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Structure of acrosome reaction-inducing substance in the jelly coat of starfish eggs: A mini review ☆

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ABSTRACT

Our knowledge at present on the structure of acrosome-reaction inducing substance (ARIS) in the jelly coat of starfish eggs is summarized. ARIS ia a proteoglycan-like molecule consisting of very long, linear, and highly sulfated glycans and three ARIS proteins, ARIS1-3. Detailed structures of the major glycan of ARIS and of ARIS1-3 are discussed. 3D-models of ARIS glycans are also presented. Phylogenetic distribution of ARIS proteins and/or genes indicates that ARIS genes are well preserved from the *Ctenophore* to *Cephalochordata*. In the *Echinodermata*, ARIS1–3 and ARIS genes were detected in all classes except for sea urchins.

Fertilization is achieved through a series of sequentially programed steps such as the acquisition of sperm motility, sperm chemotaxis to the egg, the induction of acrosome reaction (AR) by egg surface components, sperm binding to and penetration through the egg coat(s), sperm–egg fusion, and egg activation to initiate the developmental program [1]. Egg surface/coat glycoconjugates serve as a key signal for the induction of AR and for sperm binding to the egg coat [1].

The AR was discovered by Dan first in starfish [2,3] and is generally perceived as an obligatory step for fertilization in most metazoans. Three different molecules in the jelly coat of eggs act in concert upon sperm for the induction of AR in starfish [3,4]. These molecules are AR-inducing substance (ARIS), a highly sulfated proteoglycan-like molecule of a molecular size over 10^4 kDa [5]; Co-ARIS, a group of steroid saponins [6]; and asterosap, a group of sperm activating peptides [7]. The AR is artificially triggered by a combination of either ARIS and Co-ARIS or ARIS and asterosap in normal seawater [3], and even solely by ARIS in high Ca^{2+} or high pH seawater, whereas it is never induced by either Co-ARIS or asterosap or any combinations of them. Thus, ARIS is the major player among the three. This paper summarizes our knowledge at present on the structure of ARIS of the starfish, *Asteria amurensis*.

1. The AR in starfish

Upon the AR, starfish sperm extrudes a process, the acrosomal process (AP), of ca. 25 μm long, which makes AR assays much easier in this animal than most systems used for fertilization studies like sea urchins, amphibians and mammals. It is known that simultaneous increases in intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) by Ca^{2+}-uptake from seawater and in intracellular pH ($[pH]_i$) trigger the AR [8] . When ARIS and asterosap bind simultaneously to their respective receptors, signal transduction cascades leading to the prerequisite situation for AR are triggered, which in turn culminates in the AR as summarized in Fig. 1. ARIS receptors are localized in a restricted area of the plasma membrane of sperm head [9,10], and asterosap receptor, namely guanylate cyclase, is distributed over the plasma membrane of sperm tail [11,12] . Co-ARIS seems to be inserted into the lipid bilayer and induce structural changes in microdomains of the plasma membrane [13].

2. ARIS glycans

Pronase digest of ARIS (P-ARIS) retained the full biological activity of ARIS and about a half amount of its proteins. However, periodate oxidation or desulfation of P-ARIS and ARIS completely destroyed the biological activity, indicating sulfated glycans are essential for the activity [8,14]. The minimum functional unit of sulfated glycan named Fragment 1 was isolated from P-ARIS,

Abbreviations: AR, acrosome reaction; ARIS, AR-inducing substance; P-ARIS, pronase digest of ARIS; AP, acrosomal process; $[Ca^{2+}]_i$, intracellular concentration of Ca^{2+}; $[pH]_i$, intracellular pH; FA5/8C, coagulation factor 5/8 type C motif.

☆ This paper is a tribute of praise to the great contribution made by Dr. William J. Lennarz to biological sciences, particularly to the glycobiology of fertilization, upon his retirement.

