Structural Analysis of the Predicted Coiled-coil Rod Domain of the Cytoplasmic Bullous Pemphigoid Antigen (BPAG1)

EMPIRICAL LOCALIZATION OF THE N-TERMINAL GLOBULAR DOMAIN-ROD BOUNDARY*

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The bullous pemphigoid antigen BPAG1 is required for keratin filament linkage to the hemidesmosome, an adhesion complex in epithelial basal cells. BPAG1 structural organization is similar to the intermediate filament-associated proteins desmplakin I (DPI) and plectin. All three proteins have predicted dumbbell-like structure with central a-helical coiled-coil rod and regions of N- and C-terminal homology. To characterize the size of the N-terminal globular domain in BPAG1, two polypeptides spanning possible boundaries with the coiled-coil rod domain of BPAG1 were expressed in Escherichia coli. BP-1 (M, ~111,000), containing amino acids 663–1581 of BPAG1 (Sawamura, D., Li, K., Chu, M.-L., and Uitto, J. (1991) J. Biol. Chem. 266, 17784–17790), and BP-1A, with a 186 amino acid N-terminal deletion, were purified. BP-1 and BP-1A behave as highly asymmetric dimers in aqueous solution according to velocity sedimentation and gel filtration. Both have globular heads with rod-like tails of roughly equal length, 55–60 nm, upon rotary shadowing. BP-1A content of a-helix, determined by circular dichroism, is ~90% consistent with a-helical coiled-coil formation in the rod-like tails. The estimated rod length, 383 ± 57 amino acids (0.15 nm/amino acid), implies that globular folding in the BPAG1 N-terminal extends to the end of N-terminal homology with DPI and plectin. These findings support the existence of a common domain structure in the N-terminal regions of the BPAG1/DPI/plectin family.

The intracellular bullous pemphigoid antigen (BPAG1) is a part of the cytoplasmic plaque of the hemidesmosome, a supramolecular structure that links keratin intermediate filaments to extracellular matrix in a number of epithelial cell types, including epidermal keratinocytes (1, 2). BPAG1 (or the 230-kDa bullous pemphigoid antigen) was identified by autoimmune antibodies of patients with the dermal-epidermal blistering disease, bullous pemphigoid (3, 4). BPAG1 is rapidly assembled into a stable anchoring contact, or prehemidesmosome, at the ventral surface of freshly plated keratinocytes in culture (5–7). Disruption of the BPAG1 gene in mice by homologous recombination prevents keratin filament attachment to the hemidesmosome and consequently weakens dermal-epidermal adhesion but does not affect formation of the membrane-associated dense plaque of the hemidesmosome (8). The hemidesmosome also contains the high molecular weight cytoplasmic component HD-1 (9) and two transmembrane proteins, BPAG2, which contains an extracellular collagenous domain (10–13), and the integrin a6b4 (5, 14).

BPAG1 is homologous to two proteins which associate with intermediate filaments (IF): desmplakin I (DPI), which is part of the desmosome, a cell-cell junction; and plectin, a ubiquitous cytoskeletal protein that binds IF subunits in vitro (15–20). The predicted molecular masses of the three proteins are >300 kDa, and all three have homologous N- and C-terminal domains. Cell transfection and molecular studies have demonstrated that the C-terminal domains for plectin and DPI specifically interact with several IF types (21–23). The central regions of all three proteins, which lack homology to one another, nonetheless contain highly significant heptad repeats characteristic of a-helical coiled-coil rods, similar to the rod domain of myosin or to the core of IF heterodimers (20, 24). Purified DPI dimerizes and has an extended dumbbell-like shape in which terminal globular regions are separated by a central rod (25). Plectin and BPAG1 appear to take similar but less well-characterized conformations (26, 27).

