# ISOLATION AND CHARACTERIZATION OF ACTIN FROM HUMAN HAIR FOLLICLES

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## 1. Introduction

Proteins of the hair follicle have been studied extensively [1,2]. However, attention has mainly been focused on keratin, a giant molecule that forms >90% of the dry weight of hair, and which is insoluble in aqueous salt solutions. The water-soluble components of the hair have been generally neglected. In the hair follicle, ~10% of the protein is water-soluble [3]. A new, simple method for hair protein fractionation was developed [3]. Combination of this procedure with scintillation autoradiography enabled detection of newly synthesized polypeptides in human hair follicles. Here, we show that one of the major components of both the water-soluble and -insoluble fraction from hair follicles is actin. We demonstrate that actin is newly synthesized when hair follicles are incubated under appropriate conditions.

## 2. Materials and methods

### 2.1. *Chemicals*

L-[$^{35}$S]Methionine (spec. act. 1000 Ci/mmol) was purchased from the Radiochemical Centre (Amersham). Fetal calf serum was obtained from Sera Labs. (Sussex). DNase I recrystallized, from bovine pancreas (Sigma Chem. Co.), was used. Guanidine–HCl was acquired from Merck. Sepharose 4B was purchased from Pharmacia (Uppsala). Cyanogenbromide was obtained from Kodak.

### 2.2. *Hair protein fractionation*

Human hair follicles were plucked from the scalp and only those in the anagen phase with visible bulb and sheath were used. They were labeled with [$^{35}$S]-methionine (10 μCi/ml labeling medium: Dulbecco's Modified Eagles Medium with amino acids except for methionine, supplemented with 50 μg/ml gentamycin and 10% dialyzed fetal calf serum). A water-soluble protein fraction was extracted from the hair follicles by 5-times freezing (−20°C) and thawing in water. The obtained suspension was centrifuged at 15 000 × *g* and the supernatant was used as watersoluble protein fraction. A urea-soluble protein fraction was obtained by extracting the remaining material with 8 M urea for 15 min at room temperature. The suspension was centrifuged at 50 000 × *g* for 30 min and the supernatant was used.

### 2.3. *Affinity chromatography of hair follicle actin on DNase I–Sepharose 4B*

A DNase I–Sepharose 4B column was prepared by activation of 25 g Sepharose 4B with 4 g cyanogenbromide. DNase I (20 mg) was added to the activated slurry and stirred overnight. After a thorough washing of the DNase-substituted Sepharose, 5 ml slurry was poured in a column and prewashed with 4 M guanidine–HCl, 0.5 M sodium acetate, 30% glycerol, followed by 0.01 M Tris–HCl (pH 7.5), 5 mM $CaCl_2$ [4]. The water-soluble fraction of hair follicle proteins was applied to the column. Unabsorbed material was washed off at a flow rate of 2 ml/5 min with 0.01 M Tris–HCl (pH 7.5), 5 mM $CaCl_2$. The absorbed proteins were eluted from the affinity column in two steps; 0.75 M guanidine–HCl, 0.5 M sodium acetate, 30% glycerol and 3.0 M guanidine–HCl, 1.0 M sodium acetate, 30% glycerol, respectively. The fractions obtained from the column were dialyzed against distilled water and, after lyophilization, analyzed by polyacrylamide gel electrophoresis as for hair follicle proteins [3].

### 2.4. *Two-dimensional polyacrylamide gel electrophoresis*

Water- and urea-soluble proteins extracted from hair follicles were separated by two-dimensional gel electrophoresis as in [5] with slight modifications. Isoelectric focusing was done in 120 × 2 mm gels for 16 h at 400 V and 1 h at 1000 V. Gels were removed from the glass tubes, equilibrated for 30 min at room temperature in 1 ml sample buffer (2% sodium dodecylsulphate (SDS), 5% β-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue), and transferred onto a SDS–polyacrylamide gel slab (120 × 150 × 0.5 mm) [6].

## 3. Results

The water-soluble protein fraction from isolated human scalp hair follicles comprises ~10% of their total protein content. Treatment of the remaining water-insoluble material with 8 M urea solubilizes a further 30% of the protein. The SDS–polyacrylamide gel electrophoretic pattern of both fractions is shown in fig.1(a,b). The water-soluble fraction is characterized by 2 major components with app. $M_r$ 31 000 and

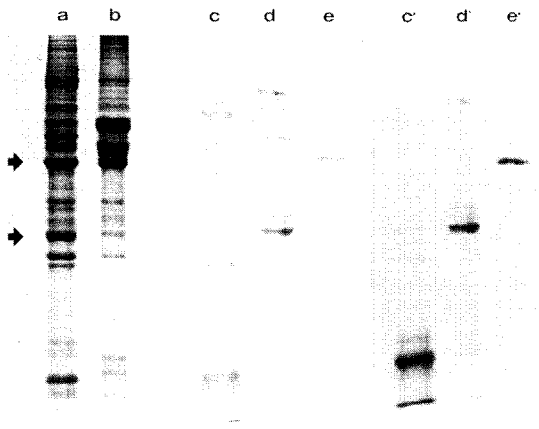


Fig.1. SDS gel electrophoretic pattern of water-soluble hair follicle protein (a) and urea-soluble hair follicle protein (b). Hair follicles were incubated in the presence of [$^{35}$S]methionine and extracted as in section 2. (c–e) Hair follicle proteins (water-soluble) at subsequent steps of actin purification by means of DNase I–Sepharose affinity chromatography: (c) unabsorbed material; (d) absorbed proteins, eluted by 0.75 M guanidine–HCl, 0.5 M sodium acetate, 30% glycerol; (e) absorbed protein, eluted by 3.0 M guanidine–HCl, 1.0 M sodium acetate, 30% glycerol. (c'–e') Picture of an autoradiograph of (c–e). Arrows indicate the position of the 45 000 and 31 000 $M_r$ hair follicle proteins.

