Intermediates in the conversion of prekeratin into keratin molecules in human epidermis

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In order to elucidate the relationship between prekeratin and keratin, we performed pulse-chase experiments using [^{35}S]methionine (^{35}S-Met) in vitro. Of 6 prekeratin molecules (49, 52, 55, 62, 69 and 71 kDa) that incorporated ^{35}S-Met, the 55-kDa prekeratin incorporated the most ^{35}S-Met. In 3 molecules (52, 55 and 62 kDa) incorporation was decreased at 30 min after being chased; however, incorporation of only two molecules (55 and 62 kDa) of the 6 prekeratins was increased at 60 min. From these results and our previous data, we conclude that the initial stage of processing is as follows: 3 prekeratin molecules (52, 55 and 62 kDa) are first cleaved in the N-terminal region, then two prekeratin molecules (55 and 69 kDa) are processed to intermediates (52 and 62 kDa) by some proteolytic enzyme(s).

Prekeratin *Keratin* *Intermediate* *Conversion* *Keratinization*

1. INTRODUCTION

It is known that keratin (the filament protein of the stratum corneum) is converted from prekeratin (the tonofilament protein of the living epidermis) to cause post-translational modification in the granular cell layer in the epidermis [1–4]. Although the biochemical and biophysical properties of both prekeratin and keratin molecules have been studied [5–11], the relationship between prekeratin and keratin is poorly understood. In this paper, we performed pulse-chase experiments to determine the initial stage of the processing of prekeratin into keratin molecules during the terminal differentiation.

2. MATERIALS AND METHODS

L-[^{35}S]Methionine (1070 Ci/mmol) and EN^{3}HANCE were purchased from New England Nuclear, Boston, MA. Dulbecco MEM in powdered form and fetal calf serum were purchased from Osaka Dainihon, Osaka, Japan.

Abbreviations: SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; 2-ME, 2-mercaptoethanol; MEM, minimum essential medium; PMSF, phenylmethylsulfonyl fluoride

2.1. *Incorporation of ^{35}S-Met into prekeratin*

Fresh, whole human skin was obtained from surgical operations and used immediately. In order to obtain the epidermis, the sample was sliced with a keratotome into 100-μm thick sections and the epidermis was minced with scissors into fragments of about 3 mm^2 × 0.1 mm. The specimens were incubated in a flask containing 500 μCi of ^{35}S-Met in 2 ml of Dulbecco MEM supplemented with 30% fetal calf serum (pH 7.4), for 4 h at 37°C with gentle shaking.

2.2. *Pulse-chase experiment*

After incubation for 60 min at 37°C in the above medium containing ^{35}S-Met, the labelled intact specimens were chased for various lengths of time by incubation in the same medium containing 20 mM unlabelled L-methionine (about 400-fold excess). At each time point (30 min–6 h), one specimen was removed and frozen immediately at −80°C.

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2.3. *SDS–PAGE and fluorography*

To extract the prekeratin and keratin from the epidermis, the specimen was homogenized with 1 ml of 10 mM Tris–HCl (pH 7.6), 10 mM EDTA containing 10 mM PMSF. The homogenate was centrifuged at 20000 × *g* for 30 min at 4°C. The resulting pellet obtained was washed 5 times with the same buffer and the pellet obtained was incubated with 8 M urea, 50 mM 2-ME, 50 mM Tris–HCl (pH 9) for 3 h at 37°C. After centrifugation at 20000 × *g* for 30 min, the supernatant was dialyzed against 5 mM Tris–HCl (pH 9), 25 mM 2-ME for 48 h at 4°C, and then lyophilized. The samples were extracted with SDS–buffer as described [12] at 37°C overnight and then subjected to SDS–PAGE (8.5% acrylamide) as in [12]. After staining with Coomassie brilliant blue R-250, fluorography was performed as in [13], using EN^{3}HANCE. Each band of SDS–PAGE gel which corresponded to one in the fluorographic X-ray film was cut out and solubilized with 30% H_2O_2 at 60°C for 6 h in a tightly closed vial, and counted in 6 ml of NT scintillator [14].

3. RESULTS AND DISCUSSION

The time course of incorporation of ^{35}S-Met into prekeratin in the pulse-chase labelling experiments is shown (fig.1). Pulse labelling for 60 min followed by chase for various time periods with unlabelled methionine showed that no further ^{35}S-Met incorporation occurred after the cold methionine chase in the fractions of tissue homogenate and prekeratin.