The actual size of the N-terminal globular domains in BPAG1, DPI, or plectin have not been measured directly. In the case of BPAG1, this is of particular interest since the presence of heptad repeats in its N-terminal homology domain has suggested that coiled-coil rod formation may extend as far as 450 amino acids into this domain from the central, nonhomologous rod region (16, 18, 20). In plectin and DPI, the N-terminal homology region lacks certain characteristics generally associated with coiled-coil structures, such as a high (>1.0) ratio of charged/apolar amino acids, and, thus, rod formation in these domains is considered unlikely (19, 20). In N-terminal conserved regions of BPAG1, however, the presence of heptad repeats along with a high level of predicted interchain charge interactions has led to predictions that a-helical coiled-coil formation initiates at residue 708 or 875 (16, 18) according to the numbering of Sawamura et al. (18) rather than near the boundary of the N-terminal homology domain (residue 1145) as is the case with plectin and DPI (20). In order to directly establish the boundary of the N-terminal globular and central rod domains in BPAG1, two BPAG1-derived polypeptides containing the predicted N-terminal rod domain transitions were expressed in Escherichia coli and purified. The renatured polypeptides dimerize and, by rotary shadowing, are shown to...
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have highly asymmetric structures in which a globular N-terminal canyon is defined by complementation with plecin and dentin.

EXPERIMENTAL PROCEDURES

Materials—DNA clones II-1 and III-1, representing a portion of the BPAG1 sequence, were obtained from J. R. Stanley (NIH) and are described in Fig. 1 of Tanaka et al. (16). The pET series of protein expression vectors (28) were supplied by Novagen (Madison, WI). Purified human fibronectin was a kind gift of S. I. Chung (NIH), while rabbit tropomyosin was donated by F. Whitby and G. N. Phillips (Rice University). The 3-16 (n-hexadecanoyl-N-diethylamino-1-propanesulfonate) was purchased from Calbiochem.

Construction of Protein Expression Vectors—Plasmids were carried in E. coli strains DH5a and BL21(DE3) transformed with pET vectors was selected in 50 mg/ml ampicillin and cultured at 37°C. At an optical density of 0.6–0.8, isopropyl-1-thio-

BP-1 and BP-1A were verified as correct by dideoxy sequencing using specific primer pairs for bp-2 and bp-1, but protein could not be expressed in significant quantities. The fragment comprising the C-terminal 1,000 residues of bp-1 coding sequence was excised from pET-BP-1 with fragment in pET-BP1, was ligated into pET-5c, such that the fragment comprised of a 220- and a 240-kDa polypeptide. BP-1 and BP-1A were excised at the Asn site overlapping clone III-1 and at PstI in the polylinker region of the vector. The fragments from clone III-1 and III-1 were ligated simultaneously into pEX-2 (29) to create pEX-BP-1, a β-galactosidase fusion protein. The 3' and 5' ends of BP-1 were verified as correct by deoxy sequencing using specific primers for pEX-2, but protein could not be expressed in significant quantities. BP-1 cloning vector itself (see Table I). BP-1A was created by insertion of the sequence (18) and includes N- and C-terminal peptides derived from the BP-1 spans amino acids 663-1581 of the predicted BPAG1 amino acid sequence, were obtained from J. R. Stanley (NIH) and are

The partially purified protein was renatured by dialysis into 20 mM Tris-Cl, pH 7.4, 1 mM DTT for 14–18 h at 48,000 rpm. Marker proteins were probed in the polylinker region of the vector. The fragments from clone III-1 and III-1 were ligated simultaneously into pEX-2 (29) to create pEX-BP-1, a β-galactosidase fusion protein. The 3' and 5' ends of BP-1 were verified as correct by deoxy sequencing using specific primers for pEX-2, but protein could not be expressed in significant quantities. BP-1 cloning vector itself (see Table I). BP-1A was created by insertion of the sequence (18) and includes N- and C-terminal peptides derived from the
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TABLE I

