INHIBITION BY CYSTEINE OF CARBOHYDRATE-BINDING ACTIVITY OF LECTINS FROM *Ricinus communis*, *Canavalia ensiformis*, AND *Euonymus europaeus*

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In recent years lectins have been used on an ever-increasing scale as a tool with which to study biolgoical processes such as cell differentiation, agglutination, tumor growth, and the nature and distribution of carbohydrates on the cell surface. In all these cases the property of lectins of binding carbohydrates is used. However, very little is known about the mechanism of binding of sugars by lectins. For some lectins in particular, such as concanavalin A (Con A), even the tertiary structure of the carbohydrate-binding active center has been studied, although the problem of which amino acids of the lectin participate in the binding of sugars remains unsolved. In the literature there are only a few papers on the subject which show that cysteine residues of lectin from Lima bean are necessary for it to exhibit N-acetylgalactosamine-binding activity [4, 6, 7].

In the investigation described below it was shown that cysteine inhibited the carbohydrate-binding activity of three different lectins: from *Ricinus communis* (RCA_1), *Canavalia ensiformis* (Con A), and *Euonymus europaeus* (Eel), interacting specifically with galactose, mannose, and fructose respectively. These data, in our opinion, are indirect evidence that the cysteine residues of these lectins are essential for specific binding of sugars.

EXPERIMENTAL METHOD

The following amino acids of USSR origin were used: DL-α-alanine, L-arginine hydrochloride, L-asparagine, L-histidine hydrochloride, DL-lysine hydrochloride, DL-threonine, DL-serine, and L-cysteine hydrochloride; DL-glycine (from Reanal, Hungary) also was used. The concentration of the solution of each of these amino acids was 3 M in 0.15 M NaCl. Lactosylceramide (Lac-Cer) was generously provided by E. V. Detlovitskaya (Institute of Bio-organic Chemistry, Academy of Medical Sciences of the USSR, Moscow). Lectin RCA_1, isolated by the method described previously [2] was homogeneous on disk-electrophoresis. Precipitation of liposomes containing Lac-Cer in the composition of their membrane, and obtained by the method described previously [1], was studied spectrophotometrically, using a Specord M-40 spectrophotometer (East Germany). Development of the precipitation reaction was studied by measuring changes in the absorption time of the test preparations at 360 nm. Lectin Con A was generously provided by J. Kosourek (Prague, Czechoslovakia). Precipitation induced by this lectin was

TABLE 1. Carbohydrate-Specific Precipitation of Lectins Con A and Eel in the Presence of Various Amino Acids

Experimental conditions	Without amino acids	Amino acid								Specific inhibitor	
		Alanine	Arginine	Asparagine	Histidine	Lysine	Threonine	Serine	Cysteine	Methyl-mannoside	Fucosyl-lactose
Con A + amylogluco-sides	+	+	+	+	+	+	+	+	—	—	
Eel + blood group substances	+	+	+	+	+	+	+	+	—		—

Legend. +) Distinct precipitate, —) no precipitate formed.

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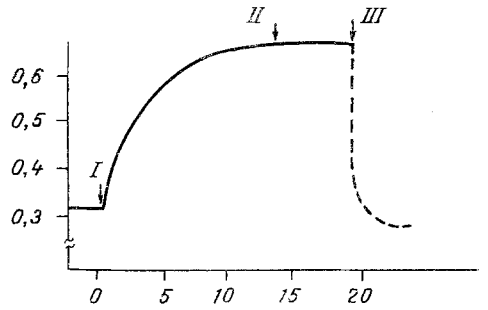

Fig. 1. Absorbance (A_{360}) of suspension of lectin liposomes, containing 10 moles % of Lac-Cer in their membrane, before and after addition of lectin RCA_1 in the presence of various amino acids. Abscissa, incubation time (in min); ordinate, A_{360} (in relative units). To liposomes (1.4 ml) in a concentration of 0.2 mg lecithin/ml were added: 100 µl of RCA_1 up to a final concentration of 125 µg/ml, followed by amino acids up to a final concentration of 0.5 M, or lactose, to 0.1 M. Arrows: I) addition of RCA_1; II) addition of any of the amino acids studied except cysteine; III) addition of cysteine or lactose.

studied on amyloglucosidase (AG) from *Aspergillus* (from Koch-Light, England), and α-methyl-D-mannoside (from Chemapol, Czechslovakia) was used as precipitation inhibitor. Lectin Eel, obtained from J. Petryniak (Wroclaw, Poland), was isolated by the method described previously [8]. Blood group substances (BGS), prescipitating in the presence of this lectin, were generously provided by V. A. Derevitskaya. Precipitation of AG and BGS by Con A and Eel lectins respectively in the presence of various amino acids was studied visually on slides. Equal volumes (20 µl) of solutions of the lectin (Con A in a concentration of 2 mg/ml or Eel in a concentration of 1.6 mg/ml), of one of the amino acids, and of AG or BGS in a concentration of 1 mg/ml, were placed on the slide. The α-D-methylmannoside or fucosyl-lactose, used as inhibitors of the precipitation process, were added to the drop on the glass in the form of a dry weighed sample.

