ALTERATIONS OF POST-TRANSLATIONAL MODIFICATIONS OF PROCOLLAGEN BY SV40-TRANSFORMED HUMAN FIBROBLASTS

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1. Introduction

It is now well known that collagen is initially synthesized as a precursor called procollagen, which then undergoes a variety of post-translational modifications to yield collagen. These modifications include hydroxylation of proline and lysine residues, glycosylation of certain hydroxylysine residues, and cleavage of the carboxy- and amino-terminal peptide extensions from the procollagen molecule. Although the procollagen intermediates are short-lived in vivo, their conversion into collagen is relatively slow in cells growing in culture [1]. Normal human dermal fibroblasts growing in culture are known to secrete procollagen into the medium [1–6]. These cells also secrete procollagen peptidase(s) which cleave the terminal peptide extensions of procollagen to form collagen [7]. Viral transformation of cells in culture is known to result in changes in growth patterns, specific activity of enzymes, cell surface, and nuclear and membrane associated antigens [8]. This communication gives evidence that viral transformation also affects the postsynthetic modification of procollagen. Those activities that appear to be altered by transformation include procollagen peptidase activity as well as the extent of lysine hydroxylation and glycosylation in the procollagen molecule.

2. Materials and methods

An SV40-transformed human fibroblast line (GM637) and its untransformed normal diploid fibroblast parent (GM37) were obtained from the Inst. Med. Res. Camden, NJ. The cells were grown as monolayers either in plastic tissue-culture dishes or roller bottles in Waymouth medium 752/1 (Gibco) supplemented with 10% fetal-calf serum and 100 μg/ml ascorbic acid, without antibiotics. When the cells formed monolayers, the medium was changed to either serum-free 752/1 supplemented with 100 μg/ml ascorbic acid and 20 ng/ml growth tripeptide (Calbiochem) or with Dulbecco's modified Eagle's medium (DME) minus serum, plus 100 μg/ml ascorbic acid, 20 ng/ml growth tripeptide and 1 μCi/ml [^{3}H]proline. The culture medium was removed from the cells after 2–3 days incubation and the procollagens and collagens were purified through DEAE-cellulose chromatography as reported [9].

3. Results and discussion

DEAE-cellulose chromatography of procollagen isolated from the culture medium of normal fibroblasts (GM37) is seen in fig.1A. Fractions pooled as indicated were further analyzed by SDS polyacrylamide gel electrophoresis. Sensitivity of these fractions to collagenase and pepsin, and their cross-reactivity with antisera prepared against purified type I and type III human procollagens were also tested. Peak 1, which eluted from the column in the buffer wash, was found to contain collagen molecules composed of α1 and α2 chains approx. 95 000 mol. wt. Peak 2 contained type I procollagen having a chain composition of [pro α1(I)]$_2$pro α2 at a purity of more than 95% as judged by collagenase sensitivity (fig.2A).

