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Secretion and intracellular degradation of collagen in cultures of normal and SV-40-transformed human fibroblasts

A.E. Berman and G.E. Morozevich

Institute of Biological and Medical Chemistry, USSR Academy of Medical Sciences, Moscow 119832, USSR

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With a pulse-chase technique, secretion and intracellular degradation of collagen were investigated in human cultured normal and SV-40-transformed fibroblasts. Normal cells at a proliferative phase of growth secreted collagen more actively than the stationary phase (resting) cells. Transformed fibroblasts secreted protein at a lower rate than both normal cell types. Resting and dividing normal cells displayed no differences in the rates of intracellular collagen degradation at the various stages of the chase period. Transformed cells did not differ from the normal ones in collagen degradation rates at the first hour of the chase period while at later times in the chase period, the total amount of degraded collagen was reduced by 20–30% in the transformed vs normal cultures. The data are discussed in the view of possible relations between the various mechanisms of intracellular transport and degradation of collagen.

Fibroblast; Oncotransformation; Collagen; Secretion; Intracellular degradation

1. INTRODUCTION

Intracellular degradation of proteins is one of the stages of their posttranslational processing that appears to play an important role in the maintaining of the total balance of secretory proteins including collagens [1]. It was established that oncogene transformation of cultured fibroblasts is accompanied by a sharp decrease in collagen synthesis that results in a marked reduction of the total amount of collagen produced by these cells [2,3]. The role of intracellular degradation of collagen in alterations of its production upon cell transformation is actually unclear.

At present, data are available indicating that a correlation exists between the rates of intracellular degradation and secretion of proteins [1,4]. The parameters of this correlation are different for distinct proteins and depend to a marked degree on the types and physiological conditions of cells.

In the present study, it was established that oncovirally transformed fibroblasts differed from normal ones in the rates of secretion and intracellular degradation of collagen.

2. MATERIALS AND METHODS

Strains of normal diploid human embryo fibroblasts between 18 and 22 passages and established lines of SV-40-transformed human embryo fibroblasts were supplied by the Institute of Medical Genetics of the USSR Academy of Medical Sciences and by the All Union Oncologic Centre of the USSR Academy of Medical Sciences, respectively. Cultures were grown in Dulbecco's modified Eagle's medium (DME) supplemented with 10% fetal calf serum and 1 g/ml kanamycin. For serial subcultivations, normal and transformed fibroblasts were plated at a density of 1.5×10^6 cells per 80-mm tissue culture plastic dish followed by incubation in 5 ml of DME medium with supplements at 37°C in a humidified atmosphere containing 5% CO_2. The normal cells were analyzed in both logarithmic (exponential) and stationary phases of growth for which purpose the cultures were maintained for 24 h and 4 days, respectively, before labelling the cells. Intracellular degradation of collagen was measured in 'pulse-chase' experiments as described [4]. Briefly, the cultures were incubated for 3 h in 5 ml of DME medium without serum but in the presence of ascorbate (50 g/ml), β-aminopropionitrile (50 g/ml), and L-[^{14}C]proline (24 mCi, 200 mCi/mmol). After labelling the radioactive incubation medium was removed, cell layers were washed 5 times with warm (37°C) phosphate-buffered saline and subsequently incubated with 5 ml of chase medium (identical to incubation medium except that 1 mM unlabelled proline was substituted for labelled proline). At various time points of chase, the incubation media were removed and supplemented with inhibitors of proteases [5]. Cells were scraped off the culture plates with a rubber policeman in ice-cold 1 M NaCl-Tris buffer, pH 7.6, and sonicated. Combined media and cell suspensions were heated at 100°C for 10 min to inactivate the proteases. Collagen degradation was measured by determining the amount of dialyzable [^{14}C]hydroxyproline in distinct samples and expressed as a percentage of dialyzable hydroxyproline radioactivity of total (nondialyzable and dialyzable) hydroxyproline radioactivity [4]. Secretion of collagen was calculated from the formula: secretion (%) = [(cpm) of [^{14}C]hydroxyproline in the incubation medium] × 100/[(cpm) of total [^{14}C]hydroxyproline in the sample]. Separation of radiolabelled hydroxyproline and proline was performed by ion exchange chromatography on Dowex 50 × 8 [6].

Correspondence address: A.E. Berman, Institute of Biological and Medical Chemistry, Pogodinskaya St. 10, Moscow 119832, USSR

3. RESULTS AND DISCUSSION

Data in Fig. 1A show that activity proliferating normal fibroblasts exhibited significantly higher levels of collagen secretion than resting cells. But the ranges of these differences varied at distinct steps of the chase

