Comparison of Molecularly Cloned Bullous Pemphigoid Antigen to Desmoplakin I Confirms That They Define a New Family of Cell Adhesion Junction Plaque Proteins*

(Received for publication, January 15, 1991)

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Bullous pemphigoid is a subepidermal blistering disease in which patients have autoantibodies against the plaque of the hemidesmosome. Starting with a previously isolated 2-kilobase (kb) cDNA for bullous pemphigoid antigen (BPA), we used primer extension of keratinocyte mRNA to isolate overlapping cDNAs with a combined open reading frame of 6.3 kb, encoding most (243 kDa) of the BPA, but lacking the far amino terminus. Analysis of this acid sequence revealed a carboxyl-terminal domain containing two regions of 174 and 176 residues with high sequence identity. Most of the amino-terminal two-thirds of BPA is predicted to be in α-helical conformation in which two chains would aggregate into a coiled-coil rod structure. BPA and desmoplakin I, a desmosome plaque protein, show remarkable sequence and structural homology. In its carboxyl-terminal domain, desmoplakin I also has 176 residue repeats with 49% sequence identity to those in BPA. The repeats in both molecules have a regular linear distribution of acidic and basic residues with a period of 9.5, the same as that found in the 1B segment of keratin filaments, suggesting a means of ionic interaction between keratin and these plaque proteins. Also, desmoplakin I, like BPA, is predicted to have a rod domain, which in both proteins has similar regular charge periodicities, suggesting a means of ionic self-aggregation. These findings extend those of Green et al. (Green, K. J., Parry, D. A. D., Steinert, P. S., Virata, L. A., Wagner, R. M., Angst, B. D., and Niles, L. A. (1990) J. Biol. Chem. 265, 2603–2612) which show that BPA and desmoplakin I represent the first members of a new family of adhesion junction plaque proteins.

Bullous pemphigoid is an autoimmune disease in which patients develop subepidermal blisters and antibodies directed against the epidermal basement membrane zone (1). The autoantibodies from these patients have been shown to bind to hemidesmosomes, structures thought to be important in anchoring basal cells to the basement membrane (2–4). Although bullous pemphigoid antigen may be heterogeneous (5), almost all patients have antibodies against an ~230-kDa protein, termed the major bullous pemphigoid antigen (BPA)1

We have previously cloned a partial 2-kb cDNA encoding about 76 kDa of the carboxyl domain of BPA (8). Recent cloning of desmoplakin I (DPI) (9), a desmosome plaque protein (10–12), revealed sequence homology with BPA for about 170 amino acids in the carboxyl domains of both proteins.

In this report, we used the initially isolated BPA cDNA clone to isolate overlapping cDNA clones encoding for 243 kDa of BPA. Analysis of the deduced amino acid sequence of this 6.3-kb open reading frame revealed remarkable sequence and structure homology between BPA and DPI and suggests that these proteins define a new family of adhesion plaque proteins.

EXPERIMENTAL PROCEDURES

Construction and Screening of cDNA Libraries—Fig. 1 shows from which libraries (labeled by Roman numerals) each subclone was obtained. All cDNA libraries were constructed with poly(A)–RNA from cultured human keratinocytes (8). λgt11 library I was constructed by oligo(dT) priming, and clone I-1, identified by screening with bullous pemphigoid patient sera, is the one previously described (8). The other libraries were made by primer extension of keratinocyte poly(A)–RNA with reverse transcriptase, followed by RNase H and DNA polymerase I, according to the basic method of Gubler and Hoffman (13) and the specific methods of suppliers (Boehringer Mannheim cDNA Synthesis Kit, Clontech c-Clone II cDNA Synthesis Kit, and Invitrogen λ Librarian). Primers used were 17 or 18 nucleotides and, for construction of each of the libraries indicated, were complementary to the following areas: II-5’ end of 2-kb EcoRI fragment of cDNA I-2; III-141 nucleotides downstream from the 5’ end of cDNA II-1; IV and V-215 nucleotides downstream from the 5’ end of cDNA III-1. EcoRI linkers (Bethesda Research Laboratories) or adaptors (Amersham Corp.) were ligated to the cDNA for cloning into λgt11 (14) or Lambda Zap II (Stratagene), with subsequent packaging (Gigapack II Gold, Stratagene). Libraries were screened by hybridization with probes labeled by random priming (15, 16). Hybridization was performed in 6 × SSC (1 × SSC = 150 mM NaCl, 15 mM Na citrate, pH 7), 0.5% sodium dodecyl sulfate, 12.5 × Denhardt’s solution at 68 °C for 16 h. The most stringent final wash was at 68 °C in 0.1 × SSC, 0.1% sodium dodecyl sulfate. Probes used to screen each library were as follows (see Fig. 1): I-HindIII 218-bp fragment at the 5’ end of the 1.5-kb fragment of I-1, and EcoRI 1.1-kb fragment from the 3’ end of I-2; II-300-bp EcoRI fragment at the 5’ end of cDNA I-2; III-218-bp AccI fragment at the 5’ end of cDNA II-1; IV-203-bp EcoRI-PstI fragment at the 5’ end of cDNA III-1; V-150-bp

1 The abbreviations used are: BPA, the ~230-kDa bullous pemphigoid antigen; DPI, desmoplakin I; kb, kilobase(s); bp, base pair(s).