Summary of the BPAG1-derived polypeptides

| Construct       | Vector | Amino acids (mass) | N-, C-terminal extensions | Intraacellular location in E. coli |
|-----------------|--------|-------------------|---------------------------|-----------------------------------|
| BP-1            | pET5c  | 663–1581 (111 kDa)| NH$_2$-MASMTGGQQMGRRIR GILDERAS-COOH | Inclusion body$^b$                |
| BP-1A           | pET5c  | 849–1581 (89 kDa) | NH$_2$-MASMTGGQQMGRRIR GILDERAS-COOH | Cytoplasm                          |

$^a$ Range of amino acid residues incorporated according to the predicted BP230 primary amino acid sequence (18).
$^b$ Based on resistance to extraction by nonionic detergent and deoxycholic acid.

RESULTS

Protein Purification—Two polypeptides encompassing the predicted structural transitions from the N-terminal homology domain to the central $\alpha$-helical coiled-coil rod portion of BPAG1 were expressed in E. coli (see Table I). One of these polypeptides, BP-1, could be renatured in a largely soluble form with a small number of lower molecular weight impurities (Fig. 1A) after extraction in 8 M urea from bacterial inclusion bodies (lane a), purification on DEAE-cellulose (lane b), and dialysis from urea (lane c). The migration of BP-1 on SDS gels is consistent with a predicted molecular mass of 111 kDa (Fig. 1A, lane c). A second purified polypeptide, BP-1A, was constructed by an N-terminal deletion of 186 amino acids from BP-1 (see Table I). In contrast to BP-1, BP-1A was expressed in the cytosol of lysed E. coli. BP-1A isolated following ion exchange and gel filtration appeared to be >95% pure (Fig. 1B, lane c), and this was confirmed by laser scanning densitometry of Coomassie Blue-stained gels. A high molecular weight contaminant, which represents <5% of total protein (Fig. 1B, lane c), could not be removed by ammonium sulfate precipitation or hydroxyapatite chromatography. Other polypeptides from this region of BPAG1 were expressed at high levels in E. coli, but none could be satisfactorily purified and characterized (55).

Further purification of BP-1 and BP-1A by heat treatment was attempted since many water-soluble coiled-coil proteins, such as tropomyosin and light meromyosin, renature spontaneously when cooled following heating to 95 °C (43, 44). Interestingly, both BP-1 and BP-1A precipitated out of solution when heat-denatured (Fig. 2). Horowitz and co-workers (45) have shown that a detergent, such as Zwittergent 3-16, when near its critical micelle concentration, can facilitate native protein folding during rapid renaturation by binding to exposed hydrophobic surfaces that otherwise induce aggregation. In the presence of 0.1 mg/ml Zwittergent 3-16, BP-1 and BP-1A renature primarily to soluble forms (Fig. 2) following heat treatment. While overall protein purity is not improved by this procedure, the detergent requirement for renaturation of BP-1 and BP-1A following heat treatment is consistent with the presence of complex globular folding domains in addition to the predicted $\alpha$-helical coiled-coil.

Physical Characterization—BP-1A comigrates with bovine serum albumin ($s_{20,w}^0 = 4.4$, mass $= 68$ kDa) on velocity sucrose gradients as a single homogeneous peak (Fig. 3). BP-1 has a slightly higher estimated $s_{20,w}^0$ of 4.85 than BP-1A. Traces of the two proteins were found at the bottom of both of the sucrose gradients, but at such low levels (<5%) to suggest that aggregation was not a major factor influencing their behavior. The two proteins also eluted before much higher molecular weight globular standards on Superose 6 gel filtration, suggesting that BP-1 and BP-1A have highly asymmetric or elongated structures. The Stokes radii of BP-1 and BP-1A are greater than both thyroglobulin and fibrinogen but smaller than the spec-
trin dimer (Fig. 4) and were estimated to be 115-121 Å and 111 Å, respectively. Solution molecular weights of BP-1 and BP-1A were inferred from the measured sedimentation coefficients and Stokes radii as 235 kDa and 195 kDa. Both proteins thus appear to associate in solution as dimers, consistent with formation of α-helical coiled-coil. In addition, each is predicted to have an axial ratio of >20:1 (Table II). Gel filtration of highly asymmetric proteins such as these on Sepharose 4B has been reported to underestimate actual Stokes radii when globular standards are used for calibration (46). However, the elution of the asymmetric protein standards fibrinogen and dimeric human erythrocyte spectrin correlated well with the three globular protein standards on Superose 6 (Fig. 4), and, therefore, the estimates of Stokes radius for BP-1 and BP-1A on Superose 6 appear to be valid.

