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# Dermal glands of *Xenopus laevis* contain a polypeptide with a highly repetitive amino acid sequence

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Mature dermal glands of *Xenopus laevis* contain storage granules with a characteristic ellipsoid shape. These granules contain, as a minor component, a heat-stable, acidic polypeptide with an apparent molecular mass of 75 kDa. Using antibodies against this protein, positive clones were isolated from a cDNA expression library prepared from skin of *X. leaevis*. One of the cloned cDNAs encodes a pre-protein with a typical signal sequence and a mature part of 396 amino acids. The protein contains 33 copies of the sequence Gly-Gly/Glu-(Ala-Pro)$_{2-4}$-Ala-Glu. Using the single-letter code for the four predominant amino acids, we have termed this polypeptide the APEG protein. Near its carboxy-terminus, one segment has been found with an amino acid sequence similar to that of spasmolytic polypeptide from porcine pancreas and to the human protein pS2.

cDNA cloning; Nucleotide sequence; Amino acid sequence; Amphibian skin; Secretory gland

## 1. INTRODUCTION

Dermal glands of many amphibians are a rich source of biologically active peptides [1,2]. In the case of *Xenopus laevis*, detailed studies have shown that its skin secretion contains at least two dozen peptides with diverse properties [3–5]. Moreover, from the sequence of cloned cDNAs, the structure of several precursors has been established, from which the final products are derived by the consecutive action of processing enzymes [6–8]. This complex mixture of peptides, together with biogenic amines and the processing enzymes [9,10], are stored in dermal glands which release their content upon adrenergic stimulation. After depletion of the skin, these glands regenerate within about three weeks. Mature glands are composed of a single syncytial compartment filled with ellipsoid storage granules [11,12]. Electron micrographs of isolated granules from skin secretions of *X. laevis* have shown that these contain an ordered scaffold structure [12] to which the other constituents may be bound. These granules lyse easily in distilled water, but it is at present not clear whether they are surrounded by a phospholipid bilayer. After separation of the protein constituents of these granules by SDS-PAGE, three major and some minor proteins can be detected [12]. Of these putative scaffold proteins, one representative of a small group of proteins with apparent molecular masses of 26–28 kDa has recently been investigated by cDNA cloning [13]. In the course of the purification of these heat-stable, basic proteins, another protein was isolated, which was also stable to boiling. As an amino acid analysis of the purified protein indicated a very unusual composition, we wanted to establish its structure via cDNA cloning.

Here we present the amino acid sequence deduced from two cloned cDNAs of a protein with an apparent molecular mass of about 75 kDa. This unusual polypeptide contains many repeats of the type G-G/E-(A-P)$_n$-A-E, with $n$ being 2, 3 or 4. As this protein is composed mostly of alanine, proline, glutamic acid and glycine, we propose to call it the APEG protein, using the single-letter code for these amino acids.

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*Abbreviation:* SDS-PAGE, SDS-polyacrylamide gel electrophoresis

The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number no.Y07528

## 2. EXPERIMENTAL

### 2.1. *Isolation of proteins from skin secretion*

Storage granules were isolated from skin secretions of *X. laevis* as described previously [13]. These were lysed with distilled water and the solution was heated to 95°C for several minutes. Insoluble material was then removed by centrifugation and the supernatant was passed through a CM-Sepharose (Pharmacia) column. Proteins not retained by this column were collected and fractionated twice by HPLC over a TSK 250 column to remove low-molecular-weight compounds. This yielded a preparation, which upon SDS-PAGE on a 12% gel, gave a single band with an apparent molecular mass of about 75 kDa (data not shown).

### 2.2. *Protein characterization and preparation of antibodies*

A sample of the purified protein was hydrolyzed in 6 N HCl at

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105°C for 15 h and used for amino acid analysis. Polyclonal antibodies against the 75 kDa protein were obtained by injecting two rabbits with the protein in complete Freunds adjuvant.

2.3. *Cloning procedures*

Using total mRNA prepared from dorsal skin of *X. laevis* [14], a cDNA expression library was constructed [15,16] and screened with a polyclonal antibody as described by Young and Davis [17]. From immunopositive phages, DNA was isolated, digested with *Eco*RI and subcloned into Bluescript vectors using standard procedures [18]. Finally, DNA inserts were sequenced using the enzymatic [19] as well as the chemical degradation method [20].

For Northern blots, 15 $\mu$g of total skin RNA were separated in a 1% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred to nitrocellulose sheets and hybridized with the randomly primed APEG-cDNA insert [21].

## 3. RESULTS AND DISCUSSION

The storage granules present in skin glands of *X. laevis* contain three major and a few minor proteins. Of these, a family of basic proteins with a molecular mass of 26–28 kDa and one acidic polypeptide with an apparent molecular mass of about 75 kDa were found to be heat stable. The 75-kDa protein could be purified by a three-step procedure (see section 2). After total hydrolysis, amino acid analysis of this protein has shown that at least 80% of its mass were alanine, proline, glutamic acid and glycine in an approximate ratio of 2:2:1:1, while all other amino acids were present in much lower amounts (table 1). Using the one-letter abbreviations for these four amino acids, we have thus called it the APEG protein.

