of bundle is distinct. The DNEQ filament bundles are usually composed of three to five tightly joined filaments. Additionally, crossstriations are often observed, indicating that the filaments are in register (an example is indicated with an open arrowhead in Fig. 5C). The ΔDSE filament bundles, on the other hand, are consistently larger and appear to be more loosely packed than the DNEQ bundles, with no appearance of crossstriations. The end of a large bundle is shown in Fig. 5E. Often, the filaments appear to be woven together, as indicated with filled triangles in Fig. 5, D and F. It appears, therefore, that the lack of NH₂-terminal negative charges promotes the association of actin filaments into bundles. Since the ΔDSE bundles are larger than the DNEQ bundles, it would also appear that a lack of negative charges, coupled with a positively charged NH₂ terminus, further promotes this bundling effect. Since it has been previously reported that Ca²⁺ has an aggregating effect in wild type yeast actin (32), we also prepared F-actins in the absence of CaCl₂. Bundles were still evident in samples of the mutant actins (data not shown), indicating that this effect is not due to the presence of Ca²⁺.

To determine if this bundling effect could be decreased by the presence of wild type actin, an equimolar mixture of wild

![Fig. 3. Thermolysin digestions of the NH₂-terminal tryptic peptides of wild type and mutant actins. The NH₂-terminal tryptic peptides were isolated and digested with thermolysin. The digestion products were analyzed by electrophoresis at pH 6.3 and autoradiography. A, wild type actin; B, DNEQ actin; C, ΔDSE actin. The positions of the Nt and Ct fragments, described in Fig. 1, are indicated. O, origin.](image1)

![Fig. 4. Chemical acetylation of actin NH₂-terminal peptides. Following reaction of these compounds with acetic anhydride, the reaction mixture was analyzed by thin layer electrophoresis and autoradiography. Lanes A and B show a portion of the electrophoretogram in greater detail, whereas lanes C and D show the entire electrophoretogram. A, NH₂-terminal tryptic peptide from DNEQ actin, showing the original peptide (arrow) and a single chemically acetylated species (star). B, NH₂-terminal tryptic peptide from ΔDSE actin showing the original peptide (arrow) and the mono- and diacetylated species (one and two stars, respectively). C, wild type Nt fragment, unreacted with acetic anhydride. D, wild type Ct fragment, reacted with acetic anhydride and showing no chemically acetylated species. O, origin; og, position of the marker dye orange G.](image2)
type and ΔDSE actin was made and the actins co-polymerized. Fig. 5G shows a typical view of such filaments. Clearly, the bundles are composed of fewer filaments when compared with the ΔDSE sample and appear similar to those seen with the DNEQ actin alone, as the filaments are tightly bundled and appear in some instances to be in register (open arrowhead). This result would suggest that this bundling effect is not the result of a contaminating “bundling protein” but is rather inherent in the mutant actins themselves.

**Activation of Myosin ATPase**—To further characterize the in vitro effects of removing all negative charges at the NH2 terminus of actin, we examined the ability of these mutant actins to activate the ATPase activity of rabbit skeletal muscle myosin S1. Fig. 6 shows the results of an assay done with 20 mM wild type, DNEQ, and ΔDSE actins. The actin-dependent ATPase activity of S1, based on three determinations, is 43 ± 0.5 nmmol of Pi/min·nmol of S1 with wild type actin, 5 ± 0.2 nmmol of P/min·nmol of S1 with DNEQ, and 3 ± 0.5 nmmol of P/min·nmol of S1 with ΔDSE. This reflects a -fold activation of S1 activity of 15.3, 2.7, and 2.0, respectively. These results indicate that the mutant actins activate myosin only poorly. When a 1:1 co-polymer of wild type and ΔDSE actin was used in this assay at a total actin concentration of 20 μM, the activity of the S1 improved to 8 ± 0.3 nmmol of P/min·nmol of S1. This result is consistent with that obtained in the electron microscopic studies of these actins; the DNEQ actin, which has a neutral NH2 terminus and which forms smaller bundles, can activate myosin S1 poorly but to a greater extent than can ΔDSE actin. Additionally, a mixture of wild type and ΔDSE actin exhibits smaller bundles and activates myosin S1 better than ΔDSE alone. In collaboration with D. Root and E. Reisler at UCLA, we tested DNEQ F-actin for its ability to move along rabbit myosin filaments in an in vitro motility assay (33). Neither the bundled filaments nor individual filaments are able to move, showing that the lack of S1 activation described above is not entirely due to the presence of bundles but must also be related to the lack of the NH2-terminal charge density as well.

**Analysis of Invertase Secretion of Yeast Actin Mutants**—It has been previously reported that temperature-sensitive yeast actin mutants are unable to secrete vesicles efficiently at the restrictive temperature (34). More recently, the link between actin and secretion in yeast has been supported further by the discovery that sac1, a suppressor of actin mutant act1–1,
is also a suppressor of sec14, a secretion mutant with a Golgi defect (35). These results and those of others (36) have led to the development of a model in which secretory vesicles move to the periphery of the cell along actin filaments.

We utilized this relationship to more closely examine the ability of our mutant actins to function in vivo. In particular, we wished to determine if these mutant actins, lacking all NH2-terminal negative charges, could promote the movement of secretory vesicles as well as wild type actin. We thus examined the ability of these actins to carry out secretory functions in a sec18-1" background. sec18 protein, or N-

ethylmaleimide-sensitive factor, is a cytoplasmic protein important in the movement of vesicles from the endoplasmic reticulum to the Golgi, within Golgi compartments, and from the Golgi to the plasma membrane (37). At restrictive temperatures, vesicles within sec18-1" cells accumulate in the endoplasmic reticulum. However, these vesicles are quickly secreted when the temperature is lowered (27). Hence, if the mutant actins in our study have even a minor defect in their ability to promote movement of vesicles, we should be able to detect it by examining the ability of sec18-1"DNEQ or ΔDSE cells to recover from a temperature restriction.