Rotary Shadowing—To evaluate both the existence and the extent of the predicted α-helical coiled-coil domain in BPAG1, we performed rotary shadowing on both BP-1 and BP-1A. Rotary-shadowed images of BP-1 and BP-1A were rod-like with an apparent globular structure at one end; a sample field of the BP-1A is shown in Fig. 5a. The knob-like or globular end is more readily visible on BP-1 (Fig. 5, b and c) than on BP-1A (Fig. 5, d, e, and f) as expected since BP-1A is larger and contains a greater proportion of the N-terminal homology region. The lengths of the rod-like regions of both proteins were measured from images having a clearly defined knob at one end and are distributed in a Gaussian fashion: 60 ± 9 nm (N = 185) for BP-1 and 55 ± 8 nm (N = 159) for BP-1A (Fig. 6). The length of rabbit tropomyosin was measured to be 44 ± 4 nm (N = 111), which gives a predicted length that is within the range determined recently by x-ray diffraction (47) and confirms the accuracy of our technique. The knob-like regions were variable in size, however, due perhaps to variability in spreading of the proteins, and their size could not be quantitated directly. A prominent kink was also observed in the tail region of both proteins (Fig. 5, c and f). The length from the end of the tail to the kink in BP-1A was measured to be 26 ± 5 nm (n = 61).

Circular Dichroism—The content of α-helical secondary structure in BP-1A was directly estimated from its CD spectrum in several preparations. As shown in Fig. 7, a prominent positive peak at 193 nm, a pair of peaks of negative ellipticity at 208 and 222 nm, and a 2:1 ratio of [θ]222 to [θ]208 could be observed, all of which are characteristics of α-helices (48). The value of [θ]222 was −40,000, based on an estimation of protein concentration in 6 M guanidine HCl, and is consistent with 100% α-helix content. According to the fitting method of Yang et al. (48), BP-1A is estimated to contain 90% α-helix and 10% random coil.

