Antigenic cross-reactivity between electrolectins

G. Levi and V.I. Teichberg*

Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel

Received 17 September 1982

The antigenic cross-reactivity between purified chick, eel and mouse electrolectins (endogenous β-D-galactoside specific lectins) have been studied using a solid phase radioimmunoassay. The immune serum raised against the eel electrolectin crossreacts both with the chick and the mouse electrolectins, while the anti-chick electrolectin anti-serum recognizes only the eel but not the mouse electrolectin. These findings are analyzed in terms of the phylogenetic distance separating the species considered; they suggest that electrolectins fulfil a fundamental biological function.

Animal lectin *β-D-Galactoside specificity* *Crossed immunoreactivity* *Radioimmunoassay*

1. INTRODUCTION

Electrolectins are endogenous β-D-galactoside binding lectins that are found in vertebrates [1–7]. They are particularly abundant in skeletal muscle but are also present in other organs such as the spleen, the thymus and the lungs [1,2,7]. Their tissue concentration is often developmentally regulated reaching a maximum concomitantly with the processes of myoblast fusion and synaptogenesis [8,9]. Several attempts have been made to demonstrate their involvement in cellular recognition and in particular in the process of myoblast fusion, but these studies have led to conflicting results [10–12]. We have observed that eel electrolectin has prophylactic and therapeutical effects toward the experimental autoimmune myasthenia gravis induced by immunization against the purified acetylcholine receptor protein [13]. This experimental autoimmune disease appears to be an appropriate model for the human disease myasthenia gravis [14]. The prophylactic effect of electrolectin was accompanied by a reduced ability of the immune system to produce antibodies directed against the acetylcholine receptor.

In view of the possible role of electrolectins in the development of tolerance, we were interested in determining whether electrolectins are phylogenetically related or are distinct proteins sharing only a similar saccharide-binding specificity. β-D-Galactoside binding lectins from different tissues of the same animal display a full antigenic cross-reactivity [3,4]. However, the study of the interspecies cross-reactivity of electrolectins shows no antigenic similarity between the chick heart lectin and the calf lectin [3] and between the chick and the rat electrolectins [15]. On the other hand, an antigenic similarity between the calf, human and monkey electrolectins has been reported [6].

We have established here the existence of an immunological crossreactivity between the electrolectins purified from the electric organ of the electric eel *Electrophorus electricus* (teleost), from the chick pectoral muscle (avian) and from the mouse thymus (mammalian). The quantitative results that we have obtained are in agreement with what can be expected considering the phylogenetic distance that separates these species. These results support the hypothesis that all electrolectins share a common function.

2. METHODS

2.1. *Tissues*

Live electric eels, *Electrophorus electricus*, were obtained from Worldwide Paramount aquarium (Ardsley NY). They were decapitated and the

*To whom correspondence should be addressed

Abbreviations: BSA, bovine serum albumin; PBS, phosphate buffered saline

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

main electric organ was cut in small cubes and frozen at −20°C. Fertilized white Leghorn chicken eggs were obtained from kibbutz Yavne. The pectoral muscle of 16-day-old embryos was excised and kept frozen at −20°C. Thymuses were excised from 2-month-old C57BL/6J mice and kept frozen at −20°C.

2.2. *Purification of electrolectins*

All the lectins were isolated from the homogenates of the respective tissues by affinity chromatography on a lactosyl–Sepharose column as in [16]. The eluting buffer consisted of PBS supplemented with 100 mM lactose and 14 mM 2-mercaptoethanol. The elution peak of the lectins was monitored by fluorescence (exitation 285 nm, emission 327 nm). The purifity of the lectins was checked by SDS gel electrophoresis as in [16]. The complete characterization of the mouse thymus lectin will be reported separately.

2.3. *Assay of electrolectin activity*

The agglutinating activity of the lectins was assayed on rabbit trypsinized erythrocytes. A quantitative hemagglutination assay was performed in microtiter plates as in [16], 0.1 M lactose was added in control assays to inhibit the hemaglutination.

2.4. *Preparation of rabbit antisera*

Rabbits (New Zealand white) received a primary immunization with 100 μg of purified electrolectins (either eel or chick) emulsified in complete Freund adjuvant. Each rabbit was injected subcutaneously in several sites on the back. A secondary immunization was administered 2 weeks later using the same procedure. The rabbits were bled 4 weeks after the secondary immunization. The antisera, divided in small aliquots, were kept frozen at −20°C.

