Hybridization between the Heterologous Chains of Ricin D and Castor Bean Hemagglutinin†

Gunki FUNATSU, Shigetoshi UENO
and Masaru FUNATSU

Laboratory of Biochemistry, Faculty of
Agriculture, Kyushu University,
Fukuoka, Japan

Received April 18, 1977

Despite the fact that the two lectins—ricin and hemagglutinin (CBH)—present in castor bean seeds (*Ricinus communis*) consist of Ile and Ala chains having similar physicochemical properties, they are remarkably different in their toxicity and hemagglutinating activity. In the preceding paper,¹⁾ we have shown that ricin D and CBH-A have subunit structures represented as $\alpha\beta$ and $(\alpha'\beta')_2$, respectively and that the high hemagglutinating activity of CBH is due to its tetramer structure possessing two binding sites for red blood cells.

With respect to the toxicity of ricin, on the other hand, it has been shown that the high toxicity of ricin is exhibited only by a cooperative action of the Ile chain which inhibits protein synthesis and the Ala chain which binds to galactose-like residues on cell surface.^{2,3)} Therefore, it is interesting to know whether the extremely low toxicity of CBH is due to a functional difference in the Ile chain, the Ala chain or both chains. One way to clarify this problem is to prepare hybrid molecules between heterologous chains of ricin D and CBH-A, and determine their toxicities.

The two constituent chains of ricin D were prepared as described previously⁴⁾ and those of CBH-A were also isolated principally according to the same method. One hundred mg of CBH-A were dissolved in 20 ml of 0.15 M NaCl–0.1 M phosphate buffer, pH 8.0, containing 1% β -mercaptoethanol, and allowed to stand for 1 hr at room temperature with stirring. After dialysis against 0.15 M NaCl–0.1 M phosphate buffer, pH 8.0, containing 0.1% β -mercaptoethanol for 3 hr, the reduced CBH-A were applied to a Sepharose 4B column (3×30 cm) which had been washed previously with the same buffer. When the column was developed with the same buffer, the Ile chain was eluted while the Ala chain was adsorbed on the column with unreacted CBH-A. The adsorbed protein was eluted with 0.1 M lactose in the buffer, dialyzed against 5 mM

FIG. 1. SDS-polyacrylamide Gel Electrophoresis of the Isolated Chains of Ricin D and CBH-A, and Their Hybrid Molecules.

(A), ricin-Ile chain; (B), CBH-Ala chain; (C), hybrid ricin-Ile chain/CBH-Ala chain; (D), hybrid ricin-Ile chain/CBH-Ala chain after treatment with β -mercaptoethanol at 50°C for 3 hr; (E), CBH-Ile chain; (F), ricin-Ala chain; (G), hybrid ricin-Ala chain/CBH-Ile chain; (H), hybrid ricin-Ala chain/CBH-Ile chain after treatment with β -mercaptoethanol at 50°C for 3 hr. The concentration of acrylamide was 10%.

Tris-HCl buffer, pH 8.5, and applied to a DEAE-cellulose column pre-equilibrated with the same buffer. The Ala chain of CBH-A was passed through the column without adsorption, whereas the unreacted CBH-A was adsorbed on the column.

The hybrid molecules between heterologous chains of ricin D and CBH-A were prepared using the isolated chains as described previously.⁴⁾ When the product obtained by reduction-reoxidation of the mixture of ricin-Ala chain and CBH-Ile chain was applied to a Sepharose 4B column (2.2×10 cm) pre-washed with 0.2 M NaCl–5 mM phosphate buffer, pH 7.1, most of the protein was adsorbed on Sepharose and eluted with 0.1 M lactose. After dialysis against 5 mM Tris-HCl buffer, pH 8.5, the protein was applied to a DEAE-cellulose column which had been equilibrated with the same buffer, and eluted as a single peak by a linear gradient of NaCl from 0.02 M to 0.2 M. SDS-polyacrylamide gel electrophoresis of the hybrid product thus obtained gave two bands corresponding to mol. wt. near 65,000 and 130,000 as shown in Fig. 1G. On the other hand, the product from the ricin-Ile chain and the CBH-Ala chain was eluted without lactose from a Sepharose 4B column as a broad peak, due to its weak affinity to the column. SDS-polyacrylamide gel electrophoresis of the hybrid product gave essentially one band corresponding to mol. wt. near 65,000 as shown in Fig. 1C. After treating with β -mercaptoethanol, both hybrid molecules gave two bands—Ile and Ala chains—on SDS-polyacrylamide gel electrophoresis (Figs. 1D and 1H). These results indicate that the Ile chains and the Ala chains of ricin D and CBH-A

