Galactans and Anti-Galactans from Invertebrates

G. Uhlenbruck, G. Steinhausen

Medizinische Universitätsklinik Köln, Abteilung für Immunbiologie

and

B. A. Baldo

Princess Margaret Hospital, Subiaco, University of Western Australia

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Antibody-like substances with anti-carbohydrate specificities directed against different structures of galactans have been detected in several invertebrates.

An anti-galactan from invertebrates was first detected by us in the haemolymph of *Limulus polyphemus* [1]. Subsequently, another very strong precipating anti-galactan (Tridacnin) was discovered in the haemolymph of the bivalve clam *Tridacna maxima* (Röding) [2–5]. A wide distribution of antibody-like anti-galactans amongst the invertebrates is indicated by these findings and the occurrence of anti-galactan precipitin in the marine sponge *Axinella polypoides* [6] and the coelenterate *Cerianthus membranaceus* [8]. The anti-galactosyl specificity of the agglutinins from *Axinella* has first been described by Gold *et al.* [7]. Galactans which are complementary to the natural anti-galactan reagents occur widely in micro-organisms, plants, invertebrates and vertebrates [9]. In this communication we would like to emphasize three essential points, which have to be considered, when investigating galactans and anti-galactans from invertebrates.

First it must be mentioned that the invertebrate anti-galactans demonstrate different specificities. This has already been demonstrated using three different invertebrate anti-galactan preparations [10] and can be seen from the results summarized in Table I and Fig. 1 a *. When *Lymnaea stagnalis* galactan was used in Ouchterlony gel diffusion, precipitation lines which formed with the *Cerianthus* and *Tridacna* (Tridacnin) precipitins did not show complete identity. No precipitation was observed with the *Limulus* extract (Fig. 1 a). These and other results demonstrate that the specificities of invertebrate anti-galactans may differ but that these specificities may be very similar to anti-galactans of vertebrate origin [10], for instance mouse myeloma proteins [10].

Secondly, unspecific reactions may occur in this system [1]. We found by agar-gel electrophoresis and subsequent precipitation with basic polymers (trough), that Tridacnin is an acidic molecule. Accordingly, it can give unspecific precipitin reactions with basic proteins. This is of importance, when studying the precipitating properties of Tridacnin with crude material and this may lead to false conclusions, as the precipitation lines may not be due to a galactan-anti-galactan reaction. On the other side, this observation can be used to purify Tridacnin by adsorption on basic polymers. In this regard we have found, that polylysine and polybrene behave in a similar manner. The fact that the precipitated material is identical with Tridacnin can be demonstrated by agar electrophoresis [3] and is also shown in Fig. 1 b, where different galactans from snails precipitate with *Tridacna maxima* haemolymph and fuse with the precipitation line produced by the basic protein lysozyme, indicating that acidic and specific receptors are located on the same molecule, namely Tridacnin.

Table I. Comparison of different anti-galactans and galactans from invertebrates.

Precipitin derived from	Galactans from *Helix pomatia*	*Lymnaea stagnalis*	*Helix aspersa*	Assumed specificity	Author
Tridacna maxima	+	+	+	Gal $\frac{1-6}{\beta}$ Gal	2, 3, 5
Limulus polyphemus	+	–	–	Gal $\frac{1-3}{\beta}$ Gal (?)	1
Cerianthus spec.	–	+	–	unknown	8
Axinella polypoides	+	+	+	Gal $\frac{1-6}{\beta}$ Gal	6, 7, 10

+, Precipitin reaction. –, No precipitin reaction.

Requests for reprints should be sent to Prof. Dr. G. Uhlenbruck, Abteilung für Experimentelle Innere Medizin, Kerpenerstraße 15, *D-5000 Köln 41*.

* Figs 1a–1c see Plate on page 206 a.

Finally, using "immunoelectrophoresis" with anti-galactans, one can distinguish several different forms of galactans from different invertebrate sources. This is clearly evident from Fig. 1 c, where several purified galactans behave in a very characteristic manner with respect to their electrophoretic mobility. Thus, anti-galactans may prove useful as tools in search and research of galactans and vice versa, a hitherto neglected field of carbohydrate biochemistry.

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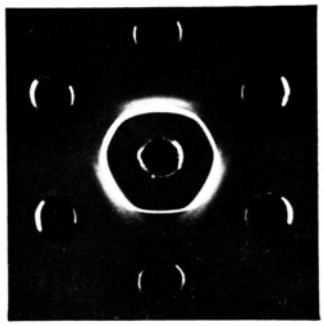
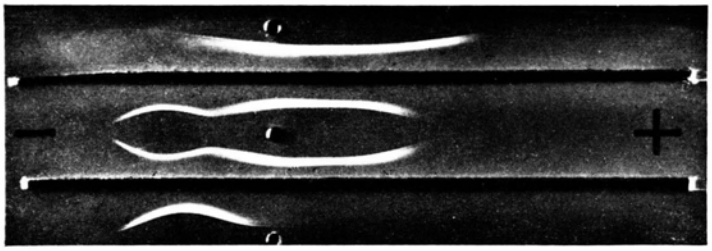
a. Agar-gel diffusion experiment with *Lymnaea stagnalis* galactan in the middle and then clockwise from the top:
1, 4, Tridacnin
2, 5, *Cerianthus spec.* extract
3, 6, the non-reactive *Limulus polyphemus* haemolymph.



b. Centre well: Tridacnin.
1, 3, 5, Lysozyme
2, *Helix pomatia* galactan
4, *Lymnaea stagnalis* galactan
6, *Achatina spec.* galactan



c. Agar-gel immunoelectrophoresis of different invertebrate galactans. Upper well: *Helix pomatia* galactan; middle well: *Achatina spec.* galactan. Lower well: *Lymnaea stagnalis* galactan; top and bottom trough: Tridacnin.

Fig. 1. Gel diffusion pictures of different galactans from invertebrates.