A cDNA expression library in λgt11 was prepared from dorsal skin of *X. laevis* and screened with a polyclonal antibody directed against the APEG protein. Of 11 positive clones, two were sequenced on both strands. The sequence of the clone with the longest insert is shown in fig.1. This clone has a single open reading frame coding for a polypeptide which comprises 416 amino acids. At the 3′-end there is a poly(A) tail preceded by an AATAAA polyadenylation signal. The second clone had an insert about 400 nucleotides shorter at the 5′-end. Its sequence was identical except for two extra bases immediately preceding the poly(A) tail.

Table 1

Amino acid composition of the APEG protein

| | Analysis | cDNA sequence |
|---|---|---|
| Asp + Asn | 7 | 7 |
| Thr | 8 | 5 |
| Ser | 6 | 5 |
| Glu + Gln | 55 | 61 |
| Pro | ~100 | 99 |
| Gly | 49 | 51 |
| Ala | >100 | 130 |
| Cys | 7 | 6 |
| Val | 8 | 10 |
| Met | 0 | 0 |
| Leu | 1 | 0 |
| Ile | 3 | 3 |
| Leu | 2 | 2 |
| Tyr | 3 | 2 |
| Phe | 5 | 4 |
| His | 3 | 0 |
| Lys | 7 | 6 |
| Arg | 3 | 3 |
| Trp | – | 2 |

The predicted polypeptide starts with a typical signal sequence from which the initiating methionine and probably only a few amino acids are missing. The mature protein most likely starts with the glutamine residue after the arrow in fig.1. The sequence of the predicted polypeptide then contains 33 copies of the peptide Gly-Gly/Glu-(Ala-Pro)$_{2-4}$-Ala-Glu. Six of these copies differ by single amino acid replacements from the standard sequence. The repeats are numbered in fig.1. It is noteworthy that this repetitive part has the unusual amino acid composition of Ala-124, Pro-92, Glu-54, Gly-43, Val-3, Asp-1, Ser-1 and a net charge of −55. On the contrary, the 75 residues of the carboxy-terminal region of this polypeptide have an average amino acid composition and a positive net charge. With the exception of histidine and threonine, the amino acid composition determined for the purified protein agrees quite well with the one deduced from the cloned cDNA (see table 1).

The predicted amino acid sequence of the carboxy-terminal region reveals similarity to sequences found in another precursor from the skin of *X. laevis* [22], as well as to spasmolytic polypeptide from porcine pancreas [23] and to protein pS2 secreted by human breast cancer cells as well as by normal stomach mucosa [24–26]. The protein from porcine pancreas inhibits gastric secretion and gastrointestinal mobility and apparently also has growth factor activity [26–28]. The sequence homology is shown in fig.2. The spacing of the six cysteines as well as several other amino acids are highly conserved between this predicted skin peptide and the other sequences mentioned above (fig.2). The APEG protein also contains a single Lys-Arg sequence, but it is currently not known whether cleavage at this site is taking place in dermal glands.

The mature APEG protein comprises 396 amino acids and has a calculated molecular mass of 39200. This is much lower than the apparent molecular mass of 75 kDa estimated from the mobility of this protein on SDS-PAGE.

The cloned cDNA was radioactively labeled by random priming and used for a Northern blot analysis of total RNA from skin of *X. laevis*. A single mRNA containing about 1600 nucleotides could be detected (data not shown). The cloned insert of 1471 nucleotides, excluding the poly(A) tail, thus encompasses more than 90% of the mRNA for the APEG protein.

Sequences with some similarity to the repetitive part of the APEG protein have been found in other

