COMPARATIVE STUDIES ON THE STRUCTURES OF COLICINS E2 AND E3

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1. Introduction

Colicins E2 and E3 share a common receptor on the surface of sensitive cells though the final targets are DNA and ribosomes, respectively [1–6]. Recent studies have shown that colicins E2 and E3 have some similar molecular features: each consists of two components, A and B [2,7,8] where A is the active component and B is the so-called immunity protein [7], which neutralizes the in vitro activity of protein A of homologous but not of heterologous colicin. Digestion of protein A with trypsin leads in each case to two fragments, E2-T1, E2-T2A and E3-T1, E3-T2A, respectively [9,10]. Each T2A fragment is the active fragment of the corresponding colicin, having the activity equivalent to protein A, when assayed in the in vitro system. The activity of T2A is neutralized by the corresponding protein B. The amino acid sequence of E3-T2A has been determined in our laboratory [11]. Since T2A fragments have no bacteriocidal activity it is expected that the function of attachment to or penetration through the cell membrane resides in T1 fragments. On the other hand, amino acid and immunochemical analyses of these colicins suggested that there are regions of similar structure in the two proteins [12].

On the basis of these findings we have proposed the following model for these colicins. Protein A is composed mainly of two regions, T1 and T2A. The T1 regions of E2 and E3 should be almost identical, assuming that this region participates in binding to receptor. The T2A regions of these two colicins, however, are quite different from each other, explaining the functional difference of these colicins. The present communication provides some supporting data for this model.

2. Materials and methods

Intact colicins, components A and B, and tryptic fragments T1 and T2A were prepared as in [9,10], except that T1 and T2A were purified by ion-exchange column chromatography.

Electrophoresis on 12.5% acrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) was carried out by the Weber and Osborn method [13].

Amino acid compositions of colicin preparations were analyzed on a Hitachi 835 amino acid analyzer after hydrolysis with 6 N HCl in vacuo at 110°C for 24–72 h.

Antisera against purified colicin fragments and immunity protein were prepared from New Zealand white rabbits. Protein (1–2 mg) in Freund's adjuvant was used as a primary subcutaneous injection. Each rabbit was thereafter given 3 subcutaneous injections of ~0.5–2 mg protein at monthly intervals. Antisera were prepared and stored at −20°C. Antigen–antibody reactions were analyzed by double-diffusion in 1.2% agarose dissolved in 0.15 M NaCl and spread on glass slides.

3. Results

3.1. *Chemical properties*

Figure 1 shows the result of SDS–polyacrylamide gel electrophoresis of the isolated tryptic fragments

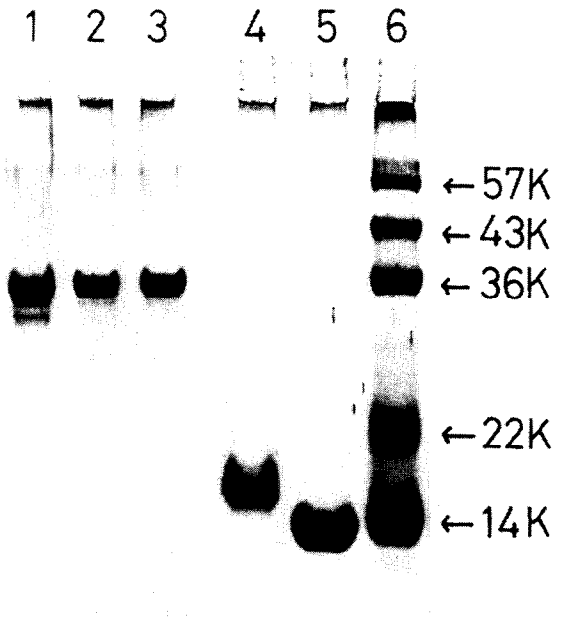

Fig.1. SDS–polyacrylamide gel electrophoresis of isolated fragments of colicins E2 and E3. (1)E2-T1, (2)E2-T1 + E3-T1, (3)E3-T1, (4)E2-T2A, (5)E3-T2A, (6)standard markers: pyruvate kinase (mol. wt 57 000), ovalbumin, (43 000), lactate dehydrogenase (36 000), soybean trypsin inhibitor (22 000) and lysozyme (14 000).

of colicins E2 and E3. E2-T1 and E3-T1 have exactly the same molecular weight.

Table 1 shows the results of amino acid analyses of these fragments. The amino acid composition determined for E2-T1 is almost identical to that of E3-T1. The C-terminal amino acid of both T1 fragments was determined to be arginine. On the other hand, the amino acid composition of E2-T2A is significantly different from that of E3-T2A. In addition, the C-terminal part of E2-T2A was determined to be –(His,Gly,Arg)–Lys–COOH using carboxypeptidases A and B, which does not resemble the C-terminal sequence of E3-T2A, –Asn–Ile–Lys–Lys–Tyr–Leu–COOH [11,14].

3.2. *Immunochemical properties*

Purified colicin fragments and immunity proteins were examined by the Ouchterlony immunodiffusion technique using homologous and heterologous antisera.

