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Short Communication

Localization of Transglutaminase in Adult Chicken Epidermis*

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Transglutaminases (E.C. 2.3.2.13) have been described in the epidermis of humans [1], cows [2] and chicken [3]. Since most transglutaminases are found in the cytosol, and are soluble in neutral buffers [4], the soluble transglutaminases have been studied extensively. We report that in adult chicken, feather-free epidermis (leg scales) transglutaminase occurs predominantly in an insoluble form and that it can be solubilized with a chaotropic solvent.

Transglutaminase in chicken leg epidermis is associated with the insoluble pellet (Table 1). Direct microtome separation of the epidermis from the dermis produces the same pattern of enzyme distribution as epidermis separated from dermis by heat separation (data not shown). Further homogenization of the pellet, followed by recentrifugation, releases very little additional transglutaminase activity into the second supernatant. Incubation of pellet material in 250 mM KSCN results in solubilization without significant loss of enzyme activity as noted in Table 2. Attempts to solubilize the enzyme from the pellet in 0.1 % and 1 % Triton X-100, 0.5 M sucrose, 0.15 M NaCl, 0.5 M n-butanol, and 0.001 % trypsin were unsuccessful.

The KSCN-solubilized enzyme is a transglutaminase as shown by its ability to covalently incorporate [1,4-^{14}C] putrescine into casein, and by the inhibition of the solubilized enzyme by EDTA and iodoacetamide. The effect of varying concentrations of iodoacetamide on the solubilized enzyme is shown in Table 3. 15.6 mM iodoacetamide completely inhibited enzyme activity. Preincubation of the KSCN solubilized enzyme with an equal volume of 200 mM EDTA at 37° C for time periods ranging from 5 to 30 min results in a complete loss of activity; a 25.5 mM excess of EDTA was present in the final assay mixture under these conditions. Assay of the KSCN-solubilized enzyme, in the absence of exogenous calcium, resulted in 52 % of the activity when compared to a control assayed in the presence of the 11 mM calcium (standard assay). Dialysis (24 h, 4° C) of the KSCN-

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Table 1. Distribution of transglutaminase activity in feather-free chicken epidermis

	Supernatant		Pellet	
	(cpm)	% of Activity	(cpm)	% of Activity
Exp. I	3916	3.8	98,700[a]	96.2[b]
Exp. II	–	–	195,675	100[b]
Exp. III	–	–	389,880	100[c]
Exp. IV	712	1.0	73,950	99[c]

[a] In a parallel experiment beginning with an identical weight of epidermis, the KSCN treated pellet had a total activity of 91,650 cpm

[b] Direct assay of pellet suspension

[c] Assay of KSCN solubilized pellet enzyme prepared as described below

Chicken skin was removed from the feather-free dorsal portion of chicken feet by cutting the skin from the bone. The dermis and epidermis were separated by heating the skin in 56° C tap water of 30 s, placing it on ice for 60 s, and removing the epidermis with a scalpel. The weighed epidermis was homogenized in 10 mM tris (pH 7.4, 5 ml/g of epidermis) with a Polytron (Brinkman Instruments) on ice, at speed 4, for 30 s, four times. The homogenate was centrifuged at 17,300 × g for 30 min at 4° C. The supernatant was decanted and reserved. The pellet was resuspended in 10 mM Tris (pH 7.4, 5 ml/g epidermis) by homogenization with the Polytron on ice, at speed 4, for 30 s, two times. The resulting suspension was used in further experiments.Transglutaminase activity was measured by the incorporation of [^{14}C] putrescine into α-casein (Worthington) by the method of Goldsmith and Martin [5]. The assay mix contained 1.65 mg α-casein per 0.5 ml assay, in 0.111 M glycine, 0.011 M $CaCl_2$, 4.8 mM dithiothreitol with 5 μM [1,4-^{14}C] putrescine (19.5 mCi/mmole, New England Nuclear). 0.5 ml of assay mix was added to 100 μl of the enzyme, and the mixture was incubated at 37° C for 30 min. The reaction was stopped by the addition of 0.6 ml 10% TCA, and the precipitate was collected on Whatman GF/C filters. The radioactivity was measured in 5 ml of Aquasol-2 (New England Nuclear) on a Packard 3375 scintillation counter. Activity is reported as counts per minute (cpm) after the subtraction of background. Values are the means of duplicate determinations unless otherwise noted.

Table 2. Effect of KSCN on chicken epidermal transglutaminase

Final Concentration of KSCN (mM)	Activity (cpm)
0	1974[a]
5	762
10	540
25	2120
50	1814
250	3666
500	2662

[a] 0.1 ml of suspended pellet assayed

A constant volume (0.3 ml) of homogenized enzyme pellet, resuspended in 10 mM Tris (pH 7.4) by rehomogenization on ice with a Beckman polytron at speed 4, for 30 s, two times, was added to 0.3 ml of KSCN to give the above final concentrations of KSCN. The mixtures were pre-incubated at 37° C for 30 min, the insoluble matter aggregated at the bottom of the tube, and the supernatants were decanted. 0.1 ml of each supernatant was assayed as previously described; since this 0.1 ml was derived from 0.05 ml of pellet, the cpm were multipled by 2 to derive the figures for activity in 5 – 500 mM KSCN concentration which are in the Table.

Table 3. Effect of iodoacetamide on chicken epidermal transglutaminase

Final Iodoacetamide Concentration (mM)	Activity (cpm)
0	1318
1.4	1121
3.3	1085
3.6	952
8.3	206
15.6	36

KSCN solubilized enzyme (100 µl) was added to 0.5 ml of standard transglutaminase assay mix [5] that did not contain [1,4-^{14}C] putrescine. Varying amounts of concentrated iodoacetamide were added, and the reaction allowed to pre-incubate at 25° C for 5 min. 10 µl of [1,4-^{14}C] putrescine was then added to each tube, and the reaction mixtures were incubated for 30 min at 37° C using the standard transglutaminase assay procedure [5].

solubilized enzyme against 10 mM Tris (pH 7.4) resulted in activity that was only 22% of the undialyzed activity, suggesting instability of the KSCN-solubilized enzyme under the given storage conditions or the removal of tightly-bound calcium needed for activity into the dialysis buffer.

Most tissues contain easily solubilized transglutaminase [4]; however, particulate transglutaminase has been described by Birkbichler et al. [6] in normal and virus-transformed human and hamster cells and in chemically transformed mouse cells. It is suggested that insoluble transglutaminase may be related to the role this enzyme has in stabilizing or cross-linking intracellular proteins or membrane bound proteins [7]. Our findings of insoluble transglutaminase activity in adult chicken epidermis indicates that previous studies on the induction of chicken embryo epidermal transglutaminase [3] may need to be reininterpreted since the reported increased activities of soluble transglutaminase may reflect translocation of enzyme activity within the cell rather than new synthesis. Insoluble-soluble translocations may be a mechanism for compartmentalizing or controlling transglutaminase activity in vivo. In addition, since almost the entire scale of the chicken foot is composed of cells which produce a β-feather-like keratin [8], the presence of considerable transglutaminase activity in this tissue suggests that epidermal transglutaminases are not restricted to tissues synthesizing α-fibrous proteins.

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