DISCUSSION

Location of N-terminal to Rod Boundary in BPAG1—The BPAG1-derived polypeptides BP-1 and BP-1A were designed to span a junction between the predicted globular N-terminal and the central α-helical coiled-coil rod domains of BPAG1. The molecular weights of both polypeptides in solution, based on velocity sedimentation and gel filtration, are consistent with dimer formation. The very high percentage of α-helix in BP-1A, as determined by circular dichroism, confirms predictions that the central region of BPAG1 will form an α-helical coiled-coil dimeric rod, characteristic of myosin, tropomyosin, and other members of the intermediate filament family (16, 18, 20). The calculated axial ratios of BP-1 and BP-1A in solution are greater than 20 (Table II), and a substantial rod-like extension is observed in both molecules by rotary shadowing electron
microscopy. Significantly, the measured tail lengths of BP-1 and BP-1A are nearly identical (60 and 55 nm, respectively), and, if forming a-helical coiled-coil, are predicted to be 383 ± 65 amino acids in length, based on the average of the measured tail lengths for BP-1 and BP-1A, 57.5 ± 8.5 nm, and a 1.5 Å rise per amino acid for a-helical coiled-coils (47). The observed junction of the rod domain with adjacent globular structures in BP-1 and BP-1A corresponds to amino acid residues 1197 ± 57 of the complete BPAG1 molecule. Earlier predictions for the boundary of the coiled-coil, at residue 708 in DPI and plectin, correspond to amino acid residue 1145 (16, 27), are not supported by these data, and would imply much longer tails as imaged by rotary shadowing. The hypothesis (20) that the coiled-coil rod begins at the end of the N-terminal homology with DPI and plectin, approximately at residue 1145, appears to be correct. The kink observed in the tail region of some molecules of BP-1A was estimated to be 26 ± 5 nm from the end of the rod, corresponding to residues 1408 ± 33. This is in rough agreement with a predicted interruption within the BPAG1 rod domain that occurs from residue 1327 to 1354 (20). Our estimate of the N-terminal rod boundary is also supported by the predictive algorithm of Lupas et al. (42), which is based on a statistical analysis of amino acid distribution in each position of the heptad repeat in known coiled-coil proteins. When BPAG1 is analyzed by this algorithm, residues 1137 to 1870 form a continuous coiled-coil with a short interruption at residues 1325 to 1360 (Fig. 8). Similar analysis of DPI and plectin (not shown) also aligns the start of rod formation with the end of N-terminal homology, as predicted previously (20). Interestingly, two short regions in the N-terminal homology domain, none more than four heptad repeats in length, show greater than 99% probability of coiled-coil formation, but these are shorter than required for coiled-coil formation in solution and, in the context of other secondary structure motifs, are not likely to assemble as rods (49).

Previous estimates that coiled-coil rod extends into the N-terminal homology domain of BPAG1 were based upon the high frequency (70%) of hydrophobic amino acids in the a and d positions of heptad repeats (a, b, c, d, e, and f) found in this domain as well as higher than average intrachain charge interactions consistent with coiled-coil formation (17, 20). These characteristics may instead give rise to non-rod a-helix (such as...
BPAG1 Structure and Function—A model for BPAG1 structure based on our results is shown in Fig. 9. The N-terminal globular domain occupies a position homologous to that predicted for both plectin and DPI. Specific C-terminal repeats also found in plectin and DPI (16, 18, 20) may mediate IF interactions (21–23), and these repeats would demarcate a central rod of 107 nm. The measured rod length of purified DPI, 130 nm (25), is consistent a rod bounded by the same N- and C-terminal homology domains as in BPAG1 (20). A rotary shadowing study of purified BPAG1 from bovine tongue reported an overall rod length of more than 140 nm (27). This larger estimate was based on a very small number of images of BPAG1 renatured from 9.5 M urea, while the majority of BPAG1 was in aggregates. Since formation of additional rod in the C-terminal homology domain is very unlikely, the reported dumbbell structures with rod length of 140 nm probably represent partially denatured structures.

Although analysis of soluble BPAG1 polypeptides was a necessity in this study, the approach can be justified on the grounds that BPAG1 is expressed in cultured keratinocytes in a soluble or cytosolic form which may act as a precursor for incorporation into the detergent-resistant plaques found in culture (3, 6, 50). DPI and DPII are also soluble once purified (25), and they are present largely in soluble form in epithelial cells cultured in low (<0.05 mM) Ca\(^{2+}\); induction of desmosome formation by raising extracellular Ca\(^{2+}\) causes a shift from cytosolic to desmosome-associated forms, suggesting a precursor-product relationship for the soluble and insoluble forms of the desmosplakins as well (51, 52). We presume that once BPAG1 has been incorporated into the hemidesmosome, major tertiary folding patterns are unchanged. Ultrastructural and genetic evidence suggest that the BPAG1 C-terminal is associated with the cytoplasmic plate of the hemidesmosome, which is a junction for keratin filament attachment. In BPAG1 knockout mice, not only is the plate absent but keratin attachment to the hemidesmosome is also abrogated (8). The cytoplasmic plate is found at a distance of about 0.1 \(\mu\)m from what is the most prominent feature of the hemidesmosome, the plasma membrane-associated dense plaque (2, 8, 53). Labeling with bullous pemphigoid patient autoantibodies directed to BPAG1 shows predominance of staining at an average of 90 nm from the plasma membrane, and two rabbit antisera to the BPAG1 C-terminal also bind at the approximate location of the keratin plaque attachment (6, 12, 54). Ultrastructural localization of the BPAG1 N-terminal has not been addressed experimentally, although studies in cultured normal human keratinocytes (6) and SCC-12 cells (13) strongly suggest that BPAG1 is exclusively intracellular, based on its resistance to degradation by extracellular protease. The central discontinuous rod of BPAG1 may provide a flexible link to another cytoplasmic structure such as the membrane-associated dense plaque, where the N-terminal domain itself could in turn bind to transmembrane hemidesmosome components such as BPAG2 or the integrin \(\alpha_6\beta_4\).

