ISOLATION AND CHARACTERIZATION OF HYOSOPHORIN FROM *BUFO JAPONICUS*

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With the aim to isolate cortical alveolar-derived carbohydrate rich glycopolyprotein (hyosophorin), we applied the isolation procedures established for fish eggs to *Bufo japonicus* eggs and carried out structural determination of a glycoprotein thus obtained. In brief, after removal of jelly from the fertilized eggs of *B. japonicus*, the homogenate was treated with phenol. Following dialysis of the aqueous phase, it was chromatographed on a DEAE-Sephadex A-25 column. When the fraction eluted at ~0.15 *M* NaCl was then subjected to Sephacryl S-200 chromatography, we obtained a glycoprotein which eluted at position slightly after breakthrough. Interestingly, this glycoprotein was found to have amino acid and carbohydrate compositions closely similar to those of hyosophorin of *Oryzias latipes*. Sequence analysis of the core protein of the *Bufo* glycoprotein revealed that it comprises tandem repeats of nonapeptide exactly identical with apo-H-hyosophorin of *O. latipes*. Furthermore, methylation analysis of the huge carbohydrate chains showed the presence of a number of partially methylated alditol acetates which mostly share with those formed upon methylation analysis of the *O. latipes* hyosophorin, strongly indicating that the glycan units of the *Bufo* hyosophorin has a *N*-linked bulky pentaantennary structure. The possible physiological significance of the present findings of closely similar hyosophorin molecules in toad and medaka is considered in the light of their unique structural features.

VITELLIN COAT LYSINS FROM *Mytilus edulis* SPERM.

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The acrosomal proteins obtained from *Mytilus edulis* were separated by a reverse phase HPLC. They were separated into 11 peaks and 3 of them (M3, M6, M7) showed strong vitellin coat lysin activity. The amino acid sequences of these proteins were determined by peptide sequence analyses. The sequence of M7 was confirmed by cDNA sequence analysis and revealed to have a signal peptide of 38 residues. M6 and M7 were composed of 180 amino acid residues and sequences were 76 % identical. On the other hand, M3 was composed of 148 residues and the sequence was different from M6 or M7. No sequence homology with lysins of abalone and *Tegula* was observed. Although the sequence of M3 was different from those of M6 and M7, all three proteins have a typical C-type lectin structure. No lectin activity was observed, but they were coprecipitate with isolated egg membrane. *Mytilus* lysins, M3, M6 and M7 are supposed to recognize the carbohydrate moieties of proteins involved in vitellin coat membrane and bind them and destroy the the structure of membrane. These processes can explain that stoichiometrical amount of lysin is necessary to destroy membrane.

IMMUNOELECTRON MICROSCOPE OBSERVATIONS ON THE FORMATION AND DEVELOPMENT OF CORTICAL GRANULES IN XENOPUS LAEVIS OOCYTES.

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The origin and development of cortical granules were observed by treating sections of Xenopus laevis oocytes with a rabbit antiserum against cortical granule lectins and with a gold-conjugated goat antiserum against rabbit IgG. In stage I oocytes, gold particles were present on small numbers of cortical granules of 200-600 nm size. In stage II and III oocytes, they appeared on granules in the Golgi complexes as well as on large numbers of cortical granules, ranging in size from 200 nm to 1.4 μm, in the cortical cytoplasm. Some of these cortical granules showed an irregular shape, indicating fusion of small granules into a large one. Gold-labeled granules disappeared from the Golgi complexes at stage IV. Cortical granules in stage V oocytes consisted exclusively of large granules and they were alined beneath the oolemma at stage VI. These results suggest cortical granules form in the Golgi complexes of stage I to III oocytes and coalesce in the cortical cytoplasm of stage II to IV oocytes.

SPECIES-SPECIFIC SEQUENCES OF TEGULA VITELLINE COAT LYSINS.

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The vitelline coat lysin (VCL) of Tegula, a marine Mollusca genus, is released from acrosomal vesicles of the sperm during acrosome reaction and can lyze the vitelline coats of only the same species. Thus, the lysin action is extremely species-specific.

cDNA libraries were constructed from the testicular poly(A) RNAs of Tegula rustica (Owase) and T. lischkei with lambda gt10 phage as a vector. A clone bearing the mRNA sequence for each VCL was isolated by screening the corresponding libraries with a 5'-terminal region; 400 bp (ORF-370) was obtained from the cDNA clone for VCL of T. pfeifferi. The cDNAs of T. rustica (Owase) and T. lischkei contained an open reading frame encoding 162 and 173 amino acid residues, respectively, and indicated the occurrence of 22 residues of signal sequence at the amino terminal region of the nascent peptide. Both deduced amino- and carboxyl-proximal domains were virtually identical to those determined for the VCL of T. pfeifferi. However, the deduced sequences at the central domain (position 77-92) were different among the three species. This variable domain may account for the species specificity of the lysin.