Table 1
Amino acid compositions of fragments of colicins E2 and E3

Amino acid	E3-T1	E2-T1	E3-T2A[a]	E2-T2A
Lys	11	13	16	20
His	6	6	4	5
Arg	11	11	5	10
Asp	46	46	12	26
Thr[b]	19	18	4	2
Ser[b]	36	36	3	6
Glu	23	23	8	11
Pro	30	30	8	9
Gly	55	55	16	13
Ala	34	33	2	6
Val[c]	37	36	0	6
Met	4	4	0	1
Ile[c]	13	13	4	8
Leu[c]	17	17	6	5
Tyr	2	2	5	1
Phe	8	8	2	8
Trp	n.d.[d]	n.d.	2	n.d.
Total	352	351	97	137
Mol. wt[e]	35 000	35 000	10 917	15 500

[a] Calculated from its amino acid sequence [11]
[b] Extrapolated value to zero time of hydrolysis
[c] Average of 72 h values
[d] n.d., not determined
[e] Estimated by SDS–gel electrophoresis

Values are the average of 2 determinations of 24 h, 48 h and 72 h hydrolyzates

Each of the anti-E2-T1 and anti-E3-T1 sera gave strong single precipitin lines when reacted with E2-T1 and E3-T1 (fig.2). The lines produced by the homologous T1–anti-T1 system, fused without spurring with the lines produced by heterologous systems (fig.2). These indicated that E2-T1 and E3-T1 are serologically identical, suggesting a close similarity in higher order structure. The higher order structure of the T1 region was not influenced by fragmentation of protein A, since the line from reaction of E3-A–anti-E3-T1 completely fused with the line from reaction of E3-T1–anti-E3-T1 (data not shown).

When reacted with antiserum against E3-T2A, homologous T2A and protein A gave strong precipitin lines, but heterologous ones did not (fig.3b). It is very interesting that neither E3 protein nor the complex of T2A and protein B gave precipitin lines when reacted with antiserum against E3-T2A. This might

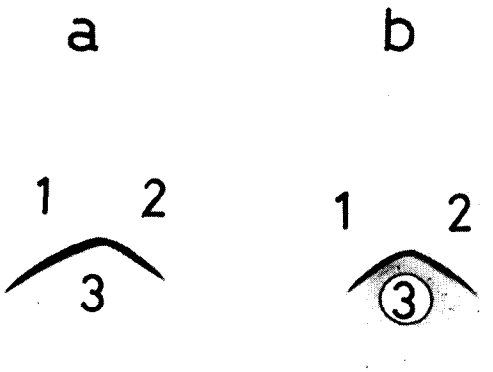

Fig.2. Immunochemical analysis of T1 fragments. (1)E3-T1 (1.4 A_{280}); (2)E2-T1 (1.3 A_{280}); (3)anti-E3-T1 serum (a) or anti-E2-T1 serum (b). Antisera were undiluted. The antibody and antigens were allowed to diffuse for 48 h at 4°C before fixing and staining.

indicate that antigenic sites on T2A are covered with protein B or, alternatively, conformational changes induced by protein B alter the antigenicity of T2A fragment. E3-B but not E2-B produced a precipitin line with antiserum against E3-B (fig.3a).

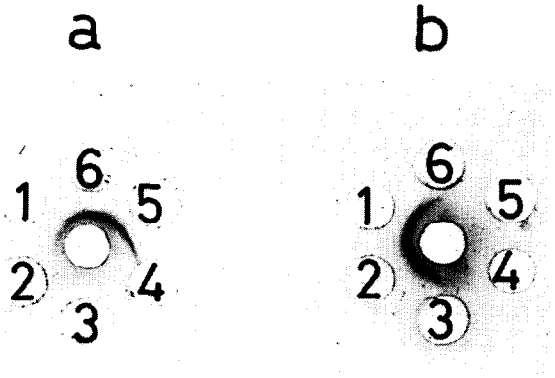

Fig.3. Immunochemical analysis of T2A and protein B. a : (1)colicin E3 (0.43 A_{280}); (2)colicin E2 (0.4 A_{280}); (3)E2-B (0.005 A_{280}); (4)E2-B (0.021 A_{280}); (5)E3-B (0.007 A_{280}); (6)E3-B (0.029 A_{280}). Anti-E3-B serum was layered in the central hole. b : (1)E3-T2A (0.038 A_{280}); (2)E3-A (0.82 A_{280}); (3)E2-A (0.76 A_{280}); (4)colicin E2 (0.4 A_{280}); (5)colicin E3 (0.43 A_{280}); (6)E2-T2A (0.062 A_{280}). Anti-E3-T2A serum was layered in the central hole.

4. Discussion

These results can explain the functional similarity and dissimilarity of colicins E2 and E3 on a structural basis. Colicins E2 and E3 share a very similar T1 region which may participate in binding to a common receptor, providing penetration into a cell. On the other hand, the structural differences observed for the T2A regions could explain the differences of intracellular functions of the two colicins. E2-B and E3-B, which are responsible for the immunity of respective colicinogenic cells, also have different structures.

Since the T1 and T2A regions and protein B are segregated in the molecular architecture of colicin E2 or E3 we may explain the above results on a genetic basis. Col E2 and Col E3 DNAs could arise from a common ancestor which differentiated by integrating different genes of nucleases and their inhibitors through recombination and insertion. It has been reported that heteroduplex of E2 and E3 DNAs contains only one heterologous region corresponding to 10–20% of total DNA [15]. It is expected that this heterologous region will code T2A and protein B.

Interestingly, colicins B and D share the common receptor, have similar amino acid compositions, and partially cross in serological reaction. But they are distinct in their modes of action and bacterial immunity [16]. Likewise, colicins Ia and Ib are very similar except that their serological cross is not complete and they never cross in bacterial immunity [17]. It is our hope that the similarity and dissimilarity of these pairs of colicins will be explained in a way similar to that noted here for colicins E2 and E3.

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