In summary, we have carried out high resolution mapping of the N-terminal homology region of BPAG1, empirically localizing a boundary between the N-terminal globular and central rod domain. Comparison of two polypeptides differing only in an N-terminal deletion has enabled us to specify the absolute orientation of the rotary shadowing images of these molecules unambiguously. These data on BPAG1 domain structure should facilitate more rational design of polypeptides for in vitro analysis and cell transfection studies of N-terminal globular domain function in all members of the BPAG1/DPI/plectin protein family.

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REFERENCES
1. Klatte, D. H., Kurpakus, M. A., Grelling, K. A., and Jones, J. C. R. (1989) J. Cell Biol. 105, 3377–3390
2. Owarike, K., Kartenbeck, J., Stumpf, S., Magin, T. M., Krieg, T., Diaz, L. A., and Steinert, P. M. (1991) J. Cell Biol. 115, 1537–1550
3. Stanley, J. R., Hawley-Nelson, P., Yusu, S. H., Shevah, E. M., and Katz, S. I. (1981) Cell 24, 897–903
4. Stanley, J. R., Tanaka, T., Mueller, S., Klaus-Kovtun, V., and Roop, D. (1988) J. Clin. Invest. 82, 1864–1870
5. Carter, W. G., Kaur, P., Gil, S. G., Gahr, P. J., and Wayner, E. A. (1990) J. Cell Biol. 111, 3141–3154
6. Thacher, S. M., Malone, K. L., Dave, K., and Zhao, S.-M. (1991) Exp. Cell Res. 194, 238–247
7. Kurpakus, M. A., Quarranta, V., and Jones, J. C. R. (1991) J. Cell Biol. 115, 1737–1750
8. Guo, L., Degenstein, L., Dowling, J., Yu, O.-C., Wollmann, R., Perelman, B., and Fuchs, E. (1995) Cell 81, 233–243
9. Hieda, Y., Nishizawa, Y., Uematsu, J., and Owarike, K. (1992) J. Cell Biol. 116, 1497–1506
10. Giudice, G. J., Emery, D. J., and Diaz, L. A. (1992) J. Invest. Dermatol. 99, 243–250
11. Li, K., Sawamura, D., Giudice, G. J., Diaz, L. A., Mattei, M.-G., Chu, M.-L., and Uitto, J. (1991) J. Biol. Chem. 266, 24064–24069
12. Ishida, Y., Shimizu, H., Kikuchi, A., Ebihara, T., Hashimoto, T., and Nishikawa, T. (1990) J. Clin. Invest. 85, 1608–1615
13. Hopkinson, S. B., Riddle, K. S., and Jones, J. C. R. (1991) J. Invest. Dermatol. 99, 264–270
14. Shio, M. A., Spurr-Michaud, S., Tisdale, A., Ewel, J., and Gipson, I. K. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8970–8974
15. Green, K. J., Parry, D. A. D., Steinert, P., Virata, M. L. A., Wagner, R. M., Angst, B. D., and Nilles, L. A. (1990) J. Biol. Chem. 265, 2603–2612
16. Tanaka, T., Parry, D. A. D., Klaus-Kovtun, V., Steinert, P. M., and Stanley, J. R. (1991) J. Biol. Chem. 266, 12555–12559

