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STUDY OF THE INFLUENCE OF CHEMICAL MODIFICATION OF THE TRYPTOPHAN RESIDUES IN THE MOLECULES OF *Datura innoxia* LECTINS ON THEIR HEMAGGLUTINATING ACTIVITY

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It has been found that in Datura innoxia *lectins 21% of the tryptophan residues are selectively oxidized by N-bromosuccinimide (NBS) under nondenaturing conditions, which shows that one or two residues are exposed on the surface of the molecule. At the same time the hemagglutinating activity (HA) decreases by 50%. When half of all the Trp is oxidized only 30% of the HA is retained. A sharp fall in the HA on the oxidation of ~1/7 of all the Trp indicates the great importance of at least one residue for the interaction of the lectin with erythrocytes. In the hydrophobic region of the molecule 79% of the Trp is screened, and this is exposed in 8 M urea.*

In a study of the carbohydrate-binding sites of lectins a special importance of tryptophan residues for the manifestation of lectin activity has been found [1-3]. Since this amino acid is present in the molecules of the lectins of the seeds of Hindu datura *Datura innoxia,* DIL1 and DIL2, it was of interest to determine how the tryptophan residues are localized in the molecules and whether they are responsible for the manifestation of lectin activity.

As is known, useful information on whether tryptophan residues are localized on the surface of a protein molecule or are "hidden" in the hydrophobic region can be obtained by comparing titration with N-bromosuccinimide in the presence of concentrated urea and in its absence. In the presence of 8 M urea those tryptophan residues may be exposed that do not undergo oxidation with N-bromosuccinimide under the usual conditions. Titration in urea has an extra advantage, since in this medium the reactivity of tyrosine residues in relation to N-bromosuccinimide is sharply diminished [5].

We performed titration with N-bromosuccinimide in 0.05 Na acetate buffer at pH 7.0 and in 8 M urea in the same buffer at pH 4.0 by Spande and Witkop's method [6]. Figures 1 and 2 give curves of the titration of lectins DIL1 and DIL2 under the conditions described above. From the titration curves of the lectins in the absence of urea (curves 1) it is possible to deduce the fact that oxidation took place slowly and discretely and that complete oxidation required more than the theoretically necessary M_{NBS}/M_{Trp} ratio. The opinion exists that if an oxidation process takes place in this way there are "hidden" tryptophan residues in the protein, as in the cases of serum albumin [7] and creatine phosphokinase [8]. The correctness of this was shown by the titration of the lectins in 8 M urea (curve *2* in Figs. 1 and 2).

Taking the minimum molecular masses of lectins DIL1 and DIL2 (found by ultracentrifugation) to be 77 and 85.2 kDa, respectively, we calculated the numbers of titratable tryptophan residues (Table 1).

In this way, seven tryptophan residues were determined in lectin DIL1. Of these residues, 21% were reactive under normal (physiological) conditions. It may be assumed that one or two Trp residues are exposed on the surface of the native lectin molecule. In lectin DIL2 four Trp residues were found, of which one was exposed. It can be seen from Table 1 that titration without urea took place over wide intervals of consumption of the oxidizing agent. Apparently, after the oxidation of the first, most reactive, residue, a conformational change takes place in the molecule which retards the oxidation process.

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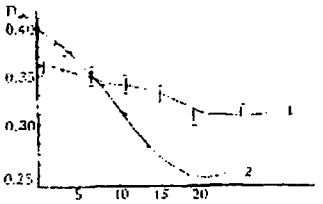
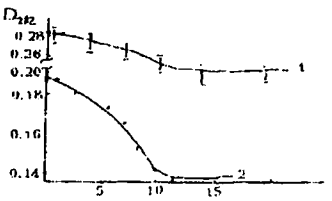
TABLE 1. Tryptophan Contents of the Lectins of *Datura innoxia*

Sample	ΔD_{282}	$\Delta D_{282corr}$	n_{Trp}	%Trp
DIL1	0.060	0.038	1.96	0.5
	0.054	0.023	1.27	0.3
DIL1, 8 M urea	0.145	0.131	7.2	1.74
DIL2	0.030	0.024	1.24	0.33
	0.029	0.018	1.0	0.23
DIL2, 8 M urea	0.072	0.062	3.94	0.9



Number of aliquots of NBS

Fig. 1



Number of aliquots of NBS

Fig. 2

Fig. 1. Curves of the titration of lectin DIL1 with N-bromosuccinimide: *1*) titration without urea; *2*) titration in the presence of 8 M urea.

Fig. 2. Curves of the titration of lectin DIL2 with N-bromosuccinimide: *1*) titration without urea; *2*) titration in the presence of 8 M urea.

