Protein and lipid composition of lipoprotein fractions of A-positive pig serum. A activity of lipids and nonlipids prepared from those lipoprotein fractions

Fraction	Protein (mg/100 ml original serum)	Lipids	Protein (% of the respective lipoprotein class)	Approximate A activity in lipids in nonlipids (% of original serum)	
A-positive serum	5,120	296.5	—	20	80
VLDL	4.6	41.5	10	5	0
LDL	53.0	169.0	24	5	0
HDL	75.8	80.0	49	10	0
Lipoprotein-free residue	4,871	—	—	—	80

in the lipid-free protein. No J activity was found in the apoproteins of the lipoprotein fractions.

A similar study was carried out on A active pig serum. Among 23 miniature pigs (Göttingen bred), 16 (i.e. 70%) turned out to have A positive serum as examined by agglutination-inhibition tests in the homologous A system. All of these A positive animals carried the A substance in the total lipid fraction of serum, while the lipid-free residue precipitated by the lipid extraction procedure was A active in 2 animals only. Among 6 A postive pigs, 5 animals carried also the A substance on their erythrocyte membranes. This cellular A activity was found in the total lipids extracted from the stroma, while the nonlipid residue was highly A active in one case only; weak activities were observed in the remaining 4 cases.

The distribution of A activity on various fractions of serum was studied in a pig whose serum was A active in both the total lipids and the nonlipid residue obtained after lipid extraction of serum. Semiquantitative assays of A activity were performed by agglutination-inhibition tests and calculated on the basis of the volume of original serum from which the respective sample was derived. Compared with the quantitative hemolysis-inhibition tests of bovine J activity described eralier[11], the agglutination-inhibition tests of porcine A activity gives relatively rough results. We found about 20% of the original total serum A activity to be present in the total lipids, and 80% in the non-lipid fraction.

The serum was fractionated by ultracentrifugal flotation at different densities as described previously[10] for bovine serum. 4 fractions were obtained by this procedure: VLDL, LDL, HDL, and lipoprotein-free residue. These fractions were characterized by their protein and lipid contents. The values shown in the Table are in agreement with the data obtained in pig serum by JANADO et al.[12] Lipids were extracted from all fractions obtained. Lipid and non-lipid fractions were checked for Ac activity. As shown in the Table, no A activity was detected in the apoproteins of any lipoprotein class, thus being in agreement with the distribution of bovine J activity. In contrast to bovine J activity, the major lipidic A activity (roughly 50%) is carried with the HDL class of serum, while the VLDL and LDL classes contain roughly 25% each.

The above results show that the distribution of procine A activity on lipid and nonlipid fractions of serum and erythrocyte membranes, and that of the lipidic A activity on various serum lipoprotein classes, is in sharp contrast to the distribution of bovine J activity on the respective fractions.

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Preparation of Specific Antiserum against *Rana esculenta* pre-α Lens Crystallin

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Summary. Specific antiserum against *Rana esculenta* lens pre-α crystallin was prepared in a rabbit by injecting antigen-antibody precipitate of this crystallin obtained from immunoelectrophoresis of *esculenta* total soluble lens proteins against homologous antiserum.

Major heterogeneous soluble crystallins from vertebrate lens are now classified into α-, β-, γ-, and δ-crystallins; VAN DAM[2] observed in bovine lens another crystallin fraction, which migrated faster than α-crystallin during electrophoresis but was eluted with a peak which also contained some β-, and γ-crystallins during gel filtration with Sephadex G 200. According to SWANBORN[3], this pre-α fraction is restricted to mammals only, but later this fraction was also found to be present in some anuran and urodele amphibians[4–6].

BOURS and BRAHMA[7] reported that the elution profiles of the pre-α crystallin from bovine and *Rana esculenta* lens were identical in gel permeation chromatography with Sepharose 6B. VAN DEN BROEK, LEGET and BLOEMENDAL[8] isolated pre-α crystallin from bovine lens by chromatography and preparative isoelectric-focusing. They found that at an alkaline pH this fraction had relatively high mobility and the molecular weight was about 14,500. Amino acid analysis showed no relationship with the α-crystallin, so they named this fraction as

F(ast) M(igrating) or FM-crystallin to distinguish it from another rather high molecular weight fraction in cow lens, which emerged in front of the α-crystallin from DEAE column chromatography and was also called pre-α[9].

