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The constituents of storage granules in the dermal glands of *Xenopus laevis*

Structure of a basic polypeptide deduced from cloned cDNA

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Mature dermal glands of *Xenopus laevis* contain storage granules with a characteristic ellipsoid shape. A few major proteins are present in these granules, including two with the same amino-terminal sequence and apparent molecular masses of 26 and 28 kDa. Using antibodies against these proteins, positive clones were isolated from a cDNA expression library prepared from skin of *X. laevis*. One cDNA encodes a preprotein with a typical signal sequence and a mature part of 187 amino acids. The protein shows internal homology at both the amino and carboxyl end. The latter part has a very high content of basic amino acids.

cDNA cloning; Nucleotide sequence; Amino acid sequence; (Amphibian skin, Secretory gland)

1. INTRODUCTION

The skin of numerous amphibians contains special storage glands that are rich in peptides and biogenic amines [1-3]. Many of these peptides are homologous or even identical to mammalian hormones or neurotransmitters. The content of these skin glands is released upon stress in an apparently defensive reaction [4]. Peptides with bactericidal and hemolytic activity have also been detected in frog skin secretions [5,6].

The skin glands of *Xenopus laevis* have been studied in some detail [7-9]. These undergo characteristic changes during development and show the ability to regenerate, within about 3 weeks, after depletion, e.g. by electric shock. Regeneration is marked by an accumulation of secretory cells which subsequently fuse to form a syncytial compartment filled with ellipsoid granules [9]. Interestingly, these granules appear to be devoid of a phospholipid bilayer even though phospholipids and lysophospholipids can be detected [10]. Electron micrographs of isolated granules reveal an ordered scaffold structure [9], to which the peptides and biogenic amines may be bound. SDS-PAGE has demonstrated the presence of only a few major polypeptides in these granules, including two with apparent molecular masses of 26 and 28 kDa [10]. Here, we present the amino acid sequence of one of these proteins as deduced from a cloned cDNA.

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Abbrevation: SDS-PAGE, SDS-polyacrylamide gel electrophoresis

The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y07507

2. EXPERIMENTAL

2.1. *Protein purification from skin secretion*

X. laevis (Herpetologisches Institut DeRover, The Netherlands) were kept in aerated tanks and fed with chopped porcine liver. At intervals ranging from one to several weeks, the animals received a mild electric shock and skin secretion was collected in 0.9% NaCl as described [11]. The granule fraction was separated from the soluble part by centrifugation at 6000 × *g* or standing overnight. Granules were lysed with distilled water and the solution was then heated to 95°C for 5 min. After centrifugation, the supernatant was applied to a CM-

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Sepharose column (Pharmacia) and proteins were eluted with a gradient of 0–1 M NaCl in 50 mM ammonium acetate at pH 6.8. The 26 and 28 kDa proteins eluted at about 0.5 M NaCl from this column. This fraction was dialyzed against distilled water, lyophilized and then used for the subsequent experiments.

2.2. *Protein characterization and preparation of antibodies*

The protein fractions were analyzed by SDS-PAGE using a 12% gel. For analysis of the amino-terminal sequences in a gas phase sequencer, individual bands were eluted after SDS-PAGE. Polyclonal antibodies were obtained by injecting two rabbits with the protein fraction (fig.2C) in complete Freunds adjuvant.

2.3. *Cloning procedures*

Total mRNA was prepared from dorsal skin of *X. laevis* as in [12]. Double-stranded cDNA was prepared as described by Gubler and Hoffman [13] and ligated with phage λgt11 arms (Stratagene) via *Eco*RI linkers [14]. The cDNA expression library was screened with a polyclonal antibody following the procedure of Young and Davis [15]. Immunopositive phages were purified, phage DNA was isolated, digested with *Eco*RI, and subcloned into Bluescript vectors by standard procedures [16]. The positive clone with the largest insert was sequenced using both the enzymatic [17] and the chemical degradation method [18]. For the former, a Sequenase kit (US Biochemicals) was used.