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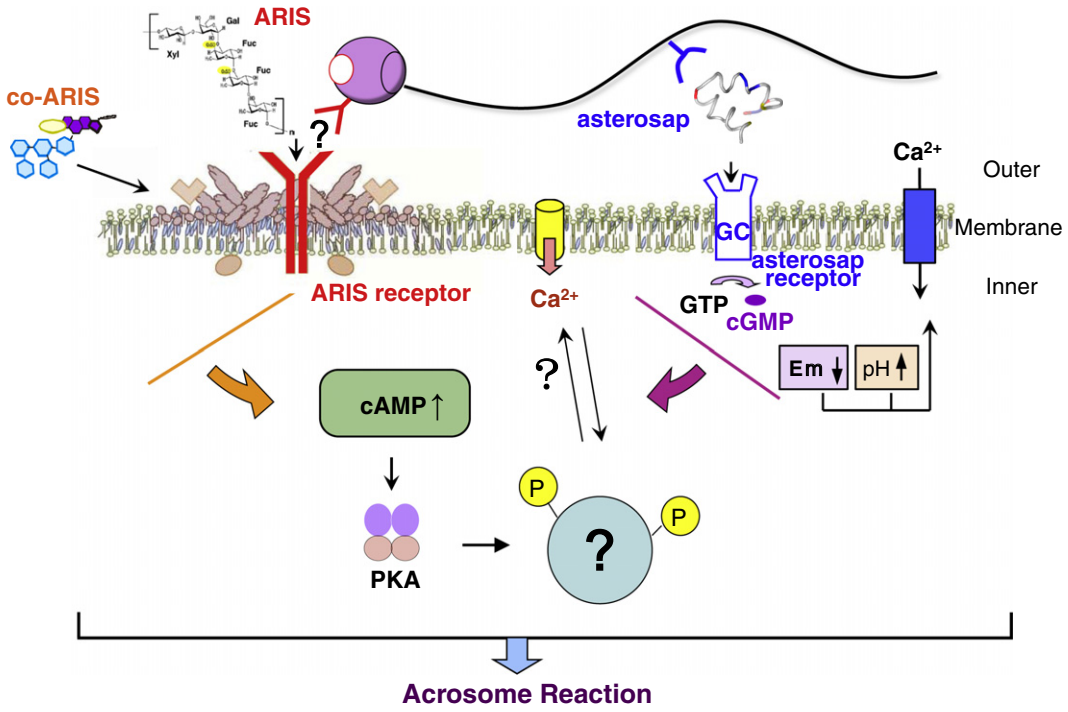

Fig. 1. Induction of the AR in starfish sperm by ARIS, Co-ARIS and asterosap. The upper drawing shows a starfish sperm having ARIS receptor in the head and asterosap receptor in the tail. The lower drawing shows changes in the sperm from simultaneous binding of ARIS and asterosap to their receptors respectively to sustained increases in $[Ca^{2+}]_i$ and in $[pH]_i$, which in turn culminates in the AR. Structural changes in microdomains of the plasma membrane by insertion of Co-ARIS into the lipid bilayer are schematically illustrated. Modified from Fig. 2 of Matsumoto et al. [4] with permission, by adding the data of Naruse et al. [13].

though the activity was much reduced. The molecular size of this glycan was about 10 kDa and did not contain amino acid residues. Its structure was revealed as ten or so repeats of the following pentasachharide unit; [→4)-β-D-Xyl*p*-(1 → 3)-α-D-Gal*p*-(1 → 3)-α-L-Fuc*p*-4(SO_3^-)-(1 → 3)-α-L-Fuc*p*4 (SO_3^-)-(1 → 4)-α-L-Fuc*p*-(1→] [14]. It is suggested that Fragment 1 is derived from a very long linear glycan, the outer layer of the major ARIS glycans, composed of hundreds of the pentasaccharide repeating units.

The second fragment isolated from ARIS was named Fragment 2 [15]. This fragment was mostly composed of sulfated glycans, but it was shown to retain about 10% (w/w) of the protein part, suggesting that its saccharide chains are located in the region of carbohydrate-protein linkage (core region). The estimated average molecular mass of Fragment 2 was over 400 kDa. Almost all of the sugar portion of Fragment 2 was released by reductive β-elimination, thus almost all, if not all, glycans of Fragment 2 are *O*-linked. Similarly, reductive β-elimination of ARIS and P-ARIS released almost all sugar portions. The *O*-glycans from Fragment 2 gave practically a single peak by gel filtration and ion-exchange chromatography, and was named Fragment 2–1, which should represent the inner layer of the major ARIS glycans. Its structure was revealed to be as follows; [→3)-Gal*p*-(1 → 3)-Fuc*p*-(1 → 3)-Gal*p*-(1 → 4)-GalNAc*p*-(1 → 4)-GlcNAc*p*-6(SO_3^-)-(1 → 6)-Gal*p*4(SO_3^-)-(1 → 4)-GalNAc*p*-(1→]$_{>100}$ [15]. The reducing terminus of Fragment 2–1 was suggested to be mostly 6-GalNAc. Thus it is concluded that the major saccharide chains of ARIS have the following structure [15].

