LACK OF CONCORDANCE BETWEEN PRIMARY STRUCTURE AND ANTIGENICITY IN THE CASE OF VARIOUS LYSOZYMES AND OF BOVINE α-LACTALBUMIN *.

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Despite the similarity of their primary structures, bovine α-lactalbumin and five different lysozymes, the structures of which differ gradually from that of hen egg-white lysozyme, present no antigenic reactivity. The respective roles of the primary and tridimensional structures concerning this absence of reactivity are shortly discussed.

1. Introduction

A large homology in amino acid sequence between hen egg-white lysozyme (EC 3.2.1.17) and bovine α-lactalbumin has been described [1]. Several comparisons have emphasized their similarities in properties reflecting molecular conformation [2–4]. However, significant differences have been observed by X-ray scattering in solution [5] and by studies concerning the reactivity of disulfide bonds [6], of tyrosine residues [6] and of free carboxylic groups [7]. Atassi et al. [6] tried to characterize eventual similarities of the tridimensional structures by immunological procedures but they were not able to detect cross reactions between these proteins and their antibodies. Jollès and coworkers published recently the primary structures of duck egg-white II [8] and human milk lysozymes [9], where 19 and around 50 amino acid replacements, respectively, were noted in comparison with hen lysozyme. Guinea-hen egg-white [10] and duck egg-white III [11] lysozymes were also nearly related enzymes, whereas goose egg-white lysozyme seemed to be different in many aspects [12]. We extended the studies of Atassi [6] to these five lysozymes of various origins and studied their immunological reactivity in relation to bovine α-lactalbumin. Two series of experiments were achieved; anti-bovine α-lactalbumin antiserum and anti-lysozyme (hen egg-white; human milk) antisera were successively employed.

* 75th communication on lysozymes. 74th communication, J.Saint-Blancard, P.Chuzel, Y.Mathieu, J.Perrot and P. Jollès, Biochim. Biophys. Acta (1970) in press.

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2. Materials and methods

Bovine α-lactalbumin and hen egg-white lysozyme were commercial samples (Mann and Worthington, respectively). Guinea-hen egg-white, duck egg-white II, duck egg-white III, goose egg-white, and human milk lysozymes were chromatographically pure samples prepared in Jollès' laboratory [9–12].

Rabbit antisera were prepared against lysozymes or bovine α-lactalbumin by injecting in the hind-foot pads 1 mg protein in the presence of 0.2–0.5 ml of complete Freund's adjuvant (Difco). One month later a booster injection was given. Immunodiffusion in

Table 1
Comparison between six lysozymes. Their reactivity against an anti-bovine α-lactalbumin antiserum

Lysozymes and α-lactalbumin	Primary structure		Antigenic similarities (see table 2)	Reactivity against an anti-α-lactalbumin antiserum	
	Amino acid replacements	Long identical sequences		Immunodiffusion	Inhibition of hemagglutination (Titer *)
Hen egg-white	reference material			0 (absence of reaction)	<1
Guinea-hen egg-white	about 10/129 [10]	numerous	very numerous	0	<1
Duck egg-white II	19/129 [8]	several, with a maximum of 17 amino acids	partial identity	0	<1
Duck egg-white III	about 20/129 [11]		partial identity	0	<1
Goose egg-white	very numerous	not determined	no cross reaction	0	<1
Human milk	52/129 1 insertion 1 deletion [9]	rare, with a maximum of 8 amino acids	no cross reaction	0	<1
Bovine α-lactalbumin	about 80/123 1 insertion 7 deletions [1]	rare, with a maximum of 3 amino acids	no cross reaction [6]	+++ (complete identity)	1024

* Titer: inverse of dilution. Titer 1 = 2 mg protein/ml. Titer $< 1 = > 2$ mg/ml.

1.5% agarose was performed at pH 7.5 (0.2 M tris-HCl buffer, containing 1.7% NaCl); the results were observed after 24 and 48 hr, before and after coloration with amidoschwartz.

The inhibition of hemagglutination was performed according to the procedure of Gordon et al. [13].

3. Results and discussion

In a first series of experiments (table 1), cross reactivities were noted between hen, guinea-hen, duck II, and duck III egg-white lysozymes, but not between hen and goose egg-white or human milk lysozymes. Furthermore no immunological reactivity could be observed between *all* the above lysozymes and an anti-bovine α-lactalbumin antiserum.

These various lysozymes with their nearly related enzymic activities and primary structures, constitute an excellent tool for the study of the immunological reactivity of several analogous proteins. Hen egg-white lysozyme possesses two main immunological areas [14, 15]: the "loop-peptide" (sequence with residues 57–107 and 2 disulfide bridges) and the fragment with the *N*- (residues 1–27) and *C*- (residues 122–129) terminal sequences connected with a disulfide bridge. In the "loop-peptides" from duck II and human milk lysozymes, 9 and 19 amino acid replacements, respectively, have been noted when compared to hen lysozyme, and a clear immunological cross reaction was only observed between hen and duck II lysozymes. Furthermore no reaction was noted between the various lysozymes and an anti-bovine α-lactalbumin antiserum. However all these proteins, with the exception of goose lysozyme, have nearly related primary structures with the same number of half-cystine residues situated in indentical positions.

Table 2
Reactivity of six lysozymes and of bovine α-lactalbumin in the presence of three different anti-lysozyme antisera.

Lysozymes or α-lactalbumin	1st anti-hen egg-white antiserum		2nd anti-hen egg-white antiserum		anti-human milk antiserum	
	I.D.	I.H.A. Titer	I.D.	I.H.A. Titer	I.D.	I.H.A. Titer
Hen egg-white	+++ (complete identity)	512	+++	2048	0 (absence of reaction)	<1
Guinea-hen egg-white	+++	128	+++	256	0	<1
Duck egg-white II	++± (partial identity)	4	++±	4	0	<1
Duck egg-white III	++±	2	++±	2	0	<1
Goose egg-white	0	<1	0	<1	0	<1
Human milk	0	<1	0	<1	+++	512
Bovine α-lactalbumin	0	<1	0	<1	0	<1

I.D. = immunodiffusion; I.H.A. = inhibition of hemagglutination. Titer, see table 1.

Table 2 indicates that no cross reactions could be observed between two anti-hen egg-white lysozyme antisera (from two rabbits) and an anti-human milk lysozyme antiserum on one side and bovine α-lactalbumin on the other side. The degree of similarity between the primary structures of bovine α-lactalbumin and hen egg-white lysozyme and of their main immunological areas are of the same order. The same order of similarity – but often in different sites – may be noted for human milk lysozyme. However the structural similarities are isolated and dispersed along the polypeptide chain; rarely longer identical fragments could be observed and these latter may be necessary to form common antigenic sites [16].

These observations might suggest two explanations: a) a common immunological reactivity does not exist between these different structures; in other words, the possible common sites are not reactive, or the tridimensional structures of the proteins are different; but the fact that no lysozyme was able to react with anti-α-lactalbumin antiserum, suggests the outstanding role of the structure in space; b) antibodies directed against tridimensional structures cannot demonstrate the presence of small common antigenic fragments. It might thus be probable that specific antibodies against a small antigenic site of one of these proteins may recognize an analogous sequence in one of the other proteins. Therefore it seems possible that experiments with peptide fragments obtained from these proteins will give an answer to this question.

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