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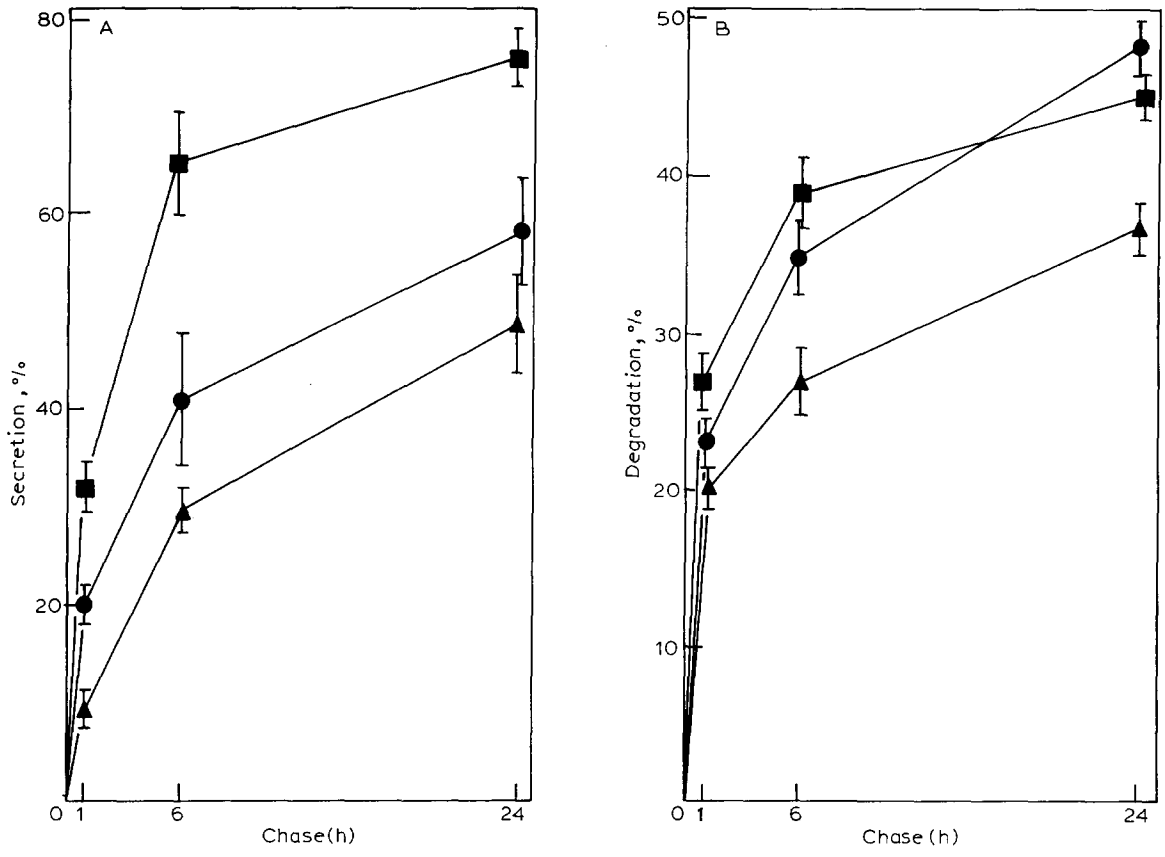

Fig. 1. Secretion (A) and intracellular degradation (B) of collagen in the cultures of normal proliferating (■) and resting (●) fibroblasts and SV-40-transformed (▲) fibroblasts. The data points represent the mean (± SEM) of 3–5 separate experiments. The degradation data are corrected for the amount of dialyzable [^{14}C]hydroxyproline remaining in the cells after pulse labelling.

period. As illustrated in Fig. 1A, the differences in the rates of collagen secretion between dividing and resting fibroblasts were less pronounced during the first hour of the chase period than at the later time interval (between 1 and 6 h). Further, exponentially growing fibroblasts actually completed secretion of collagen up to the sixth hour of the chase period whereas the resting cells appeared to continue collagen secretion for a much longer time period (Fig. 1A).

Our results are consistent with the data indicating a biphasic character of collagen secretion in cultured fibroblasts [7]. Based on the analysis of collagen secretion rates in primary cultures of chick embryo tendon fibroblasts, the author revealed quickly and slowly secreted collagen fractions of which the former was shown to be prevalent. Thus, our results suggest that of two types of normal cells, those occurring at proliferative stages are most abundant in quickly secreted collagen. This assumption is not in accord with the study cited above [7] indicating that in the primary culture of tendon fibroblasts about 25% of collagen is secreted slowly when cells are grown at low density but only 8% is secreted at a slow rate in high-density cultures. One of the reasons for these discrepancies would be that primary cell cultures perhaps differ in some way from the cell lines passed through many population doublings in mechanisms of intracellular transport and secretion of collagen. From Fig. 1A it is also evident that irrespective of the growth phase, the normal fibroblasts exhibited higher rates of collagen secretion than SV-40-transformed cells. The most pronounced differences in collagen secretion in cultures studied were those observed during the first hour of the chase period when percentages of collagen secreted from transformed fibroblasts were 3- and 2-times lower than from normal dividing and resting cells, respectively. In terms of biphasic kinetics of collagen secretion, the above results would imply that in normal proliferating cells, collagen is mostly represented by the quickly secreted fraction whereas in transformed cells, the slowly secreted fraction is apparently prevalent.