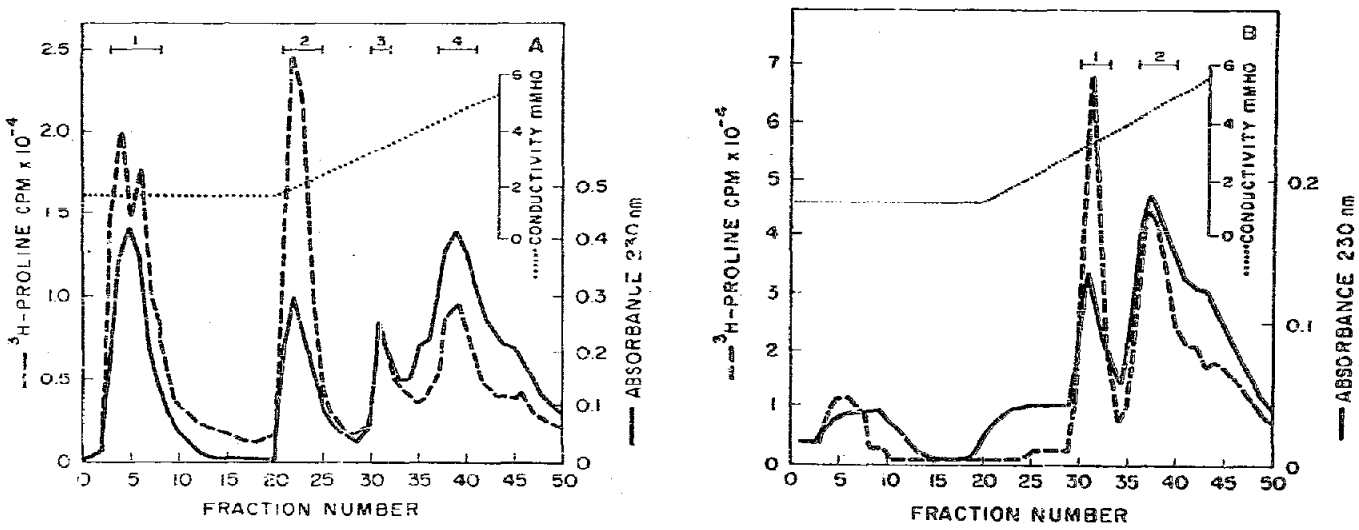

Fig.1A,B. DEAE-cellulose chromatography of partially purified procollagens obtained from (A) normal fibroblasts (GM37); (B) SV40-transformed GM37 (GM637), grown as described in the text. Procollagens were purified from serum-free culture media from the cells as described [9]. This material was dissolved in 0.05 M Tris–HCl (pH 7.4) containing 2 M urea and 0.3 M NaCl (limiting buffer) and dialyzed against 0.05 M Tris–HCl (pH 7.4) containing 2 M urea (starting buffer) and applied to a column (1.5 × 20 cm) of DEAE-cellulose equilibrated in the starting buffer. The column was developed with a linear gradient of limiting buffer (conductivity 17 milli MHO) using a Gilson Mixograd automatic gradient former. The fractions in individual peaks were separately pooled, dialyzed extensively against distilled water and lyophilized.

Pro α1 and pro α2 chains migrated with approx. mol. wt 120 000 in SDS–polyacrylamide gels. Limited pepsin digestion of the peak 2 procollagen fraction yielded α1 and α2 chains approx. 95 000 mol. wt having an α1:α2 ratio of 2:1.

More than 90% of the protein eluting in peak 3 (fig.1A) were collagenous and reacted both with type I and type III antisera. Although peak 4 had type III procollagen with no type I contamination (fig.2B), the material was only 75–80% pure and was contaminated with non-collagenous proteins. Procollagens purified from SV40-transformed cells (GM637) and chromatographed on DEAE-cellulose (fig.1B) eluted at a different ionicity on DEAE-cellulose than procollagens from the normal cells, indicating that they differed in their net charge. Also, no collagen peak was observed (the small peak which was excluded from the column, was found to be non-collagenous). Peak 1 (fig.1B) contained type I procollagen at more than 95% purity, however, the pro α chains migrated in the mol. wt 150 000 [pro α1(I)] and 120 000 (pro α2) regions on SDS–polyacrylamide gels (fig.2C). The α-chains obtained by limited pepsin hydrolysis of this procollagen migrated as chains with mol. wt 10% larger than collagen α-chains from normal cells (fig.2C). Peak 2 (fig.1B) was found to contain type III procollagen having pro α chains which migrated on SDS–gels in the β region (mol. wt 150 000). Pepsin hydrolysis produced collagen with α1 chains which appeared 10% larger in gels than normal type III collagen (fig.2D). This peak was contaminated with approx. 20% noncollagenous protein but was free from type I procollagen as judged by the absence of α1 bands on polyacrylamide gels and also the absence of any reaction with anti-type I antiserum.