2.5. *Solid phase radioimmunoassay*

The ability of the antisera to recognize each of the antigens was determined by a solid phase radioimmunoassay [17] using ^{125}I-labelled protein A. Wells of microtiter plates (Dynathec) were coated with the antigens following an incubation for 3 h at room temperature with 100 μl of a solution of the purified antigen diluted in PBS (final conc. 50 μg/ml). Control wells were incubated with a similar concentration of bovine serum albumin. The antigen solution was then removed, the plates washed 3 times with 200 μl PBS, and incubated 1 h with PBS containing 500 μg/ml bovine serum albumin. Serum in various dilutions were added to the coated wells (50 μl/well) and incubated 12 h at 37°C. The wells were then washed twice with cold PBS and ^{125}I-protein A (75 Ci/mmol) was added (25 μl/well, ~50 000 cpm) and incubated 16 h at room temperature. The plates were washed and the single wells separated and counted.

3. RESULTS AND DISCUSSION

In fig.1 and 2, we show the results obtained upon incubating, respectively, the anti-eel electrolectin and the anti chick-electrolectin immune anti-sera in wells coated with chick, eel or mouse electrolectins. The anti-eel electrolectin anti-serum recognizes all the 3 antigens, while the anti-chick electrolectin anti-serum recognizes the chick and eel electrolectins but has no significant cross-reactivity with the mouse electrolectin. The coating of the wells with the antigens was carried out using a concentration of soluble lectins (50 μg/ml) sufficient to ensure the saturation of all the available binding sites on the plastic-made wells. Under these conditions, with the sole assumption that each of the antigens has the same number of available binding sites on the plastic, one can consider that the same number of lectin molecules can possibly react with the anti-sera. Accordingly, it becomes then possible to calculate the percent of

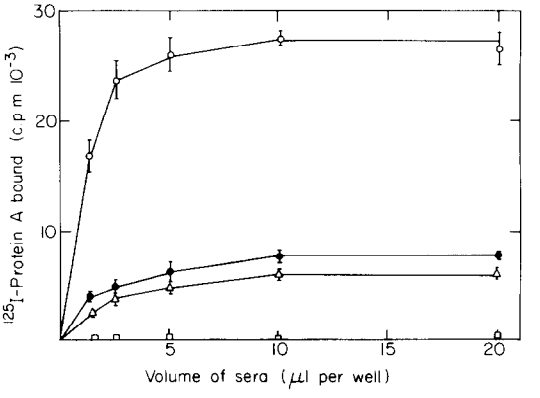

Fig.1. Anti-eel electrolectin immune anti-serum binding to microtiter wells coated with eel (○), chick (●) or mouse (△) electrolectins. Control wells were coated with BSA (□).

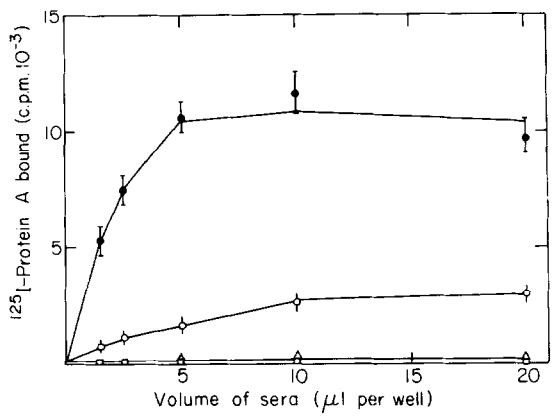

Fig.2. Anti-chick electrolectin immune anti-serum binding to microtiter wells coated with eel (○), chick (●) or mouse (△) electrolectins. Control wells are coated with BSA (□).

common antigenic determinants shared between the lectins by comparing the amounts of antibodies bound to the wells under saturating conditions; these results are summarized in table 1. The absence of cross-reactivity between the chick and the mouse electrolectins corroborates earlier findings on the lack of cross-reactivity between the chick and calf [3] and between the chick and rat electrolectins [15]. However, the fact that the anti-eel anti-serum recognizes both the chick and the mouse electrolectins emphasizes the extent of the structural conservation of the β-D-galactoside binding lectins during evolution.

We have attempted to correlate the percent of antigenic cross-reactivity that exists between the purified electrolectins and the phylogenetic distance (in millions of years) separating their originating species. These results are shown in fig.3. To calculate the phylogenetic distance between two

