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INTERACTION OF THE LECTINS OF *Datura innoxia* SEEDS WITH OVALBUMIN

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The interaction of the lectins of Datura innoxia *with ovalbumin has been investigated. The latter exhibits an inhibiting effect in relation to the lectins studied at a molar ratio of lectin to ovalbumin of 1:280 when the concentration of ovalbumin in the solution is not less than 50 mg/ml. Under these conditions a phase transition is observed in the solution, with the formation of a precipitate of the inhibitor. Characteristic features of the interaction of the lectin with ovalbumin have been studied.*

With respect to immunochemical classification, the lectins of plants of Solanaceae family are united in an individual group specific to oligomers of N-acetylglucosamine. In a study of the activity of these oligomers in the series from monomer to tetramer no appreciable inhibiting properties with relation to the lectins of *Datura innoxia* seeds were observed [1]. The titration of the lectins by M. D. Lutsik's method [2] with a 0.6 M solution of an oligochitose consisting of a mixture of 5- to 10-membered oligosaccharides (preparation provided by M. D. Lutsik) lowered the hemagglutination titer by a factor of 4, which corresponds to complete inhibition of the lectins at a ratio of lectin to oligochitose of $7.5 \cdot 10^{-5}$ mg:2.5 mg. In spite of the large excess of oligochitose on inhibition, in agar diffusion a clear band of the precipitation of an oligosaccharide–lectin complex was observed. Thus, the lectins of *Datura innoxia* are distinguished by a very low specificity to the group inhibitors of this class of lectins. Since lectins I_1 and I_2 do not differ in chemical specificity, we subsequently used lectin I_1. The study of the binding properties of the *Datura innoxia* lectin was pursued with the aid of glycoproteins.

Among the protein inhibitors widely used for lectins, ovalbumin exhibited activity in relation to the lectins under investigation. On immunodiffusion, this protein gave a sharp precipitation band with the *Datura innoxia* lectins. In a study of the ovalbumin:lectin system by the hemagglutination (HA) method, 50-μl portions of solutions of ovalbumin having different concentrations in different experiments were added to 50 μl of one of a series of twofold dilutions of the lectin (1 mg/ml) in Na phosphate buffer containing 0.9% NaCl, pH 7.4 (PBS). Inhibition of HA was observed only in an experiment with an ovalbumin concentration greater than 50 mg/ml. Here the HA titer fell from 2^{-11} in the control experiment to 2^{-6}. Thus, inhibition was observed at a molar ratio of ovalbumin to lectin of not less than 280:1, calculated for molecular masses of 45 kDa and 72 kDa for the ovalbumin and the lectin, respectively.

Inhibition of HA was observed to a greater degree (at a lower molar ration) if the system included ovalbumin reprecipitated with ammonium sulfate and freeze-dried (i.e., at a lower solubility of the ovalbumin).

Since ovalbumin is a glycoprotein and includes oligosaccharides of N-acetylglucosamine, two main reasons are possible for its manifestation of inhibiting activity in relation to the lectin under investigation: interactions through carbohydrate determinants or (and) hydrophobic interactions. It must be mentioned that neither a pronase hydrolysate of ovalbumin nor a glycopeptide isolated from the pronase hydrolysate possessed any inhibiting activity whatever in relation to this lectin. It is apparent that the oligosaccharide moiety of the inhibitor is not responsible for the inhibition process in this case.

At a certain concentration of ovalbumin in solution weak interactions occurred in the ovalbumin–lectin system, leading to structural transformations of the ovalbumin molecule followed by turbidity of the solution. At high concentrations of ovalbumin (10 mg/ml) a precipitate formed. Reversibility of these phase transition appeared even on the dilution of such systems with the initial buffer solution to an albumin concentration of less than 1 mg/ml.

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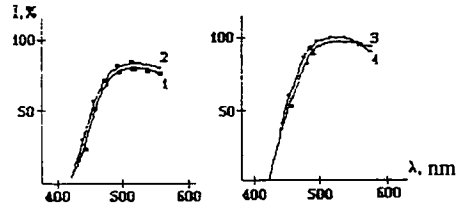

Fig. 1. Fluorescence spectra of ovalbumin–*Datura innoxia* lectin complexes: 1) ovalbumin + ANS (0.005 mg/ml): 2) ovalbumin + lectin (300:1) + ANS (0.005 mg/ml); 3) ovalbumin + ANS (0.01 mg/ml) (I = 100%); 4) ovalbumin + lectin (300:1) + ANS (0.01 mg/ml).