Amino acid composition of type I collagen of GM37 was very similar to that reported for type I collagen from skin (table 1). The composition of pepsin-treated type III collagen of GM37, differed slightly from that reported for skin type III collagen [11], probably due to the presence of minor contaminants even after pepsin treatment and re-precipitation of collagen. However, the amino acid analyses

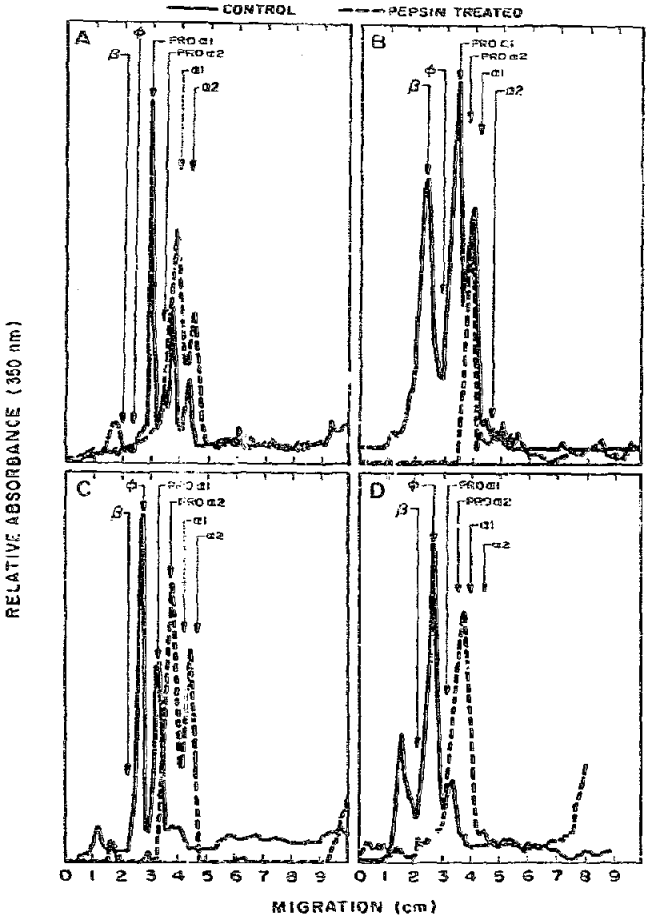

Fig.2. SDS–polyacrylamide gel electrophoresis of the procollagen fractions purified by DEAE-cellulose chromatography in fig.1. (A) Peak 2 from fig.1A; (B) peak 4 from fig.1A; (C) peak 1 from fig.1B; (D) peak 2 from fig.1B. Lyophilized samples, 50 μg (solid line), or 50 μg pepsin-treated samples (dashed line) were prepared for SDS–polyacrylamide gel electrophoresis as described [9] and electrophoresed on 4% polyacrylamide gels (Bio-Rad) until the dye marker reached the bottom of the gel. The gels were stained with Coomassie blue, destained and scanned.

indicated a significant difference in the degree of hydroxylation of lysine residues between normal and transformed cells. The transformed cells demonstrated a 2-fold increase in hydroxylysine residues both in the type I and type III collagens when compared to normal collagens. Interestingly, there was also a 2-fold increase in the total glycosylation of collagen hydroxylysine residues from the transformed cells as compared to normal (table 2). However, since the transformed cell collagens had twice as many residues of hydroxylysine as normal, the total percent glycosylation of the hydroxylysine residues did not differ significantly (table 2). The amount of hydroxylation of prolyl or lysyl residues changes the migration of α-chains on SDS polyacrylamide gels [12]. The increased hydroxylation observed in the transformed cell collagens probably accounts for the apparent higher molecular weight observed in fig.2C and 2D.

Transformed cells, unlike normal fibroblasts, exhibited a lack of procollagen peptidase activity, as judged by the accumulation of only uncleaved procollagen molecules in the culture media either with or without serum. Procollagens isolated from the trans-