We constructed a diploid strain, KC26, which, like TDyDD, contains one wild type actin allele and one that has been disrupted by LEU2*. In addition, KC26 is sec18-1"+/+ (Table 1). KC26 was transformed with pCEN-WN (carrying the wild type actin sequence), pCEN-DNEQ, or pCEN-ΔDSE and sporulated. Haploid URA*, LEU*, sec18-1" cells were isolated. Strains KC26-WN, KC26-DNEQ, and KC26-ΔDSE therefore express only the plasmid-borne wild type or mutant actin.

These cells were examined for their ability to recover from a sec18-1" temperature block at 37 °C through measurement of the secretion of invertase. Following a 30-min incubation at 37 °C in de-repressing medium for invertase (2% peptone, 1% yeast extract, 0.1% glucose), cells were shifted to 26 °C. As shown in Fig. 7, KC26-WN begins secreting invertase within 5 min of the shift to 26 °C, and additional secretion is rapid. However, in the cells expressing the mutant actins, the rate of recovery of invertase secretion is much slower. The increase in invertase activity, based on three determinations, in KC26-WN cells was 0.65 ± 0.05 μmol of glucose liberated/min, whereas that in KC26-DNEQ and KC26-ΔDSE cells was 0.41 ± 0.03 and 0.48 ± 0.05 μmol of glucose liberated/min, respectively. As described above, we observed no gross defects in yeast growth or morphology in yeast expressing these actins; yet, we do observe a defect in the ability of these cells to recover from temperature restriction in a sec18-1" background. Hence, this assay provides a very sensitive measure of the ability of these actins to function in vivo.

DISCUSSION

Recent reports have indicated that the NH2-terminal negative charges of actin play an important role in the interaction of actin with several actin-binding proteins (5-12). Our results provide new insight into the importance of these charges both in vitro and in vivo. We have expressed two mutant actins in yeast. One, DNEQ, is a double mutant in which Asp3 and Glu4 have been changed to Asn3 and Gin4. In vivo, this actin retains its initiator Met and is NH2 terminally acetylated. The second mutant, ΔDSE, is a protein in which the Asp2-Ser-Glu tripeptide has been deleted. The initiator Met of this actin is removed, but the NH2 terminus is not acetylated, giving this actin an NH2 terminus with a net positive charge. These results show that an NH2-terminal acetyl group per se is not required for yeast viability. Similar results with mutations in the actin NH2-terminal acidic residues were recently obtained by Johannes and Gallwitz (38), who showed that substitution of Val for both Asp3 and Glu4 has no observable effect on yeast viability. Further characterization of these actins in terms of myosin activation was not carried out in their study.

Our in vitro studies of these two mutant actins indicate that removal of these charges causes a bundling of the actin filaments, with the nature of the bundling effect seemingly dependent on the NH2-terminal negative charge density. This is the first report that NH2-terminal negative charge density can effect actin aggregation. Aspenstrom and Karlsson (16) reported that a β-actin in which 2 of the 3 Asp residues had been changed to Lys appeared to fragment more easily than wild type β-actin. Further, unlike the results described here, electron micrographs of the mutant β-actin displayed only individual filaments. The authors apparently did not observe the bundling effect in their mutant actin. This difference in observation could be due to differences in the actins used, although yeast and chick β-actins are 90% conserved in sequence. Alternatively, this apparent discrepancy could be accounted for by the fact that the mutant β-actin still retained 1 Asp residue, whereas neither of our mutant actins contain any NH2-terminal negative charges.

Secondly, both mutant actins examined in our study show a marked decrease in their ability to activate the ATPase activity of rabbit myosin SI, again with the loss of activity dependent on the loss of NH2-terminal negative charges and a gain of positive charge. This loss of activation is somewhat rescued by co-polymerization of the mutant actin with wild type actin to give a more negatively charged filament. This result underscores the importance of actin NH2-terminal negative charge density in muscle S1 activation suggested by the original zero-length cross-linking data. It further suggests that the data obtained by Sutoh et al. (15) with the Dictyostelium actin probably resulted from a change in the NH2-terminal charge and not the substitution of the His imidazole groups per se for the acidic residues. We were unable to dissect the effects of our mutations into K(m) and V(max) components, however, due to the difficulty in carrying out the experiments at yeast actin concentrations approaching the K(m) of wild type yeast actin (approximately 135 μM) (39).

In light of the results of the in vitro studies of these two mutant actins, it is surprising that these actins appear capable of supporting all necessary actin functions in yeast, a result that suggests that yeast actin functions do not require NH2-terminal negative charges. We see no evidence of large actin bundles in yeast stained with rhodamine-phalloidin (data not shown). It is possible that the relatively low concentration of actin in yeast or the presence of actin-binding proteins prevents its bundling. In terms of its interaction with myosin, yeast myosins have not yet been isolated in quantities sufficient for in vitro study, although a number of genes for myosin-like proteins have been identified (40-42). These myosins may be less sensitive than muscle myosins to changes in NH2-terminal charge density, since wild type S. cerevisiae actin has the lowest charge density of known actins (1-4).

The use of a more sensitive assay for in vivo actin function, utilizing cells that are sec18-deficient, demonstrates that these negative charges are indeed important in yeast, at least for the movement of vesicles required for invertase secretion upon release of cells from a restrictive temperature. This result indicates, first, that this movement of vesicles utilizes actin, as previously reported (34-36) and second, that the mechanism of transport requires actin NH2-terminal negative charges for maximal efficiency.

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