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5′-EcoRI fragment of IV-1. The cDNA inserts of positive clones were subcloned in either pGEM 3Z (Promega) or pBluescript SK (Stratagene) for double-stranded dideoxy nucleotide sequencing with Sequenase (U. S. Biochemical Corp) and primers complementary to plasmid DNA or previously determined sequence (8).

Northern analysis with isolated cDNAs was performed on 10 μg lane of total RNA from keratinocytes, as previously described (8).

Analysis of Amino Acid Sequence and Homologies—Secondary structure predictions were carried out using the methods of Chou and Fasman (17) and Garnier et al. (18). In conjunction with these methods, patterns of heptad substructure in the sequence were sought that were compatible both with an α-helical structure and a coiled-coil conformation (19, 20).

University of Wisconsin Genetics Computer Group software (21) was used as follows: (i) the BPA amino acid sequence was compared for homologies against the NBRF version 25 protein database using FASTA, and (ii) a comparison of the BPA and the DPI amino acid sequences was undertaken with COMPARE (based on the evolutionary distance between amino acids, with a window of 21 amino acids and a stringency of 15) and plotted with DOTPLOT. Other computer analyses of sequence comparisons were based on the MATCH algorithm (22).

Fast Fourier transforms were used to determine significant periodicities in the linear distribution of acidic and basic residues along the sequence of BPA using the methods described by Parry (23), Fraser and MacRae (24), and McLachlan and Stewart (25).

RESULTS

Cloning of BPA cDNA—A λgt11 cDNA library (called I), constructed by oligo(dT) priming of poly(A)-RNA from cultured keratinocytes, was screened by hybridization with restriction fragments from cDNA (called I-1 in Fig. 1) previously cloned from this library (8). This hybridization resulted in finding two overlapping cDNA sequences (Fig. 1, I-2 and I-3) containing one long open reading frame (in frame with that previously described (8)) with a stop codon near the 3′ end of cDNA I-3. (The 3′-terminal 131 nucleotides of the previously reported BPA cDNA clone I-1 were found to be different from the corresponding region of clones I-2 and I-3. Since I-2 and I-3 have identical sequences in this region, and because the stop codon defined by these clones corresponds exactly to a recently reported mouse partial BPA cDNA (26), we used their sequence to determine BPA amino acid sequence in this paper). Because no further clones could be found in the oligo(dT)-primed library, we used primer extension of keratinocyte poly(A)-RNA with primers near the 5′ ends of previously isolated clones to sequentially generate new λ cDNA libraries (called II, III, IV, and V) which were screened with probes from the furthest 5′ ends of the clones from which the primers were obtained (Fig. 1). A total of six overlapping cDNA clones (Fig. 1, I-2 through V-1) were sequenced and found to have a continuous open reading frame of 6.3 kb encoding a protein of 243 kDa. Since there is no in-frame upstream stop codon at the 5′ end there is probably more coding sequence upstream of cDNA V-1. Therefore, in the analysis below of the amino acid sequence of BPA, the extreme amino-terminal sequence is not included.

In addition to the finding of a continuous open reading frame in phase with the previously reported frame, the identity of these overlapping clones was confirmed by Northern analysis in which all hybridized to the 9-kb BPA mRNA (Fig. 2) (8, 26).