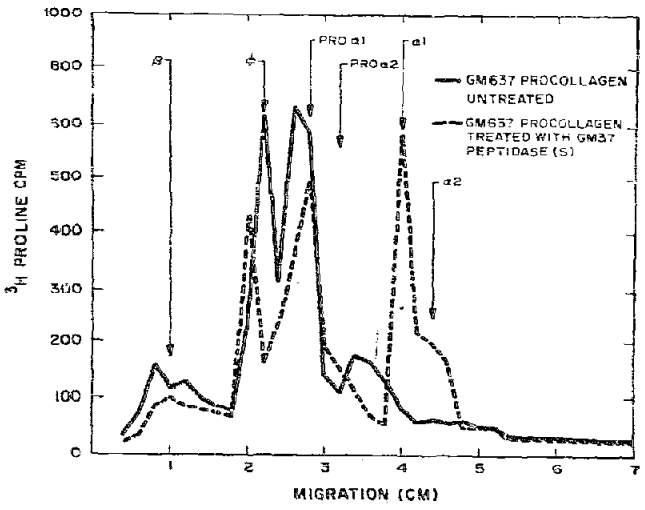

Fig.3. SDS–polyacrylamide gel electrophoresis of partially purified type I procollagen from SV40-transformed human fibroblasts (GM637 solid line) and of GM637 procollagens treated with a partially purified procollagen peptidase fraction obtained from the medium of normal human fibroblasts (GM37, dashed line). The partially purified procollagen peptidase fraction was obtained by the method [7]. The reaction mixture containing 90 μl substrate (5000 cpm procollagen from GM637) and 10 μl crude enzyme was incubated at 37°C for 2 h. The reaction was stopped by adding 10 μl 10X SDS sample buffer and heating to 100°C for 3 min. Electrophoresis of these samples were carried out as described for fig.2. Gels were fractionated into 1 mm sections using a Gilson automatic gel fractionator. Each fraction was collected and extracted in 200 μl 10% SDS and the radioactivity was determined.

Table 1
Amino acid composition of procollagens and collagens (pepsin-treated procollagens) purified from culture medium of normal human fibroblast (GM37) and SV40-transformed GM37 (GM637)

	Expressed as residues amino acids/1000 residues							
	Type I procollagen [pro α1(I)]$_2$ pro α2		Type I collagen [α1(I)]$_2$ α2			Type III collagen [α1(III)]$_3$		
	GM37	GM637	Human[a] skin	GM37	GM637	Human[b] skin	GM37	GM637
Hydroxyproline	78	83	91	98	102	121	113	116
Aspartic acid	53	55	47	45	45	48	52	53
Threonine	23	24	18	18	18	15	19	18
Serine	37	40	37	34	34	41	46	43
Glutamic acid	75	78	78	73	72	71	77	76
Proline	110	114	125	114	108	102	89	90
Glycine	300	299	324	336	327	355	336	340
Alanine	112	105	115	108	116	92	87	92
Half cystine	3.4	3.3	–	–	–	2.4	–	–
Valine	27	24	25	25	25	16	20	18
Methionine	6.4	7.2	7.0	5.2	4.7	6.8	5.8	7.1
Isoleucine	16	14	10	12	12	13	14	16
Leucine	34	34	25	27	28	21	26	27
Tyrosine	12	8.7	3.5	3.0	3.5	1.6	6.3	5.4
Phenylalanine	20	16	13	10.3	13	7.8	7.8	7.7
Histidine	7.3	7.0	5.4	5.6	5.5	6.1	11.0	8.0
Hydroxylysine	8.0	8.1	5.9	8.1	17.5	5.3	8.7	17.6
Lysine	30	30	27	26	18	30	34.4	19.0
Tryptophan	1.0	0.4	0	0	0	–	0	0
Arginine	47	48	49	48	48	46	47	47

[a] As reported [10]
[b] As reported [11]

Table 2
Hydroxylysine glycosides in purified type I collagens from normal and transformed fibroblasts

Cells	Glc–Gal–Hyl	Gal–Hyl	Total substituted Hyl	Unsubstituted Hyl	Glycosylation of Hyl (%)
Normal human fibroblast (GM37)	1.16	0.65	1.81	6.3	22.3
SV40-Transformed fibroblast (GM637)	2.3	1.57	3.87	11.97	24.2

Values are presented as residues per 1000 residues of amino acids

formed cells were converted into collagen molecules by a crude procollagen peptidase preparation (fig.3), indicating that transformed procollagens are sensitive to procollagen peptidase.

It is evident from this communication that SV40 virus transformation of human skin fibroblasts results in altered post-translational modifications in type I procollagen and probably also in type III procollagen. Transformation has been known to cause morphological as well as biochemical alterations in eukaryotic cells [8]. If biochemical alterations due to virus transformation mimic some of the connective tissue disorders, these cells will make a useful model for investigations of such metabolic disorders. Also, these alterations in procollagen post-translational modification following virus transformation may play a role in neoplasia and tumorogenesis.

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