FIG. 8. Probability of coiled-coil formation as a function of amino acid residue according to the algorithm of Lupas et al. (42). A window size of 28 amino acids was used to estimate a coiled-coil score at each residue, and a probability of coiled-coil formation was assigned based on the behavior of known proteins in this algorithm.

FIG. 9. Domain structure of BPAG1. BP-1 and BP-1A are shown as striped or shaded bars aligned with a BPAG1 open reading frame of 2,649 amino acids. The N-terminal globular domain GN is shown adjacent to the rod region (open bar) as defined in this study. B and C are homologous to one another and to comparable C-terminal repeats in plectin and the desmoplakins. Arrows 1, 2, and 3 represent start sites of the rod region according to the predictions of Tanaka et al. (16), Sawamura et al. (18), and Green et al. (20).
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17. Wiche, G., Becker, B., Luber, K., Wetzler, G., Castañón, M. J., Hauptmann, R., Stratowa, C., and Stewart, M. (1991) J. Cell Biol. 114, 83–99
18. Sawamura, D., Li, K., Chu, M.-L., and Uitto, J. (1991) J. Biol. Chem. 266, 17784–17790
19. Virata, M. L. A., Wagner, R. M., Parry, D. A. D., and Green, K. J. (1992) Proc. Natl. Acad. Sci. 89, 544–548
20. Green, K. J., Virata, M. L. A., Elgart, G. W., Stanley, J. R., and Parry, D. A. D. (1992) Int. J. Biol. Macromol. 14, 145–152
21. Stappenbeck, T. S., and Green, K. J. (1992) J. Cell Biol. 116, 1197–1209
22. Wiche, G., Gronov, D., Donovan, A., Castañón, M. J., and Fuchs, E. (1993) J. Cell Biol. 121, 607–619
23. Kouklis, P. D., Hutton, E., and Fuchs, E. (1994) J. Cell Biol. 127, 1049–1060
24. Coulombe, P. A., and Fuchs, E. (1990) J. Biol. Chem. 264, 8310–8318
25. Davis, J., and Bennett, V. (1983) J. Biol. Chem. 258, 7757–7766
26. Tanaka, T., Korman, N. J., Shimizu, H., Eady, R. A. J., Klaus-Kovtun, V., Cehrs, K., and Stanley, J. R. (1990) Invest. Dermatol. 94, 617–623
27. Tandon, S., and Horowitz, P. M. (1987) J. Biol. Chem. 262, 4486–4491
28. Nozaki, Y., Schechter, N. M., Reynolds, J. A., and Tanford, C. (1976) Biochemistry 15, 3884–3890
29. Whitby, F. G., Kent, H., Stewart, F., Stewart, M., Xie, X., Hatch, V., Cohen, C., and Phillips, G. N., Jr. (1992) J. Mol. Biol. 227, 441–452
30. Yang, J. T., Wu, C.-S. C., and Martinez, H. M. (1986) Methods Enzymol. 130, 268–263
31. Cohen, C., and Parry, D. A. D. (1990) Proteins Struct. Funct. Genet. 7, 1–15
32. Thacher, S. M., and Hefti, P. L. (1991) Invest. Dermatol. 96, 139–143
33. Pasdar, M., and Nelson, W. J. (1988) J. Cell Biol. 106, 677–685
34. Compton, C. C., Gill, J. M., Bradford, D. A., Regauer, S., Gallico, G. G., and O’Connor, N. E. (1989) Lab. Invest. 60, 600–612
35. Tang, H.-Y. (1993) Ph.D thesis, Texas A & M University
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