Previously, to determine the Trp contents of the Hindu datura lectins we used methanesulfonic acid — a known reagent in which tryptophan is not destroyed [9]. This showed 2.3 M Trp/M protein in DIL1 and 1.5 M Trp/M protein in DIL2 — less than the levels found with NBS. Liu [10] has shown that a high yield of tryptophan is obtained in the acid hydrolysis of glycoproteins containing up to 35% of hexoses. In the presence of pentoses, however, the destruction of tryptophan takes place to a greater degree than in the presence of hexoses. In the hydrolysis of a lectin from potato tubers, Allen and Neuberger [11] obtained tryptophan yields of up to 25%. Since the lectins of Hindu datura contain more than 50% of carbohydrates, and these mainly arabinose [4], the levels of Trp that we found previously may be low.

In order to ascertain whether the Trp residues of the Hindu datura lectins are or are not responsible for the manifestation of activity by them, we determined the hemagglutinating activities of samples modified with N-bromosuccinimide. Lectin DIL1 in 50 mM Na acetate buffer at pH 7.0 and in 8 M urea at pH 4.0 was oxidized with the minimum amount of NBS necessary for modifying all the tryptophan residues accessible under the given conditions. The modified samples were intensively dialyzed against water and 50 mM Na acetate buffer, pH 7.0: In the derivatives so obtained we determined hemagglutinating activity with a 2% suspension of rabbit erythrocytes. The hemagglutinating activity was determined by the use of a series of twofold dilutions of the lectin in micro test tubes. Treatment of the lectin with aliquots of NBS led to a sharp decrease in its hemagglutinating activity. When 1/7 of the whole amount of tryptophan was oxidized, 50% of the initial HA was retained. When half the tryptophan was oxidized only 30% of HA_0 was retained. On the further addition of oxidizing agent the hemagglutinating activity continued to decrease rapidly. The dependence of the decrease $\Delta A = f(M_{NBS}/M_{protein})$ is close to linear (Figs. 3 and 4); consequently, at the given pH the reagent is specific for tryptophan. However, without an amino acid analysis of the oxidized protein it is impossible to speak definitively of the intactness of the other residues.

The sharp decrease in HA on the oxidation of 21% of the Trp permits the assumption that one (or two) of the residues is essential for interaction and is oxidized selectively by NBS in the initial stages.

The number of tryptophan residues participating in binding with erythrocytes was determined as described in [12] from a graph of $\%HA_{residual} = f(n\ Trp_{oxidized})$ (Fig. 5). Extrapolation of 100% HA to the axis of abscissas showed that one residue per molecule participates in binding.

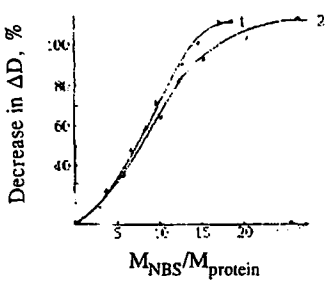


Fig. 3

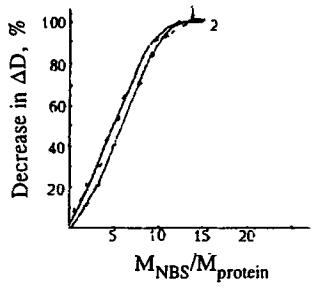


Fig. 4

Fig. 3. Titration of DIL1 with N-bromosuccinimide: *1*) in 8 M urea; *2*) without urea.

Fig. 4. Titration of DIL2 with N-bromosuccinimide: *1*) in 8 M urea; *2*) without urea.

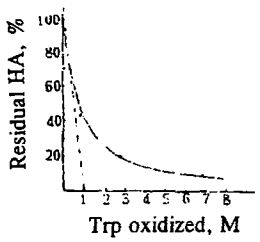


Fig. 5. Dependence of the residual hemagglutinating activity (HA) on the amount of tryptophan oxidized.

Thus, one or two tryptophan residues of a Hindu datura lectin are oxidized selectively by N-bromosuccinimide in the initial stages. The sharp fall in hemagglutinating activity on the oxidation of ~1/7 of the whole amount of tryptophan shows the possible essential nature of one tryptophan residue for the manifestation of lectin activity.

EXPERIMENTAL

The Lectins DIL1 and 2 were extracted from *Datura innoxia* seeds and purified as described in [13]. Hemagglutinating activities were determined by M. D. Lutsik's method [14]. Tryptophan was determined by the method of Spande and Witkop [6].

Solutions of the lectins (0.33 mg/ml) were titrated in 0.05 M Na acetate buffer, pH 7.0, and in 8 M urea at pH 4.0. With vigorous stirring, 10-ml aliquots of NBS (1.78×10^{-3} M) were added to the lectin solutions in quartz cells, and A_{282} values were measured in a spectrophotometer. The addition of NBS was continued until the absorption at 282 nm ceased to decrease (or until a small increase began). The minimum value of A_{282} was recorded and was corrected for dilution. The number of oxidized Trp residues was calculated as in [6].

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