[→4)-β-D-Xyl*p*-(1 → 3)-α-D-Gal*p*-(1 → 3)-α-L-Fuc*p*-4(SO_3^-)-(1 → 3)-α-L-Fuc*p*4(SO_3^-)-(1 → 4)-α-L-Fuc*p*-(1→]$_{m>200}$-(glycan X)-[→3)-Gal*p*-(1 → 3)-Fuc*p*-(1 → 3)-Gal*p*-(1 → 4)-GalNAc*p*-(1 → 4)-GlcNAc*p*-(SO_3^-)-(1 → 6)-Gal*p*4(SO_3^-)-(1 → 4)-GalNAc*p*-(1→]$_{n>100}$-(glycan Y)-(1 → 6) GalNAc-Ser/Thr (ARIS proteins). We do not have any information so far on the structures and sizes of glycans X and Y. Almost no branching, if any, was suggested to exist in ARIS glycans from the data of permethylation analysis. Neither phosphate nor sialic acid was detected in ARIS, indicating that the sulfate groups are solely liable to the anionic character of ARIS, which ought to be present in the sugar chains of ARIS for its biological activity. Taking all published data in our hands into account, we conclude that ARIS is a proteoglycan-like molecule to our best knowledge.

It may be worthy to note, as a matter of fact, that ARIS glycans have at least two unusual sugar structural features, namely in-chain Fuc residues and in-chain α-Gal residues. Also, besides the glycan structure above, ARIS has another acidic sugar chain composed of Man, GalNAc and GlcNAc (molar ratio; *ca.* 2:1:4) although it does not seem important for the biological activity.

3. Superstructure of ARIS glycans

Over the years, we have been attempting to elucidate the 3D-structure of ARIS glycans in order to address their remarkable roles in fertilization as described above. From the chemical structures, an extensive conformational freedom is present in ARIS glycans originated from the glycoside linkages. One of the primary questions about ARIS glycans is whether they have superstructure or not. Current modeling of both outer and inner glycans indicates that the ARIS sugar chains tend to adopt a sort of coiled structure (Fig. 2). Partly coiled structures have a dimension of 1.6 nm in head to tail in both models. The outer glycans can extend to 1.6 μm assuming a thousand polymerization of the unit. Since a typical thickness of the jelly coat is about 20–25 μm, further polymerizations of outer glycans are necessary to form the layer. Inner glycans seem to have condensed packing due to their lower pitch of coil than outer glycans. Since the predicted polymerization of the inner glycans is about 100, they only cover 0.2 μm.

Preliminary X-ray diffraction experiment on a stretched P-ARIS fraction suggested the ordered state with the fourfold symmetry.

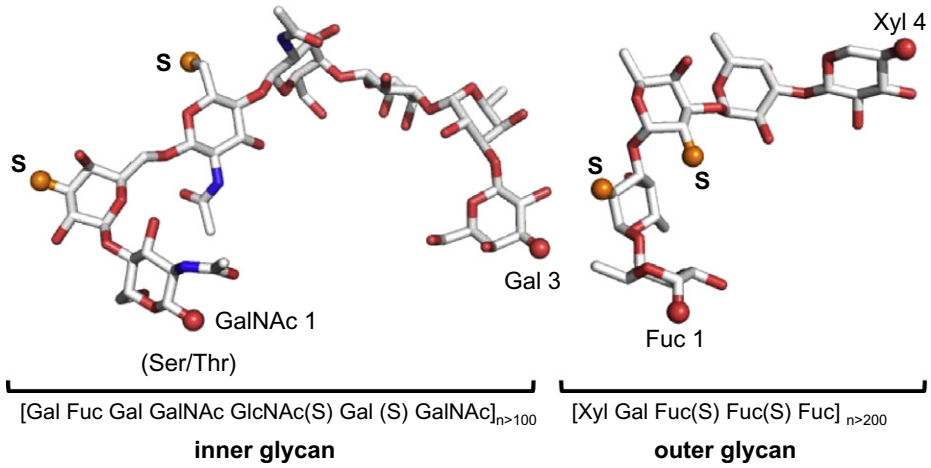

Fig. 2. Structural models of inner and outer glycan. Chemical structures of saccharides were modeled using the Sweet 2 server [16]. Stick models were colored by atoms, carbon (white), oxygen (red), nitrogen (blue). Key atoms were emphasized by small spheres, including polymerization atoms (red), and sulfate residues (orange, S).

Such ordered state was not observed with desulfated or periodate-oxidated P-ARIS which did not have any biological activity. The combination of molecular modeling and further X-ray data for a fiber ARIS glycan will illustrate the ultrastructure. Furthermore, application of neutron scattering will give better contrast in the diffraction and/or scattering of ARIS glycans.