3. RESULTS AND DISCUSSION

The synaptical compartment of the skin glands is filled with ellipsoid storage granules. A picture of these granules is shown in fig.1. SDS-PAGE of the proteins present in these granules yields the pattern shown in fig.2A. The 26 and 28 kDa proteins are marked by dots. The relative amounts of these polypeptides change during regeneration [9]. It is not known whether these proteins are encoded by separate genes or else arise by post-translational modifications. Using non-equilibrium pH gradient electrophoresis [19] it could be demonstrated that these proteins have isoelectric points above pH 8 (not shown). Both are heat-stable and elute at the same NaCl concentration from a CM-Sepharose column (see fig.2B,C).

After separation by SDS-PAGE, the 26 and 28 kDa proteins were eluted and their amino-terminal sequence was determined in a gas-phase protein sequencer. In both cases, this sequence was found to be Leu-Gln-Thr-Val-Thr-X-Phe-Arg. In addition, for the smaller of the two, residues 9–14 were identified as Thr-Gly-Leu-Lys-Pro-Ile.

A cDNA expression library in λgt11 was prepared from skin of *X. laevis* and screened with a polyclonal antibody directed against the 26 and 28 kDa proteins. Out of eight positive clones, the phage with the largest insert was investigated further. The nucleotide sequence of this cloned cDNA is shown in fig.3. It comprises 1074 bases and part of the poly(A) tail. This tail is preceded by two AATAAA polyadenylation consensus sequences. The cDNA contains a single open reading frame with an ATG initiation codon close to the 5′-end. The predicted polypeptide starts with a typical signal sequence of 26 amino acids, followed by the Leu-Gln-Thr-Val-Thr... sequence corresponding to the amino-terminal sequence of both the 26 and 28 kDa proteins. As deduced from this cDNA sequence, the mature protein comprises 187 amino acids, of which Gly, Lys, Thr and His are the most abundant, while Cys and Tyr are missing. The

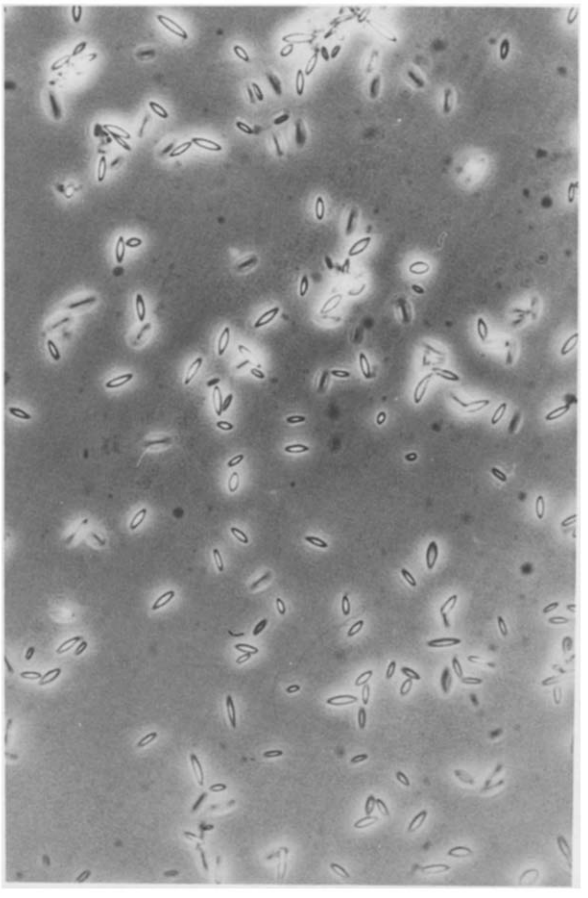

Fig.1. Microscopic picture of storage granules isolated from skin secretion of *X. laevis*. The long axis of the granules measures 1 μm to a few micrometers.

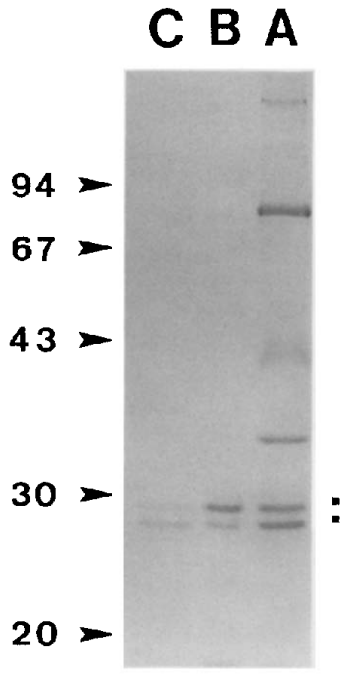

Fig.2. Purification of the 26 and 28 kDa proteins from skin secretion. Proteins were separated by SDS-PAGE in 12% gels. Lanes: (A) total skin secretion, (B) heat-stable fraction, (C) fraction eluting at about 0.5 M NaCl from CM-Sepharose (for details see section 2). On the left side molecular mass markers (in kDa) are indicated. The 26 and 28 kDa proteins are marked by dots.