Analysis of Deduced Amino Acid Sequence of BPA—Excluding the first 153 residues of the BPA sequence, which are predicted to form part of a non-α-helical amino-terminal domain, most of the next 1,162 residues are postulated to form a discontinuous coiled-coil α-helical rod of the general type found in paramyosin, myosin, and keratin molecules (Figs. 3 and 4). This conclusion is based on the following observations. 1) According to the methods of Chou and Fasman (17) and Robson (18) the sequence is predicted to be predominantly α-helical. In particular, 80-90% of the two major rod domains (lengths 67.6 and 62.0 nm, respectively; shaded in Figs. 3, 4), as well as the shorter rod domains 1 (14.3 nm) and 2 (14.4 nm) (Fig. 4) are predicted to be ω-helical. Each of the two long rod domains (in contrast to rod domains 1 and 2) have a highly significant periodic linear disposition of acidic and basic residues (Table I). 2) The rod domains all have a heptad substructure of the form (a-b-c-d-e-f-g)n, where a and d are usually filled by apolar residues (occupancy rate ~75%) (27) and are thus capable of forming a close packed hydrophobic core in a two-chain coiled-coil molecule. (Fig. 3 shows the heptad structure of the two major rod domains that are shaded in Fig. 4). 3) The rod segments have a high charged/apolar ratio (1.2–1.5 for all the rod domains in Fig. 4), a feature generally indicative of an elongated structure. 4) When lined up in parallel and without relative axial stagger, the BPA chains are capable of forming many ionic interactions between the e and g residues of different chains, thus stabilizing the coiled-coil rod. The major rod segments and rod 1 in Fig. 4 have about 0.35 and 0.7 interchain ionic interactions/heptad pair, respectively; these are highly significant values (27). However, since rod 2 lacks such interactions, it may have an enhanced flexibility relative to the other rod segments. Note also that two short regions of lengths 58 and 28 residues in the rod domain (shown in Fig. 4 as black bars) have secondary structures that are not yet clearly defined by current methods. However, the latter

Fig. 1. Isolated cDNA clones encoding BPA. Roman numerals indicate the library from which clones were obtained. Vertical lines indicate EcoRI restriction sites. Arrows indicate other restriction sites used to obtain probes for screening libraries: H, HindIII; A, AccI; P, PstI. Size of EcoRI fragments is indicated in kb.

Fig. 2. Northern blots of keratinocyte total RNA to show that BPA cDNA clones hybridize to the same mRNA (9 kb, arrow). cDNAs (as defined in Fig. 1) used as probes: lane 1, I-3; lane 2, 2-kb EcoRI fragment of I-2; lane 3, II-1; lane 4, III-1. The 0.15-kb EcoRI fragment of IV-1 also hybridized to this 9-kb RNA (data not shown).
BPI and DPI—Comparison of BPA and DPI by use of an evolutionary matrix of similarity for amino acids demonstrated remarkable homology between these proteins, especially at their carboxyl-terminal domains (Fig. 6). Searching of the NBRF protein database revealed no evolutionary homologies comparable to that seen with DPI, but did reveal slight homology of DPI to myosin (e.g., FASTA score for DPI compared to myosin was 389, whereas DPI compared to several myosins was 64-78; mean score for all sequences was 23). This minor homology to myosin, also a coiled-coil α-helical rod, was entirely contributed by the rod domain of DPI.

Like BPA, DPI has in its carboxyl domain 176 residue repeats containing internal 38 residue motifs (9). There are three of these domains in DPI, called A, B, and C (Fig. 4), and these show remarkable identity and homology (both chemically, Fig. 5, and evolutionarily, Fig. 6) to the domains in BPA. In addition, areas adjacent to these repeats (X and Y in Fig. 4) show a high degree of identity and homology. BPAs B and C domains are so-named because they show most homology to the DPI B and C domains, respectively. Analysis by fast Fourier transform indicates that these repeats in both BPA and DPI show a regular periodic charge distribution that closely approximates that exhibited by the 1B segment of the rod domain of keratin intermediate filaments (Ref. 9 and Table I), suggesting a potential means of ionic interaction between these filaments and these plaque proteins.

The rod domains of BPA and DPI also show some amino acid homology (Fig. 6) that is about the same degree as that seen between BPA and myosin, suggesting that it is due to the shared α-helical heptad substructure. As the periodicity in the acidic and basic residues in the rod domain of both BPA and DPI is similar (Table I), a potential means of self-aggregation for each of these plaque proteins through the maximization of ionic interactions becomes self-evident (9).
Bullous Pemphigoid Antigen and Desmoplakin I Homology

Charge periodicities (in amino acid residues) of bullous pemphigoid antigen (BPA) compared to desmoplakin I (DPI) and keratin

| Domains | Period | Intensity | Probability | Period | Intensity | Probability |
|---------|--------|-----------|-------------|--------|-----------|-------------|
| BPA-B   | 9.31   | 6.14      | 2.2 × 10^{-5} | 9.35   | 5.65      | 3.5 × 10^{-1} |
| BPA-C   | 3.47   | 8.90      | 1.4 × 10^{-3} | 9.50   | 5.61      | 3.7 × 10^{-1} |
| DPI-B   | 9.62   | 6.16      | 2.1 × 10^{-3} | 9.53   | 6.14      | 2.2 × 10^{-3} |
| DPI-C   | 9.44   | 8.30      | 2.5 × 10^{-4} | 9.44   | 9.61      | 6.7 × 10^{-5} |
| Keratin' (Segment 1B) | 9.48 | 9.54 | 2.2 × 10^{-8} | 10.50 | 10.83 | 2.2 × 10^{-5} |
| BPA rod (AA 318-772) | 10.16 | 7.36 | 7.0 × 10^{-10} | 10.11 | 8.28 | 2.5 × 10^{-4} |
| BPA rod (AA 888-1315) | 10.40 | 32.96 | 4.8 × 10^{-10} | 10.40 | 16.77 | 5.2 × 10^{-8} |