EXPERIMENTAL RESULTS

The following guidelines were used when setting up the experiments. Precipitation of carbohydrate-containing molecules by lectins is known to be inhibited by the addition of specific sugars [9]. Under these circumstances, the inhibitory molecules of the sugars evidently bind with certain amino acids of the lectin that are responsible for addition of these carbohydrates, and thus prevent precipitate formation. It might be supposed that inhibition of precipitation would also be observed if amino acids which directly or indirectly participate in carbohydrate-protein interaction also are used as inhibitors of this process. Accordingly it was decided to study precipitation induced by several lectins in the presence of various amino acids. Since about half of all known amino acids are sparingly soluble in water, only nine hydrophilic amino acids were used in the work.

The first lectin chosen for study was RCA_1, which specifically binds terminal galactose residues of carbohydrate-containing compounds. Precipitation induced by this lectin was investigated on liposomes containing Lac-Cer in the composition of their membrane. As Fig. 1 shows, addition of a solution of RCA_1 to liposomes with Lac-Ser built into their membrane led to a gradual increase in absorbence of the liposome suspension (A_{360}) with time, due to precipitation. By the 20th minute after addition of the lectin, the precipitation process was characterized by saturation. Addition of any of the nine amino acids chosen for testing, except cysteine, to the precipitate did not reduce absorbence of the suspension, i.e., did not destroy the precipitate. Addition of cysteine, on the other hand, like that of lactose, quickly reduced absorbence of the liposomal suspension virtually to its original value, i.e., completely destroyed the precipitate.

The next lectin studied was Con A. Mannose-specific precipitation in the presence of this lectin was studied on AG. Since Ca^{++} and Mn^{++} ions are necessary for Con A to exhibit its lectin activity, all the solutions contained 1 mM $CaCl_2$ and 1mM $MnCl_2$. As Table 1 shows, in control experiments AG and Con A in the absence of amino acids formed a distinct precipitate. In the presence of any of the amino acids chosen, except cysteine, AG and Con A also formed marked precipitates. In the presence of cysteine, however, just as in the presence of α-methyl-D-mannoside, a specific inhibitor of precipitation induced by lectin Con A, no precipitate was formed.

Data on precipitation of BGS, containing terminal fucose, by the specific lectin for fucose Eel, also are given in Table 1. It will be clear from Table 1 that BGS and Eel, in the presence of any of the amino acids used except cysteine, just as in their absence, gave a distinct precipitate. No precipitates were formed in the presence of cysteine, or in the presence of fucosyl-lactose, a specific inhibitor of fucose-specific precipitation.

Cysteine was found to inhibit the carbohydrate-binding activity of three other lectins: RCA_1, Con A, and Eel. On the one hand, this can be logically explained on the grounds that cysteine perhaps reacts chemically with certain residues of the lectins, leading to loss of carbohydrate-binding activity. However, since all the experiments were carried out at room temperature and neutral pH, this hypothesis seems unlikely [3]. On the other hand, the results can be explained by competition between cysteine and the cysteine residues of the lectins, essential for manifestation of their carbohydrate-binding activity. Considering data in the literature cited above [4, 6, 7], we regard this hypothesis as the most likely. Cysteine residues of lectins RCA_1, Con A, and Eel, just as lectin from Lima bean, are evidently essential for manifestation of carbohydrate-binding activity.

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ADENYLATE CYCLASE ACTIVITY AND cAMP CONCENTRATION IN BRAIN TISSUE OF DOGS DURING CLINICAL DEATH AND AFTER RESUSCITATION

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Neurochemical studies in the last few years have shown that cyclic nucleotides play an essential role in the pathogenesis of neurologic disorders of varied etiology [8]. The role of cyclic nucleotides in intracellular transmission of the receptor stimulus, and the connection between adenylate cyclase (AC) and synaptic sensitivity of neurons provide a firm basis for the study of these compounds in order to reveal the mechanisms of postresuscitation encephalopathy.

In the investigation described below the cAMP concentration and AC activity were investigated in tissue of the gray matter of the brain and striatum of dogs during circulatory arrest following electric shock and in the postresuscitation period.

EXPERIMENTAL METHOD

Acute and chronic experiments were carried out on 28 mongrel dogs of both sexes, weighing 10-17 kg, and anesthetized with trimeperidine (6-8 mg/kg). Circulatory arrest occurred in the animals as a result of ventricular fibrillation, induced by electric shock. Tissue of

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