† Studies on Castor Bean Hemagglutinin. Part V. For Part IV, see Reference 1).

TABLE I. TOXICITY AND CYTOAGGLUTINATING ACTIVITY OF THE HYBRID MOLECULES BETWEEN HETEROLOGOUS POLYPEPTIDE CHAINS OF RICIN D AND CBH-A

Proteins	Subunit structure	Toxicity		Cytoagglutinating activity			
		min. conc. (OD ₂₈₀)	%	Red blood cells		Tumor cells	
				min. conc. (OD ₂₈₀)	%	min. conc. (OD ₂₈₀)	%
Ricin D	$\alpha\beta$	0.002	100	0.130	2.3	0.006	100
CBH-A	$(\alpha'\beta')_2$	0.150	1.3	0.003	100	0.008	75
Hybrid							
ricin-Ile chain/ CBH-Ala chain	$\alpha\beta'$	0.110	1.8	0.15	2.0	0.15	4
Hybrid							
ricin-Ala chain/ CBH-Ile chain	$\alpha'\beta + (\alpha'\beta)_2$	0.060	3.3	0.029	10.3	0.008	75

are interchangeable and that the hybrid molecules can be easily formed.

Cytoagglutinating activities of the hybrid molecules were measured using human red blood (RB) cells and sarcoma 180 ascites tumor (SA) cells as previously described.⁸⁾ As shown in Table I, the cytoagglutinating activity of the hybrid molecule made of ricin-Ala chain/CBH-Ile chain was almost the same as that of ricin D for SA cells, whereas it was four-fold of that of ricin D for RB cells, probably due to the presence of tetramer. On the other hand, the cytoagglutinating activity of the hybrid molecule made of ricin-Ile chain/CBH-Ala chain was equal to that of the isolated Ala chain of CBH-A for both cells. These results confirm the previous findings^{1,3)} that the cytoagglutinating action of *Ricinus* lectin toward SA cells is associated exclusively with the Ala chain, the binding of the Ile chain to the Ala chain having no effect on the activity, and that the high hemagglutinating activity of CBH-A is due to its tetramer structure.

The toxicities of the hybrid molecules were determined by an intraperitoneal injection into pure-bred

mice weighing 20~25 g as previously described.⁹⁾ As shown in Table I, the toxicity of the hybrid molecule made of ricin-Ala chain/CBH-Ile chain was only 3.3% of that of ricin D which is higher than that of CBH-A, whereas the toxicity of the hybrid molecule made of ricin-Ile chain/CBH-Ala chain was less than 2% of that of ricin D. These results indicate that the Ile chain and the Ala chain of CBH-A are functionally different from the corresponding chains of ricin D and that the high toxicity of ricin D is elicited by the cooperative action of both chains.

REFERENCES

- 1) G. Funatsu, S. Ueno and M. Funatsu, *Agric. Biol. Chem.*, **41**, 171 (1977).
- 2) S. Olsnes and A. Pihl, *Biochemistry*, **12**, 3121 (1973).
- 3) G. Funatsu, S. Miyauchi and M. Funatsu, *Agric. Biol. Chem.*, **40**, 639 (1976).
- 4) G. Funatsu, S. Miyauchi, N. Yoshizuka and M. Funatsu, *ibid.*, submitted.