| AA, amino acid. | Acidic (DE)^a | Basic (KR)^a |
|-----------------|---------------|-------------|
| BPA-C           | Acidic (DE)^a | Basic (KR)^a |
| DP-C            | Acidic (DE)^a | Basic (KR)^a |
| BP-C            | Acidic (DE)^a | Basic (KR)^a |
| BPA rod (AA 318-772) | Acidic (DE)^a | Basic (KR)^a |
| Keratin' (Segment 1B) | Acidic (DE)^a | Basic (KR)^a |

TABLE II
Consensus sequence for the 38-residue motif within the B and C domains of bullous pemphigoid antigen

| No. | Consensus | % of AA that match^b | No. | Consensus | % of AA that match^b |
|-----|-----------|----------------------|-----|-----------|----------------------|
| 1   | Any AA    | 20 Apolar            | 10  | Any AA    | 20 Apolar            |
| 2   | Any AA    | 21 Apolar            | 11  | Any AA    | 20 Apolar            |
| 3   | Basic     | 60 Basic             | 12  | Basic     | 50 Apolar            |
| 4   | Apolar    | 75 Apolar            | 13  | G         | 40 Apolar            |
| 5   | S         | 60 L                  | 14  | Apolar    | 90 Apolar            |
| 6   | Apolar    | 100 E                 | 15  | Apolar    | 90 Apolar            |
| 7   | Apolar    | 50 A                  | 16  | Basic     | 60 Q                  |
| 8   | Apolar    | 30 T                  | 17  | Basic     | 35 D                  |
| 9   | Apolar    | 90 Apolar             | 18  | Basic     | 35 D                  |
| 10  | Apolar    | 90 Apolar             | 19  | Apolar    | 37 Apolar            |
| 11  | Any AA    | 30 T                  | 20  | Any AA    | 37 Apolar            |
| 12  | Basic     | 50 G                  | 21  | Apolar    | 37 Apolar            |
| 13  | G         | 40 Apolar             | 22  | Basic     | 35 D                  |
| 14  | Apolar    | 90 Apolar             | 23  | Basic     | 35 D                  |
| 15  | Apolar    | 100 Apolar            | 24  | Basic     | 35 D                  |
| 16  | D         | 60                    | 25  | Apolar    | 37 Apolar            |
| 17  | Basic     | 40                     | 26  | Apolar    | 37 Apolar            |
| 18  | Basic     | 35                     | 27  | Apolar    | 37 Apolar            |
| 19  | Apolar    | 60                     | 28  | Apolar    | 37 Apolar            |

^a AA, amino acid. (Single-letter AA codes used.)

It is important to note that sequencing data in this paper shows a difference in the 3' 131 nucleotides that we previously reported (8). Our most recent sequence of the 3' end agrees with the sequence of the mouse BPA cDNA reported by Amagai et al. (26). Whether our previous 3' sequence represents alternative splicing or a mistake in cloning is not yet clear.

The structural and sequence homology of BPA and DPI is remarkable. Both have a predicted rod domain with similar charge periodicities. Also, in their carboxyl-terminal domains there is a high degree of identity and homology of the amino acid sequences, including the organization of 176 residue blocks of repeating amino acids. Even more striking is the finding of almost identical charge periodicities in these domains of both of these molecules.

The homologies noted between BPA and DPI provide one possible explanation for the controversy regarding whether antibodies against DPI, which bind very well to the desmosome plaque, also bind the hemidesmosome plaque. Some
studies find slight binding to the hemidesmosome (10, 12, 29), whereas others do not find any binding (30). Since these proteins share significant structural and sequence homology, it is possible that some antibodies raised against DPI might cross-react, to some degree, with BPA. Antibodies raised to other components of the desmosome do not bind the hemidesmosome plaque (29).

The antibody data and the cloning data suggest that the plaque of the desmosome and hemidesmosome, although similar by ultrastructure, are biochemically distinct. However, this study confirms that DPI of the desmosome plaque and BPA of the hemidesmosome plaque are closely related proteins and can be considered the first members of a new family of adhesion junction proteins. The secondary and tertiary conformational predictions discussed here provide a framework for understanding the function of these plaque proteins, and, more importantly, suggest a basis for planning physico-chemical and biochemical studies of BPA and DPI structure, mechanisms of self-aggregation, and interactions with intermediate filaments.

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