A small amount of ^{35}S-Met was detected in the prekeratin at 30 min and the incorporation increased considerably during further incubation. The results of fluorography after SDS–PAGE are presented in fig.2. As for synthesis of prekeratin molecules, it was reported as in [15] that each prekeratin was translated from mRNAs corresponding to those of prekeratin molecules in the basal layer of the epidermis. In our case, it may be considered that rate of synthesis among the prekeratin molecules was the highest in the 62- and 55-kDa prekeratin molecules at 30 min (fig.2A). Taking these results and mRNA into account, the transla-

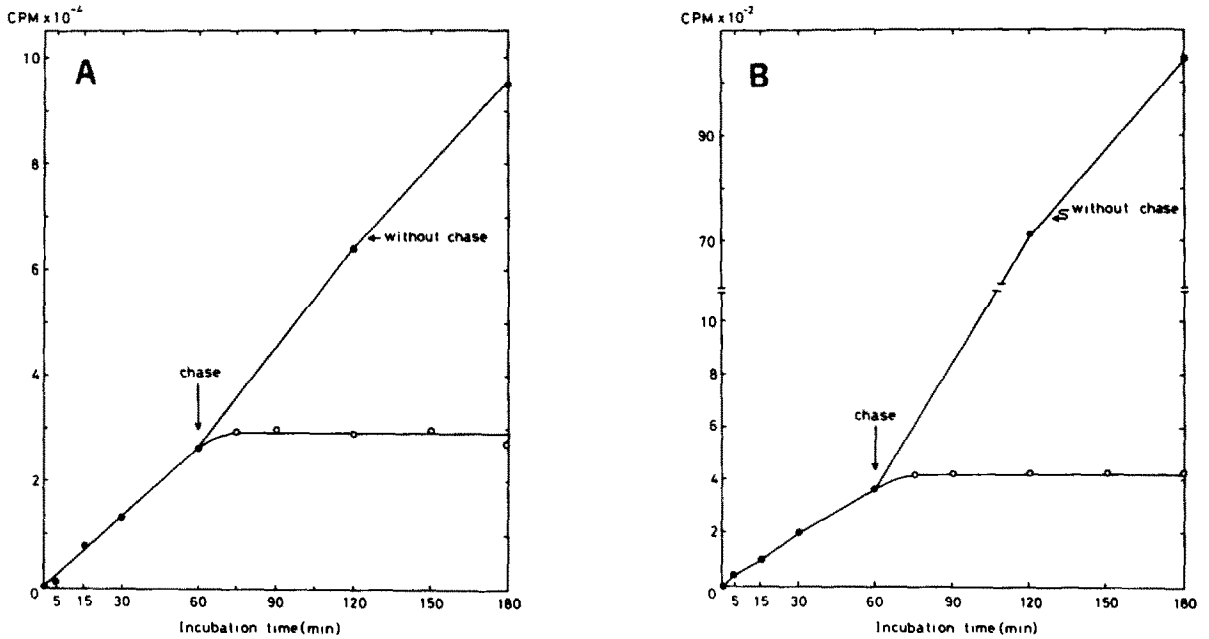

Fig.1. Time course of L-[^{35}S]methionine incorporation into prekeratin. Human epidermis specimens (about 3 mm^2 × 0.1 mm) were labelled with ^{35}S-Met (500 μCi/2 ml) at 37°C for the times indicated. For chase experiment, the specimens were transferred to fresh incubation medium containing a roughly 400-fold excess of unlabelled methionine after pulse labelling for 60 min, and incubated for the indicated times. (A) Tissue homogenate fractions of ^{35}S-Met incorporated. (B) Prekeratin fractions of ^{35}S-Met incorporated.