In this communication, we used the term pre-α crystallin to designate the fraction which showed highest mobility during electrophoresis in alkaline buffer. Since nothing is known about this fraction in amphibian lens except its presence in some species, we decided to prepare antibodies against this crystallin fraction to study its ontogeny during lens development.

To prepare antibodies, we injected agar gel antigen-antibody precipitate into a rabbit. This method has been successfully used by others with different antigens[10–14].

It was not possible to determine the exact amount of antigen injected into the rabbit, but there are reports showing that a few 100 μg of antigen-antibody complex is sufficient to produce antibody[10]. It has also been shown that immunization with specific precipitate in agar gel is a reliable method for preparing strong and remarkably pure antibody[11].

4% lyophilized total soluble lens proteins from *Rana esculenta* was tested against homologous antiserum by micro-immunoelectrophoresis[15] with high resolution buffer at pH 8.9[16]. Electrophoresis was carried out at 4 °C for 90 min with a constant current of 40 mA. Immunodiffusion was continued overnight in a humid chamber at room temperature (20 ± 2 °C). After diffusion, pre-α was observed well separated from the α-crystallin at the anodic end of the gel (Figure A). Agar block containing this precipitin line was carefully cut away from the gel and thoroughly washed by repeated change of saline for several days over a Gyrotory shaker to remove non-precipitated proteins. The blocks were then brocken up in a homogenizer in 1 ml saline and emulsified with 1 ml complete Freund's adjuvant (Difco). The complex mixture was then injected intradermally at 4 different sites on the back of a young rabbit. The injection was repeated 3 times at 3 week intervals. For each injection we used 14 antigen-antibody precipitates. 14 days after the 3rd injection, the rabbit was bled from the ear vein and the serum was tested against *R. esculenta* total soluble lens protein by immunoelectrophoresis. The slides from which the antigen-antibody precipitates were used were washed, dried and stained with Coomassie Brilliant Blue R-250[17] to check contamination.

The rabbit anti pre-α antiserum showed a single precipitin line against *R. esculenta* total soluble lens protein (Figure B) and did not show any change in its mobility when compared with the pre-α from antibodies against *R. esculenta* total soluble lens proteins (Figure A). We failed to get any immunological reaction when this anti pre-α antiserum was tested against bovine lens by Ouchterlony's[18] double diffusion experiment. It thus appears that the pre-α crystallins from amphibian and bovine lens are not antigenically related.

Work is in progress on the ontogeny of the pre-α crystallin by indirect immunofluorescence staining method.

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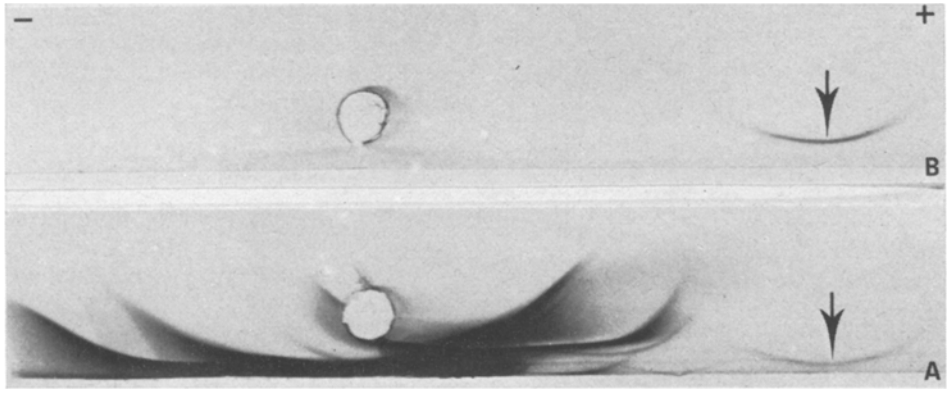

Immunoelectrophoresis of *R. esculenta* total soluble lens proteins vs. A) homologous antiserum from *R. esculenta* and B) antiserum to the antigen-antibody precipitate of the pre-α crystallin from *R. esculenta* lens. Electrophoresis was carried out for 90 min at 4 °C using 1.5% Bacto agar in high resolution buffer at pH 8.9 with a constant current of 40 mA. The slides were stained with Coomassie Brilliant Blue R-250. + = anode; − = cathode. The pre-α is indicated by the arrow. In B) only the pre-α line was visible when the antiserum was tested against *R. esculenta* total soluble lens proteins showing the specificity of the antiserum.