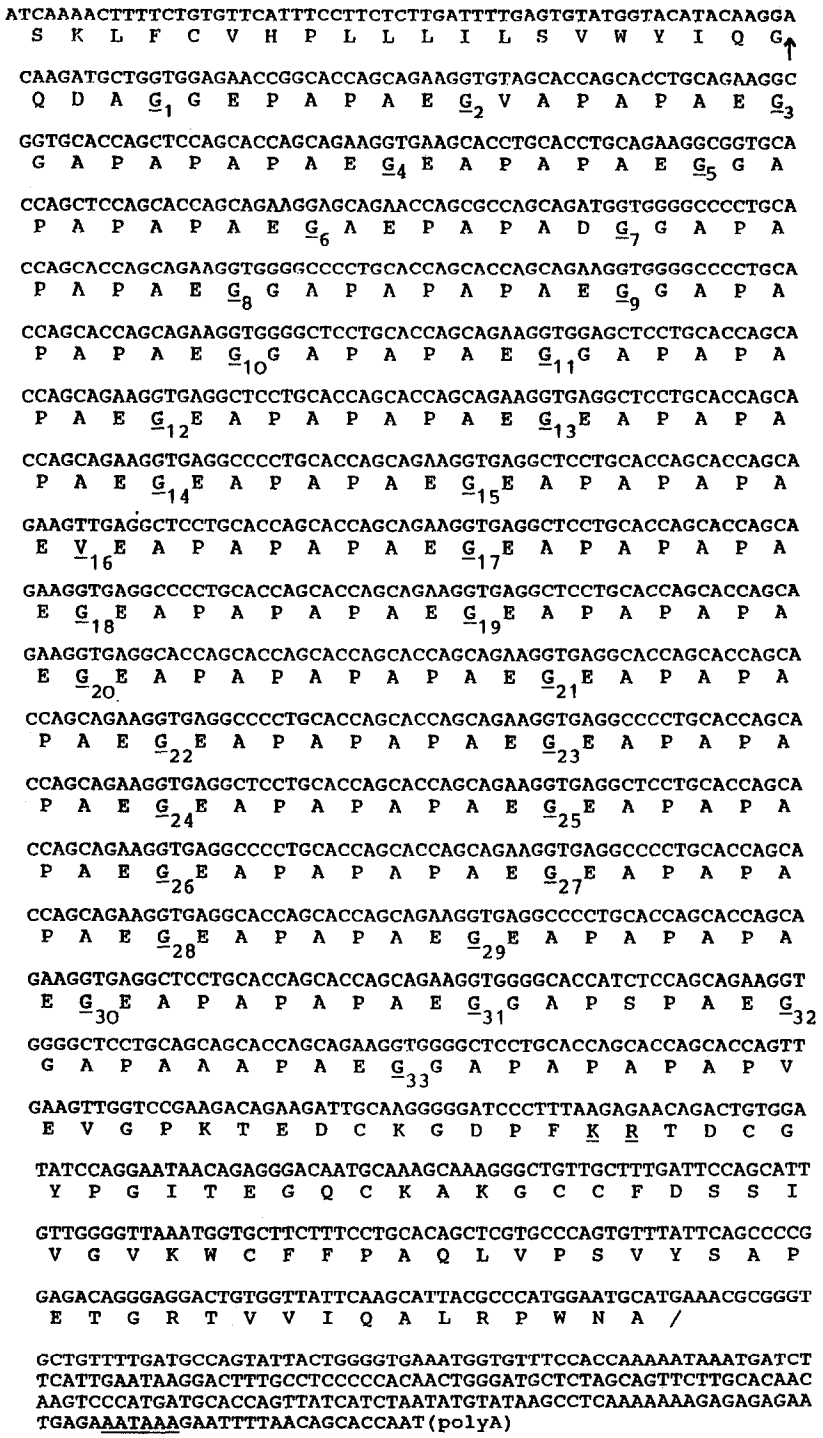


Fig.1. Nucleotide sequence of the cloned cDNA for the APEG protein. The first residues of the repeats are underlined and numbered 1–33. Also underlined are a potential Lys-Arg processing sequence and the poly-adenylation signal close to the 3'-end. The arrow marks the most likely cleavage site of signal peptidase [38].

polypeptides as well. Such regions rich in proline, alanine and/or negatively charged amino acids sometimes form exposed hinges between globular units of complex polypeptides [29]. Moreover, in surface proteins of parasites like trypanosomes [30] or *Plasmodium* strains [31], related repetitive sequences were found. It is thus possible that the amino-terminal part of the APEG protein assumes an extended structure as would be expected for a scaffold protein. Conversely, this frog skin protein also shares some properties with chromogranins/secretogranins isolated from granula of adrenal medulla and other secretory

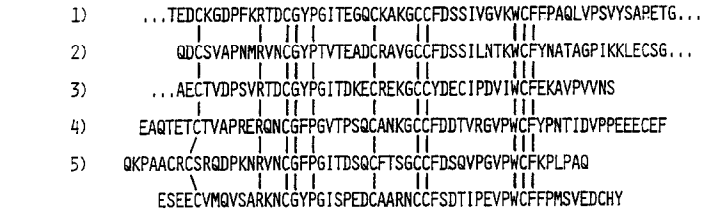


Fig.2. Comparison of fragments from *X. laevis* skin polypeptides with human pS2 and spasmolytic polypeptide from porcine pancreas. (1) Fragment of APEG protein. (2) Amino-terminal sequence of a repetitive protein from *X. laevis* skin, predicted from a cloned cDNA (taken from [22]). (3) Carboxy-terminal sequence of same protein. (4) Human pS2 polypeptide [24,25]. (5) Porcine pancreatic spasmolytic polypeptide [23,28]. This sequence is written in two lines to illustrate the internal homology. Vertical lines indicate six cysteines and other conserved amino acids. Continuation of sequences is indicated by ...

cells [32–34]. It has been proposed that these proteins may: (i) be involved in directing peptides to the regulated pathway of secretion; (ii) play a role in the organization of the granule matrix; or (iii) serve as precursors from which peptide hormones and neuropeptides are derived [35]. Like the APEG protein, the chromogranins/secretogranins are heat-stable, highly acidic proteins. They can be isolated as intact proteins, yet current evidence suggests that partial processing of chromogranins is taking place in vivo with different rates in different tissues [36,37]. The APEG protein may thus be a functional analogue of the chromogranins/secretogranins and it will be interesting to test whether homologous proteins are present in other amphibia and possibly mammals as well.

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