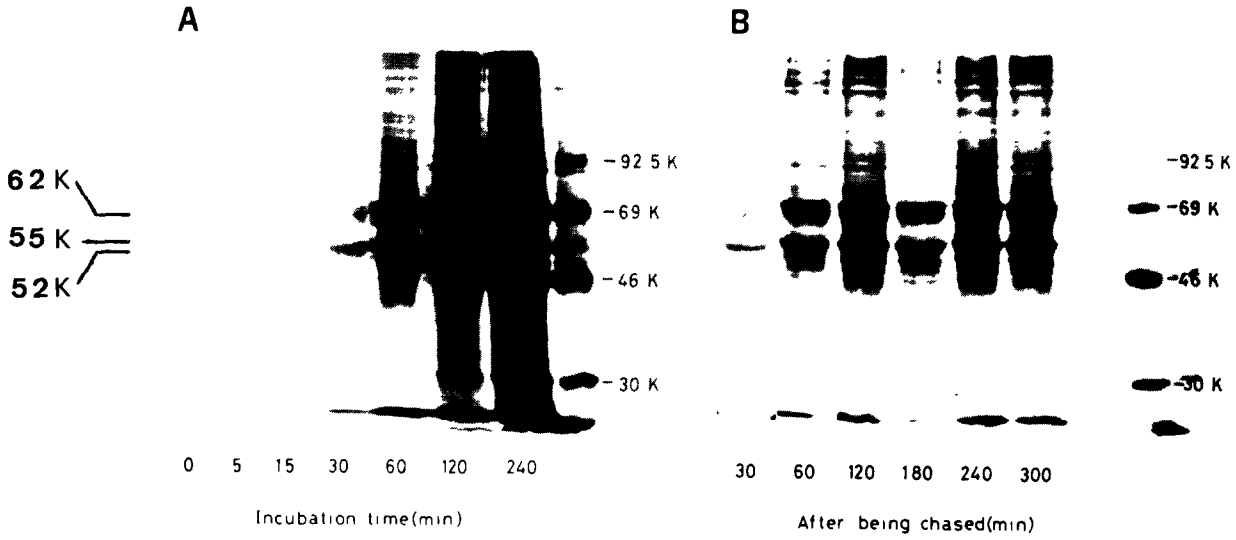

Fig.2. Fluorographs of incorporation of [^{35}S]methionine into prekeratin molecules and pulse-chase experiment. (A) Time course of incorporation of ^{35}S-Met. (B) Changes in ^{35}S-prekeratin molecules after being pulsed for 60 min.

tion product may be considered to be due to either a large quantity of the particular mRNA or a higher rate of synthesis of that mRNA.

Each band in the SDS–PAGE gel which corresponded to one in the fluorographic X-ray film was cut out and solubilized, and then counted by a liquid scintillation counter as shown (fig.3). Of 6 prekeratin molecules (71, 69, 62, 55, 52 and 49 kDa), the 55-kDa prekeratin showed the highest incorporation of ^{35}S-Met. These results and the incorporation pattern described were similar to those of [4] in which we used ^{14}C-glycine in pulse-chase experiments. Since there were many prekeratin bands in the fluorographic X-ray film, only the above 6 prekeratin bands were reacted with keratin antibody using the Western blotting technique as in [4]. The results were similar to those reported in [2] which showed that the antiserum to the 63-kDa (corresponding to our 62-kDa isolated) protein formed a precipitation line with each of 6 purified keratins of the stratum corneum. Moreover, the prekeratin molecules were also similar to each other in terms of amino acid and peptide maps as previously reported [1–3]. Recently, it was reported that the 55-kDa prekeratin molecule is absent or greatly reduced in Bullous congenital ichthyosiform erythroderma [16,17]. Therefore, the 55-kDa prekeratin molecule may play an important role during the course of terminal differentiation in the normal human epidermis.

After being chased, 3 prekeratin molecules (52, 55 and 62 kDa) were decreased at 30 min and then two (55 and 62 kDa) were increased at 60 min (fig.3B). The other 3 prekeratin molecules were changed only a little during the chase. However, we presented evidence that the two kinds of intermediates (52 and 62 kDa) might be presented at the initial stage of processing from prekeratin into keratin in pulse-chase experiments using ^{14}C-Gly [4]. In brief, two prekeratin molecules (52 and 62 kDa) were increased at 60 min after being chased, not decreased at 30 min as in the data presented here obtained by using ^{35}S-Met; moreover, the values at 60 min were higher than the starting values (pulsed values). The intermediates (52 and 62 kDa) may be converted from 55 and 69 (or 71) kDa, respectively, with human epidermal homogenates by some proteolytic enzyme during the keratinization [3,4]. From the results presented here and in [3,4], we conclude that the initial stage of processing from prekeratin into keratin consists of the following two steps: at the first step of processing, the 3 prekeratin molecules (52, 55 and 62 kDa) are cleaved at the N-terminal region (from data obtained after 30 min chasing, fig.3B), followed by modification in two prekeratin molecules (from data obtained after 60 min chasing [4]). The processing enzyme(s) is now under investigation.