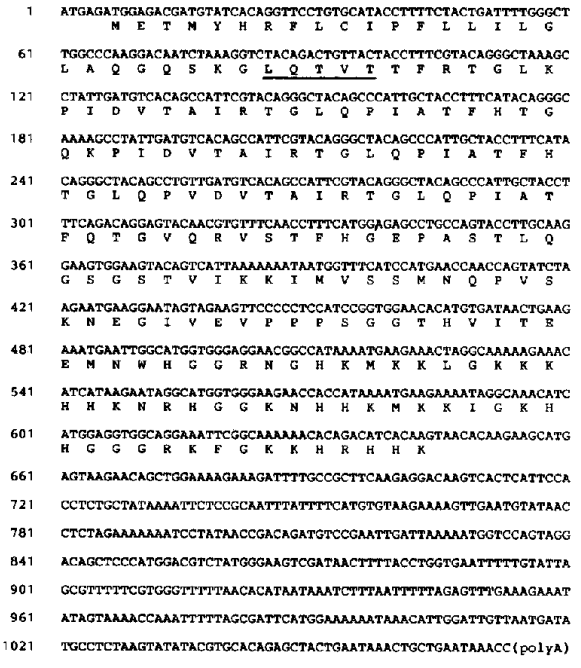

Fig.3. Nucleotide and predicted amino acid sequence of a cDNA clone. The amino-terminal sequence determined for the mature 26 and 28 kDa proteins is underlined.

calculated molecular mass is 20411 Da. The amino acid sequence of this protein has some unusual features (see fig.4). At the amino end, three complete and one incomplete copy of a 22 amino acid repeat are present and a second region showing some internal homology is found in the carboxy-terminal part. Moreover, this region is extremely basic with 16 lysines, 12 histidines and 4 arginines being present among the last 50 residues of the polypeptide chain. This highly charged region may also be the cause of the relatively low mobility upon SDS-PAGE. The amino acid sequence of this protein shows no homology to any known protein.

These data indicate that the cloned cDNA contains the genetic information for the precursor of the 26 kDa and/or 28 kDa protein. As mentioned earlier, we do not know whether these two proteins differ in amino acid sequence or only in the extent of secondary modifications. These putative modifications cannot involve *N*-linked oligosaccharides as no Asn-X-Ser/Thr sequence is present. The protein is, however, rich in serine and threonine and may thus contain *O*-linked carbohydrates.

The function of this group of basic proteins isolated from storage granules present in the skin secretion of *X. laevis* is currently known. In different mammalian cells, it has been demonstrated that peptide hormones, biogenic amines, etc. are routed into the regulated pathway of secretion

Fig.4. Internal homologies detected in the amino acid sequence of the 26/28 kDa protein. Homologous regions in the amino- and carboxy-terminal parts are underlined.

[20–22]. After sorting in the trans Golgi cisternae, acidification occurs which initiates pro-hormone processing. Ultimately, mature dense-core secretory granules are formed which release their content only upon suitable external stimuli. In skin glands, the 26/28 kDa proteins may be part of a protein scaffold analogous to structures present in dense-core granules of specialized secretory cells. Alternatively, they may be involved in directing the peptides and biogenic amines stored in these glands into the regulated pathway of secretion. In this context, it is noteworthy that a family of proteins with an apparent molecular mass of about 25 kDa has recently been isolated from dog pancreas [23]. These proteins bind to different hormones but not to proteins secreted via the constitutive pathway, such as serum albumin or immunoglobulin. The protein we have sequenced may thus be an amphibian homologue of the 'hormone binding proteins' from mammalian pancreas [23]. For any of these roles, the histidine-rich region present near the carboxyl-end of the predicted protein may be important, since on acidification below pH 6, up to a dozen positive charges would be added.

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