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LYSINE-DERIVED CROSS-LINKS IN THE EGG SHELL MEMBRANE

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Summary

Egg shell membrane protein contains significant quantities of the lysine-derived aldehyde, allysine, and its aldol condensation product. NaB^3H_4 reduction followed by alkaline hydrolysis of purified protein revealed that there were six residues/1000 of both allysine and the reduced aldol while only traces of desmosine and isodesmosine were detected. The amino acid composition of the membrane protein did not resemble that of mammalian elastin.

Several findings have suggested that the egg shell membrane is composed of significant quantities of a protein, or proteins, which relate to the known mammalian connective tissue proteins. In this regard, the presence of the desmosines in trace quantities together with hydroxylated proline and lysine residues has been reported [1—3]. Most recently, lysyl oxidase activity has been demonstrated in the isthmus of the oviduct and was thus implicated in the desmosine formation in this membranous structure [4]. This present communication reports that the aldehyde derivatives of lysine, α-aminoadipic acid-8-semialdehyde, or allysine, and its aldol condensation product are present in significant quantities in these egg shell membranes.

Egg shell membranes were isolated from fresh non-fertilized Rhode Island red chicken eggs. A 2 cm^2 area of the membrane was washed in saline and suspended in a saturated solution of Na_2EDTA for 4 h. The membranous material was washed three times with distilled H_2O, then suspended in H_2O adjusted to pH 9.0 with NaOH. To this suspension, 25 mCi of NaB^3H_4 (New England Nuclear, spec. act. 282.0 Ci/M) were quickly added. The reduction reaction, which was maintained at pH 9.0, was allowed to proceed at room temperature

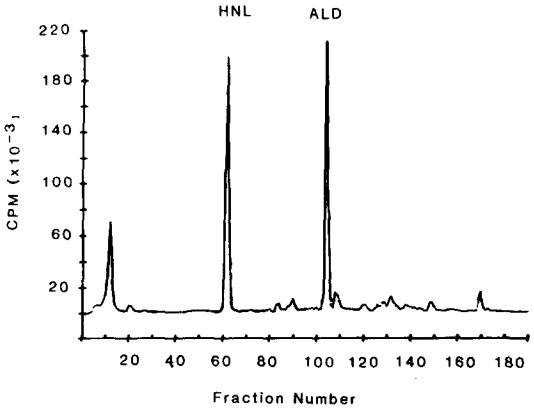

Fig. 1. The radioactive profile of NaB^3H_4-reduced egg shell membrane. HNL, ϵ-hydroxynorleucine; ALD, aldol condensation product.

for 1 h with occasional shaking. The reduced membranous material was washed three times with H_2O and then hydrolyzed in 2.0 M NaOH for 24 h at 108°C. The resulting hydrolysate was analyzed on a Technicon amino acid analyzer using a gradient system described previously for elastin [5]. A portion of the column effluent was collected and measured for radioactivity, and the radioactive profile is given in Fig. 1. The two major radioactive peaks obtained correspond to the reduced allysine and the aldol condensation product of two residues of allysine. If one assumes that a single tritium atom is incorporated into one allysine molecule to form ϵ-hydroxynorleucine or into one molecule of aldol product to form the reduced aldol, then one would conclude that approximately equal molar quantities of the two components are present in the membranous material. One of the other radioactive peaks co-elutes with lysinonorleucine (fraction number 170). The latter radioactive peak contains approx. 8% as much radioactivity as the aldol peak.

A portion of the EDTA-treated egg shell membrane (not reduced) was hydrolyzed, in vacuo, in 6 M HCl at 108°C for 20 h, and its amino acid composition is given in Table I. Also included in the analysis are the ϵ-hydroxynorleucine and aldol values as determined using ninhydrin when an aliquot of the reduced NaOH-hydrolyzed material was placed on a Beckman 119C amino acid analyzer. Again, these results indicate that there are equal amounts of the ϵ-hydroxynorleucine and aldol product. For comparison purposes, an analysis of the egg shell membrane reported by Baumgartner et al. [6] is also given in the table. While these data confirm that trace amounts of the desmosines are present, we have also detected significant amounts of allysine and its aldol condensation product. It has been suggested that collagen, elastin, or both of these proteins are present in the egg shell membrane. However, if the membrane is treated with 0.1 M NaOH at 98°C for 45 min [7], a standard procedure for purifying insoluble elastin from various tissues, the resulting residue bears no resemblance to elastin. Since the amino acid composition of the membrane is not compatible with either collagen or elastin, another protein component(s) which contains allysine and the aldol condensation product must be present.

The question of the desmosine formation in the egg shell membrane protein

TABLE I

AMINO ACID ANALYSIS OF EGG SHELL MEMBRANES

Values expressed as redisues/1000 residues. HNL, ϵ-hydroxynorleucine; ALD, aldol condensation product. The ninhydrin value has been divided by 2, since the reduced aldol has two leucine equivalents [5].

Amino acid	Shell membrane	Shell membrane (Baumgartner et al. [6])
Pro(OH)	13	—
Asp	82	81
Thr	62	62
Ser	74	68
Glu	108	110
Pro	105	105
Gly	109	103
Ala	42	40
Val	56	79
Cys	102	100
Met	36	34
Ile	26	32
Leu	44	47
Tyr	11	13
Phe	9	14
Lys(OH)	2	—
Lys	29	31
His	26	31
Arg	51	51
Desmosines	<0.2	
HNL	6	
ALD	6	

is important. It may be that the formation of desmosine is not critical to the biological functions of the protein, and it is formed as a byproduct or artifact. On the other hand, it may be that desmosine formation occurs over a relatively long period of time and the newly formed egg shell membrane will produce more desmosines with time. This will indeed be of interest, since no lysyl oxidase has been found to be associated with the membrane.

This report suggests that a membranous component produced in the chick egg shell contains lysine-derived aldehydes which may serve as cross-links by themselves or as precursors to desmosine. If the latter is true, then this membrane system would prove to be an ideal model in which to elucidate the mechanism of desmosine formation, since it is devoid of lysyl oxidase and is acellular.

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