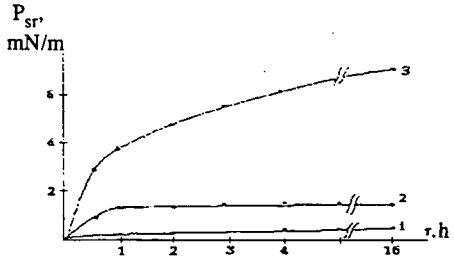

Fig. 2. Kinetics of the formation of interphase adsorption layers of *Datura innoxia* lectin at a boundary with benzene: pH 7.4; T = 293-295; I = 0.15 M; w = 0.01 rad/sec; 1) lectin, C_p = 0.02%; 2) lectin, C_p = 0.1%; 3) ovalbumin, C_p = 0.02%.

Fluorimetric investigations of ovalbumin molecules in dilute solutions (concentration 0.2 mg/ml) showed that, regardless of the initial concentration of ovalbumin in the solution on interaction with the lectin, even if a precipitate had formed the latter dissolved on dilution, and the fluorescence spectrum did not change during this process (0.2 mg/ml, λ_{max} 360 nm at λ_{exc} 275 nm). Consequently no appreciable irreversible conformational changes whatever in the ovalbumin took place on its interaction with the lectin.

A similar conclusion was made in an investigation of the surface hydrophobicities of ovalbumin and of ovalbumin–lectin complexes with the aid of the hydrophobic probe 8-anilinonaphthalene-1-sulfonic acid (ANS) (Fig. 1).

Obviously, intermolecular interactions in the ovalbumin–lectin system are weak (so that a large excess of ovalbumin is necessary for the inhibition of the lectin), which leads to its breakdown on dilution of the ovalbumin solution.

We studied the surface properties of the ovalbumin–lectin complex at a liquid/liquid phase separation boundary by the disk twisting method [3]. The results of the investigation of the influence of the lectins on the rheological properties of interphase adsorption layers (IALs) of albumin are given in Fig. 2 and Table 1.

As can be seen from Fig. 2, the lectin exhibits surface activity an order of magnitude lower (at the same concentrations) than ovalbumin, which permits an investigation of the rheology of IALs of ovalbumin in the presence of lectins (at the concentrations given above) with no appreciable contribution of the lectin to the rheology of an ovalbumin IAL.

The figures given in the table show that at high ratios (300/1 moles/mole) the strength properties of ovalbumin IALs at a water–benzene boundary remain unchanged. Only with a considerable increase in the proportion of lectin in the system was a change in the surface characteristics of the IALs observed, which shows the absence of specific interactions in the ovalbumin–lectin system under the given conditions.

Thus, for the manifestation of the inhibiting activity of ovalbumin with respect to the *Datura innoxia* lectins a considerable excess of ovalbumin over lectin is required, with a simultaneous high initial concentration of inhibitor in the solution.

TABLE 1. Influence of Additions of *Datura innoxia* Lectins on the Strength of Interphase Adsorption Layers of Ovalbumin at a Boundary with Benzene (pH 7.4; T = 293-295°C; I = 0.15 M; ω = 0.01 rad/s; C_p = 0.02%)

Ovalbumin/ lectin mass ratio	P_{sr}, mN/m as a function of T, h				
	0.25	0.5	1	3	16
1:1	4.0	4.2	4.6	6.1	6.3
1:2	3.5	3.9	4.5	5.2	5.4
1:5	–	3.1	3.7	5.1	5.3

The change in the structure of highly concentrated ovalbumin systems in the presence of lectins will be the subject of our future investigations.

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EXPERIMENTAL

Datura innoxia lectins obtained by the method of [4] and crystalline ovalbumin were used.

Hemagglutinatin activity titers were determined according to [2].

The rheological properties of the IALs of proteins at liquid phase separation boundaries were investigated by the method of [3]. Benzene purified by a standard procedure and redistilled at 80.0°C/740 mm Hg, n_d^{20} = 1.5013, was used as the nonpolar phase.

The fluorescence spectra of the ovalbumin and the lectins were taken at an excitation wavelength of 275 nm on a Hitachi UV-vis spectrometer in the interval of 290-400 nm in the case of the native proteins and 400-600 nm for the complexes with ANS. The optical density at 280 nm of the solutions was 0.1.

The surface hydrophobicity of the proteins was investigated as in [5].

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