Before discussing our data on the relationship between secretion and intracellular degradation of collagen in the normal and transformed fibroblast cultures, it is reasonable to consider the results obtained by other authors. It has been established that the rate-limiting step in the protein pathway in both normal and neoplastic cells is the translocation of proteinous products from the rough endoplasmic reticulum (ER) to the Golgi complex. A further step,

represented by the vesicular transport of secreted material from the Golgi to the extracellular space, occurs at a rate several times higher than that of the previous step [8]. Intracellular degradation of collagen in fibroblastic cells is supposedly governed by two separate mechanisms: (i) the basal degradation that occurs in either ER or the Golgi complex which does not involve lysosomes; and (ii) lysosome-dependent degradation that operates for the removal of abnormal collagen and occurs during the vesicular transport of proteins to the plasma membranes [1,9].

As may be seen from Fig. 1A,B, in all the cell types studied, 50–70% of collagen is secreted and 30–40% is degraded during the 24-h chase period. It may be suggested, therefore, that collagen degradation observed in our studies is the result of cleavage by extracellular proteases secreted into the culture medium rather than by intracellular proteases.

To test this suggestion, the pulse-labelled culture media were diluted in separate samples by an equal volume of media obtained after a 24-h incubation of distinct cell types in the presence of unlabelled proline (instead of labelled proline), the mixtures were incubated at 37°C for an additional 24-h period, and dialyzable [^{14}C]hydroxyproline was determined. Control samples were heated at 100°C immediately after mixing labelled and unlabelled media. It was found that upon prolonged incubation of labelled medium with unlabelled medium (used as a possible source of extracellular proteases), no increase in the dialyzable [^{14}C]hydroxyproline was observed (data not shown). Thus the collagen degradation revealed in this study is an intracellular event.

As may be seen from Fig. 1A and B, normal fibroblasts at various growth phases exhibited significant differences in collagen secretion rates, although they virtually displayed no differences in the rates of collagen degradation. This observation suggests that in normal cultures, the rates of collagen degradation do not depend on the rates of its secretion. This conclusion is consistent with the data obtained on the primary avian fibroblasts [9].

The close levels of collagen degradation in the normal cells at various growth phases were seen at both the earlier and the later steps of the chase period (Fig. 1B), i.e. when there were different proportions between radioactive proteins accumulated in ER and the Golgi complex and those passed through the quick step of secretion.

Other relations were found when normal and transformed cultures were compared. At an early step of the chase periods (i.e. when there was a small contribution of lysosome-dependent degradation into the overall degradation of collagen), these cells displayed significant differences in the rates of collagen secretion (Fig. 1A) but negligible differences in the rates of collagen degradation (Fig. 1B). However, as the amount of secreted collagen increased (which occurred at the later steps of the chase period), the total amount of degraded collagen in transformed cultures was reduced by 20–30% ($P < 0.05$) as compared to the normal cells. Our results may imply that those mechanisms of intracellular degradation of collagen, which occur at the quick phase of its secretion and are likely to be lysosome dependent, are less active in the transformed cells as compared to their normal counterparts.

Krieg et al. observed a 3–8-fold decrease in collagen synthesis and a slight increase in the intracellular collagen degradation in SV-40-transformed human fibroblasts as compared to their normal controls [10]. We have found in our previous experiments carried out on the same cell strains as in the present study, a 3-fold reduction in the rates of synthesis of collagenase digestible [^{14}C]peptides per cell in SV-40-transformed fibroblasts [3]. This was confirmed in the present experiments where it was indicated that the amount of [^{14}C]hydroxyproline peptides accumulated per cell in transformed cultures was 3–4 times lower than in both log and stationary normal cultures (data not shown). Discrepancies of our data on collagen degradation with those in the paper cited probably resulted from the fact that the authors determined dialyzable hydroxyproline immediately after labelling the cells, whereas in our study, a pulse-chase approach has been used. Therefore, in the cultures analyzed by the authors, the pool of [^{14}C]hydroxyproline is mainly represented by the peptides accumulating in the ER and Golgi vesicles; this accumulation would mask the differences in the degradation of collagen at a later step of each secretion between the normal and transformed cells.

In order to investigate whether differences in collagen degradation observed in the cultures studied are the consequence of structural abnormalities of collagen molecules, the levels of hydroxylation of proline residues in collagen of all cell types were determined. For this purpose, the purified radiolabelled collagen preparations were hydrolyzed and assayed for radiolabelled proline and hydroxyproline contents. There were no visible differences in the hydroxyproline/proline ratios between normal (resting and dividing) and transformed fibroblasts (data not shown). In all of the 3 cell types, the above ratios were found to be 0.9q 0.02 ($P < 0.02$), the values being close to the maximal possible degree of hydroxylation of the proline residues in collagen.

At present it is difficult to propose an unequivocal mechanism responsible for the diminished intracellular collagen degradation in SV-40-transformed fibroblasts. Of the possible explanations, one may be that the reduced production and/or altered activity of lysosomal proteases are the specific features of these particular cells. Another possibility is that intracellular transport of collagen from the ER to the plasma membranes in the transformed cells may proceed not only

via vesicular but also nonvesicular mechanisms. In this case, some of the transported molecules would escape the fusion with the lysosomes and consequently would be prevented from the cleavage by lysosomal proteases. Verification of these assumptions needs further experiments.

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