Schiff Base Formation: Demonstrated by a Precipitin Reaction in Agar

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High-molecular weight polyaldehydes, which can be obtained by periodate oxidation of glycosubstances, especially polysaccharides, do precipitate in a specific way by Schiff base formation, when diffusing in agar gel against high-molecular weight, naturally occurring polyamines.

Oxidation of lymphocytes from a variety of species with either sodium periodate, galactose-oxidase or neuraminidase and galactose-oxidase induces blastogenic transformation [1–5], or lymphocyte cytotoxicity [6]. In addition, tumor cell killing occurs, when by the same procedures artificial contacts are established between normal macrophages and tumor cells [7]. Although these intriguing phenomena are not yet completely elucidated, it is assumed that cross-linkages via Schiff bases play an important role [6–8]. The only evidence for this hypothesis is given by the fact, that sodium borohydride treatment abolishes the effect. It was therefore desirable, to have an *in vitro* model for the formation of Schiff bases in order to study the interaction of different membrane glycosubstances and proteins. As standard materials for developing a representative precipitin experiment we have first chosen periodate-treated arabinogalactan from plant origin [9] and polylysine as amino group donator [10].

In Fig. 1 it is demonstrated, that Schiff base formation between these two components indeed can be made visible in form of a precipitation line. In addition, biological substances with free amino groups, like histone [10], lysozyme [10] and haemoglobin [11] react in a similar way (Fig. 1 b), while a synthetic basic polymer without any amino groups, namely polybrene [10], does not precipitate as has been found by us. On the other hand, genuine arabinogalactan precipitates with some anti-galactosyl specific lectins like the one from *Viscum album* (mistletoe), *Ricinus communis* and from the bivalve clam *Tridacna maxima* (see Fig. 1 a). Also the plant lectins from *Ononis spinosa* and *Abrus precatorius* do give such a precipitin reaction. This precipitation phenomenon however is abolished, when the terminal D-galactose (β-1-6 linked) has been oxidized by sodium periodate (Fig. 1 b), whereas the precipitin reaction due to Schiff base formation does not occur with the genuine, untreated arabinogalactan (Fig. 1 a). Negative reactions are also seen after borohydride reduction or by blocking the carbonyl groups (or the amino groups) by other methods [11] *.

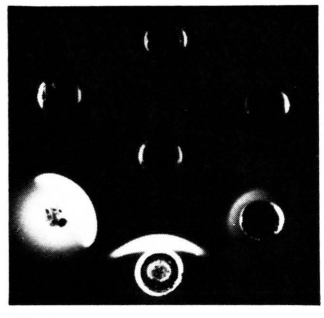

Fig. 1 a

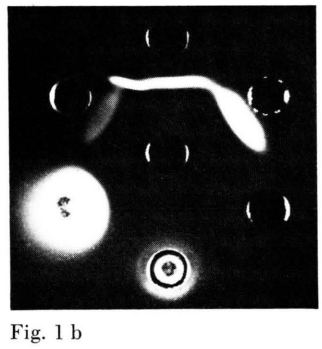

Fig. 1 b

Fig. 1. Precipitin reactions in agar gel (1%) of arabinogalactan (1 a, middle well) and periodate-treated arabinogalactan (1 b, middle well) with 5% solutions of the following pure substances (arranged clockwise from 12 o'clock on = 1): 1 = polylysine; 2 = histone; 3 = *Viscum album*; 4 = *Tridacna maxima*; 5 = *Ricinus communis*; 6 = lysozyme in Fig. 1 a, haemoglobin in Fig. 1 b. The spur formation between 6 and 1 in Fig. 1 b is due to the reaction of polylysine with the haemoglobin-(periodate-treated) arabinogalactan complex. In Fig. 1 a Schiff base formation is not possible, but the serological reactions, whereas in Fig. 1 b the reverse is the case (see text).

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* However, when the aldehyde group has been blocked by D-penicillamine, then polylysine does still react, but now because of an electrostatic interaction with the *de novo* created carboxyl group!

False or misleading results in agar gel diffusion can be obtained, if no control is made, because acid glycosubstances (membrane glycoproteins, gangliosides and sulfated galactan etc.) are also precipitated by basic polyaminoacids and polymers [10]. This exclusively electrostatic interaction is the cause for various cell aggregating properties of these basic polymers, a phenomenon, which can be partially impaired by neuraminidase treatment of the respective cell particles [10]. In contrast to polylysine, periodate-treated arabinogalactan has only a slight cell aggregating effect, which is enhanced after neuraminidase treatment of the cells (for instance erythrocytes or platelets) or by mild treatment with proteolytic enzymes, exposing Schiff base reactive amino groups [12]. Both reagents, however, do not induce mitogenic transformation in lymphocytes, probably, under the conditions used, no firm bridging of the cell surfaces occurs, because both highmolecular weight compounds may be "neutralized" by the serum proteins present in the culture.

The spur formation between 6 and 1 in Fig. 1 b is due to the reaction of polylysine with the haemoglobin-(periodate-treated)-arabinogalactan complex, which leads to a second form of precipitate, because periodate-treated arabinogalactan, bound to haemoglobin, has still enough free aldehyde groups available to react with the polylysine, a phenomenon, which can also be observed in the classical Ouchterlony test with true antigen-antibody reactions, when the free and bound antibody has receptors for "anti-antibodies", for instance a lectin or staphylococcal protein A anti-Fc reagent.

From the immunological viewpoint, our observations reveal some sources of error, as they represent "unspecific" reactions on the background of Schiff base formation. They show, that not only antigen-antibody reactions, or precipitin lines between acid and basic high molecular weight molecules [10] can be made visible in agar, but also Schiff base formation between biological substances. The same holds for the aggregation and "agglutination" results reported here, phenomena, which so far have never been taken into consideration for being relevant, except the fact, that periodate-treated cells tend to aggregate spontaneously and, as we found too, are clumped together to a high titer by adding polylysine. Our investigations underline, that Schiff base formation as well as electrostatic effects may play a role in some unexplained cell-cell adherence interactions and that this reaction can be demonstrated by using the diffusion techniques in agar gel with several (biological) macromolecules. Not only these "odd" precipitin reactions must be kept in mind and may lead to false interpretations of agar gel diffusion pictures, but also other non-immunologic precipitin lines, as they can be demonstrated with certain protease-proteaseinhibitor interactions or the C-reactive protein from human serum, which precipitates "like an antibody" with pneumococcal polysaccharide fractions [13].

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