Table 1

Percentage of antigenic cross-reactivity between electrolectins

Anti-sera	Antigen		
	Eel	Chick	Mouse
Anti-eel	100	31	26
Anti-chick	23	100	0

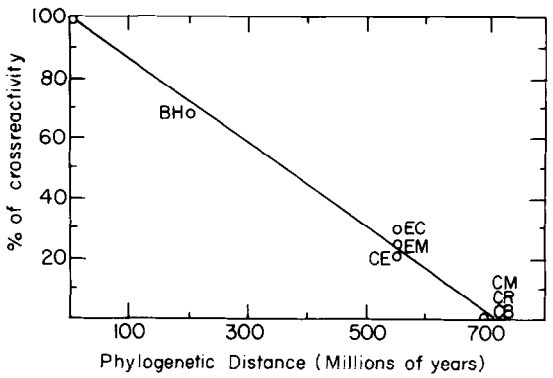

Fig.3. Correlation between the phylogenetic distance and the antigenic cross-reactivity between electrolectins of different species. The first letter represents the species against which antibodies were produced while the second letter represents the antigen used: B, beef; C, chick; E, eel; M, mouse; R, rat; H, human. The cross-reactivity data between the beef and the human lectins and between the chick and the beef lectins were derived from [3] and that between the chick and the rat lectin from [15].

species A and B that diverged from a common precursor some millions of years ago, we have summed the distances on the phylogenetic tree separating the precursor from the diverging species A and B. The points of divergence of birds and fish were placed on the phylogenetic tree, respectively, at 350 and 400 million years ago [18] and it was assumed that *Electrophorus electricus*, as a primitive teleost of the order Ostariophysi, diverged from fish during the lower Jurassic era [19]; i.e., probably after 100–200 million years. The graph reported in fig.3 should be seen only as indicative of a trend; yet, it may be useful in predicting the extent of antigenic cross-reactivity between electrolectins from different species.

The biological function of electrolectins is still unknown, yet their agglutinating properties has led several authors to propose a role in cellular recognition [20]. Their localization in different tissues has also brought up the suggestion that the endogenous lectins could exert more than one function [21]. Our results showing an antigenic crossreactivity between lectins from different tissues and different species, are certainly in line with the idea that all the β-D-galactoside binding lectins in animal tissues have a common fundamental biological

role that has been preserved through evolution. In view of our observations that the eel electrolectin displays prophylactic and therapeutical properties in an autoimmune disease [13], one may suggest that the function of electrolectins could be linked to the development of tolerance.

ACKNOWLEDGEMENTS

This research was supported by a research grant of the Muscular Dystrophy Association of America.

REFERENCES

[1] Teichberg, V.I., Silman, I., Beitsch, D.D. and Resheff, G. (1975) Proc. Natl. Acad. Sci. USA 72, 1383–1387.
[2] Teichberg, V.I. (1978) Methods Enzymol. 50, 293–302.
[3] Barak Briles, E., Gregory, W., Fletcher, P. and Kornfeld, S. (1979) J. Cell Biol. 81, 528–537.
[4] Kobiler, D., Beyer, E.C. and Barondes, S.H. (1978) Dev. Biol. 64, 265–272.
[5] Franklin, G.I., Walsh, F.S. and Thompson, E.J. (1980) FEBS Lett. 118, 200–204.
[6] Childs, R.A. and Feizi, T. (1979) Biochem. J. 183, 755–758.
[7] Powell, J.T. (1980) Biochem. J. 187, 123–129.
[8] Kobiler, D. and Barondes, S. (1977) Dev. Biol. 60, 326–330.
[9] Simpson, D.L., Thorne, D.R. and Loh, H.H. (1977) Nature 266, 367–369.
[10] Gartner, T.K. and Podleski, T.R. (1975) Biochem. Biophys. Res. Commun. 67, 3 972–978.
[11] Malinzak, H.D. and Rosenberg, A. (1976) Biochem. Biophys. Res. Commun. 69, 621–627.
[12] Gartner, T.K. and Podleski, T.R (1976) Biochim. Biophys. Res. Commun. 70, 1142–1149.
[13] Levi, G., Tarrab-Hazdai, E. and Teichberg, V.I. (1982) submitted.
[14] Fuchs, S. (1980) Trends Biochem. Sci. 5, 259–262.
[15] Barondes, S.H. (1982) personal communication.
[16] Levi, G. and Teichberg, V.I. (1981) J. Biol. Chem. 256, 11 5735–5740.
[17] Schwartz, M., Novick, D., Givol, D. and Fuchs, S. (1978) Nature 273, 543–545.
[18] Mc Laughlin, P.J. and Dayhoff, M.O. (1972) in: Atlas of protein sequence and structure 1972 (Dayhoff, M.O. ed) pp. 47–52, National Biomedical Research Foundation, Washington DC.
[19] Parker, T.J. (1964) in: Text-book of Zoology, pp. 293–334, Macmillan, London.
[20] Simpson, D.L., Thorne, D.R. and Loh, H.H. (1978) Life Sci. 22, 727–748.
[21] Beyer, E.C., Tokuyasu, K.T. and Barondes, S.H. (1979) J. Cell. Biol. 82, 565–571.