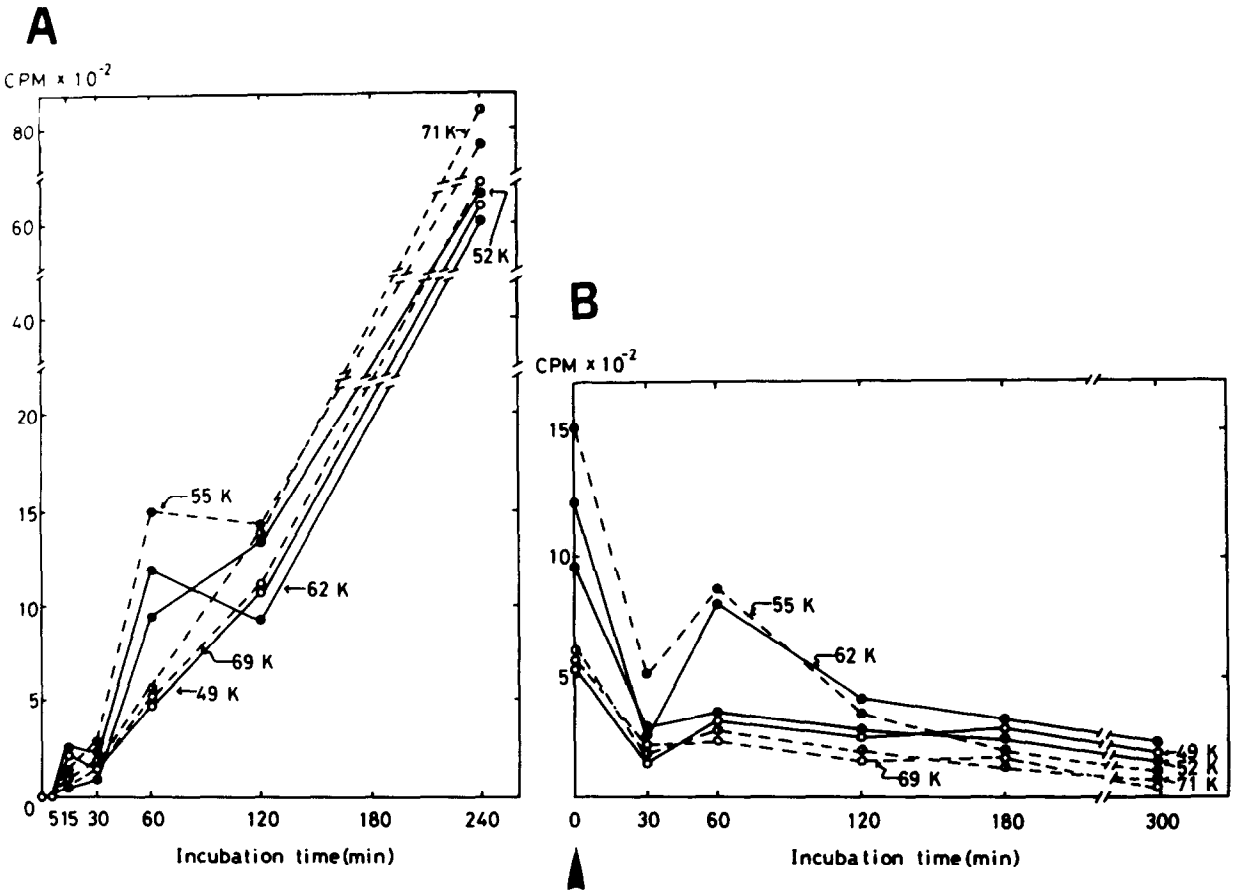

Fig.3. Radioactive changes in the time course and pulse-chase experiment (fig.2). Each band in the SDS–PAGE gel that corresponded to one in the fluorographic X-ray film was cut out and incorporation of ^{35}S-Met was estimated with a liquid scintillator. (A) Time course of incorporation of ^{35}S-Met. (B) Changes in ^{35}S-Met molecules after being pulsed for 60 min. ▲ , The value of this point employed was that of 60-min incubated (fig.3A). .

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REFERENCES

[1] Fuchs, E. and Green, H. (1980) Cell 19, 1033–1042.
[2] Fuchs, E. and Green, H. (1978) Cell 15, 887–897.
[3] Katagata, Y. and Aso, K. (1984) J. Dermatol. 11, 43–48.
[4] Katagata, Y. and Aso, K. (1984) J. Dermatol. 11, 263–268.
[5] Steinert, P.M. (1975) Biochem. J. 149, 39–48.
[6] Lee, L.D., Fleming, B.C., Waitkus, R.F. and Baden, H.P. (1975) Biochim. Biophys. Acta 412, 82–90.
[7] Bowden, P.E. and Cunliffe, W.J. (1981) Biochem. J. 199, 145–154.
[8] Fuchs, E. (1983) J. Invest. Dermatol. 81, 141s–144s.
[9] Steinert, P.M., Rice, R.H., Roop, D.R., Trus, B.L. and Steven, A.C. (1983) Nature 302, 794–800.
[10] Matoltsy, A.G., Matoltsy, M.N. and Cliffel, P.J. (1983) J. Invest. Dermatol. 80, 185–188.
[11] Marcelo, C.L. and Tong, P.S.L. (1983) J. Invest. Dermatol. 80, 37–44.

[12] Laemmli, U.K. (1970) Nature 277, 680–688.
[13] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[14] Kawakami, M. and Shimura, K. (1974) Radioisotope 23, 81–87.
[15] Fuchs, E. and Green, H. (1979) Cell 17, 573–582.
[16] Ogawa, H., Hattori, M. and Ishibashi, Y. (1979) Arch. Dermatol. Res. 226, 109–111.
[17] Yoshiike, T. (1983) Jap. J. Dermatol. 93, 755–762.