The secondary interest on the superstructure of ARIS is the mechanisms of encapsulations, in terms of formation of and degradation of layered structure. We do not have the per se structure of ARIS yet. Even so, there are two potential formats in the structures particularly to fill the outer space of egg jelly, including the globular complex like glycogen [17] and the multiple-layered structure [18]. In both cases, behaviors of sulfate group found in the glycans might take a major responsibility in assembles.

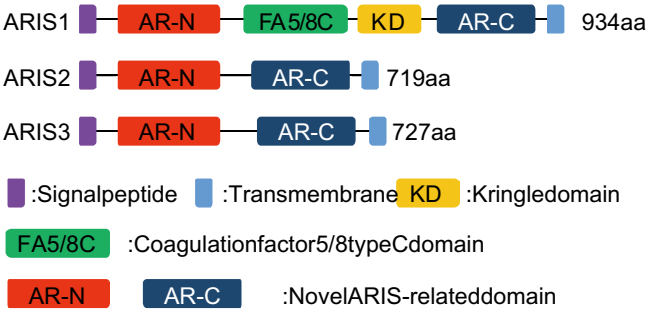

Fig. 3. Schematic diagram of the three ARIS proteins. Reproduced with permission from Fig. 2B of Naruse et al. [19]. For details, see the text.

4. ARIS proteins

It has quite recently shown that ARIS is actually composed of three distinct proteoglycan-like molecule, ARIS1–3 [19]. They are all positive to anti-Fragment 1 antiboy and seem to have very similar glycan structures. However, they are different in the structure of protein portion. The genes for ARIS1–3 proteins were cloned from *A. amurensis* ovary mRNA using RACE System. The sizes of ARIS1–3 cDNAs were 3,211 bp, 2,492 bp and 2,440 bp, respectively. All sequences of these three proteins possessed a signal peptide and C-terminal transmembrane region. The expression of ARIS proteins is restricted only to the ovary.

Psi-BLAST revealed that ARIS1 contained coagulation factor 5/8 type C motif (FA5/8C) and kringle domains. Six cysteines were found in ARIS1 as a characteristic residue in the kringle domain. ARIS2 and ARIS3 did not possess any similarity with previously classified domains. Nevertheless, all three ARIS proteins share two conserved regions named the AR-N (ARIS N-terminus) and AR-C (ARIS C-terminus) domains (Fig. 3). Particularly the serine, threonine, and cysteine residues are common in the three sequences. The highly conserved serine and threonine residues in ARIS proteins imply that these residues are target sites for *O*-glycosylation described in the Section 2. The highly conserved cysteine residues in ARIS proteins are likely to be responsible for the intra- as well as inter-molecular disulfide bonds.

FA5/8C domain is suggested to act as a binding portion on blood coagulation factors [20], and is thought to be important in endo-β-1,3-glucanase for glucan binding [21]. It is also a member of the galactose binding domain-like super family. It may be therefore not too brave to suggest a role for the FA5/8C domain of ARIS1 in its binding to either or both ARIS proteins and ARIS glycans. The presence of the kringle domain in ARIS1 also implies a role in binding of ARIS1 to another ARIS protein, because this domain in various proteins, including fibrinogen and some other members of the blood-clotting cascade, has homologous triple-loop, 3 disulfide bridges.

The sequence similarity search suggested that ARIS proteins are widely located across diverse phyla of invertebrates, from the *Ctenophora* to *Hemichordata* and *Cephalohordata*. All sequenced ARIS proteins are very similar and include both the AR-N and AR-C domains, which are likely to play an important role in fertilization. However, in the *Echinodermata*, all classes but the *Echinoidea*, namely the *Holothuroidea*, *Crinoidea* and *Ophiuroidea* besides *Asteroidea*, have ARIS proteins and/or ARIS genes. It appears that the *Echinoidea* (sea urchins) abandoned the ARIS system and adopted another protein for use as a scaffold for an AR-inducing glycans [22,23]. For this kind of discussion, more detailed studies have to be performed on the thickness and stiffness of the jelly coat, the morphology of AP, and swimming behavior of sperm.

5. Perspectives

Sixty years have passed since the discovery of the AR [2]. Yet, our knowledge on the molecular mechanism underlying the AR is quite limited, particularly the structures and their dynamic changes of signal molecules and their receptors are not well understood. ARIS is not an easy subject to study mainly because its biological activity is mostly attributable to the complicated structures

of highly sulfated glycans of ARIS. Cells recognize so subtle difference or change in glycan structures that we cannot detect. The sequencers and synthesizer are not yet available so far for glycans. However, technical and instrumental innovations have been rapidly progressing even in the field of glycobiolgy. We believe that those innovations make our conversation with cells on glycans, the third chains of life, much faster, much easier, much wider, and much deeper.

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