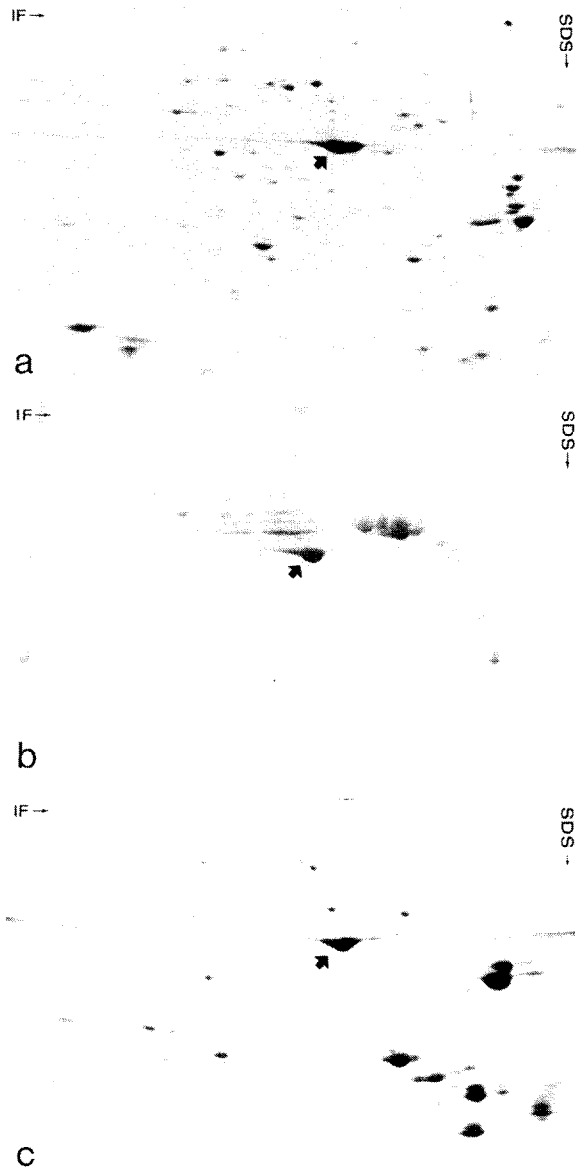


Fig.2. Two-dimensional gel electrophoresis of: (a) water-soluble hair follicle proteins; (b) urea-soluble hair follicle proteins; (c) hamster skeletal muscle proteins. The arrows indicate the position of actin. (Note the 2 isoelectric forms of G-actin in (a).)

45 000. Co-electrophoresis of this protein fraction with several purified protein preparations revealed a comparable $M_r$ for skeletal muscle actin and the 45 000 component in the water-soluble fraction. Additional characterization was performed by affinity chromatography on a DNase I–Sepharose column. The water-soluble protein fraction was applied to the

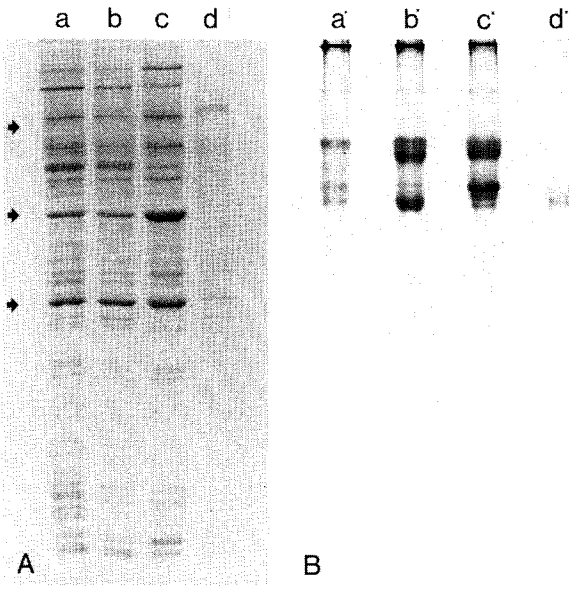


Fig.3. SDS gel electrophoretic patterns of 50 microdissected hair follicles. (A) Water-soluble proteins from hair bulb (a), elongation zone (b), hair sheaths (c) and hair shaft (d). (B) Urea-soluble proteins from the hair follicle fractions after extraction of the water-soluble proteins shown in (a). Arrows indicate the position of the following marker proteins ($M_r$): bovine serum albumin (67 000); ovalbumin (45 000); calf lens βBla (32 000).

column and eluted as in section 2. Fig.1(c–e) shows the eluted proteins at different concentrations of guanidine–HCl. It is evident (cf. fig.1(e)) that the 45 000 $M_r$ polypeptide is specifically bound, showing that it is, at least by this criterion, indistinguishable from actin. Newly synthesized hair follicle actin behaves identically (cf. fig.1(e) and 1(e')) as the native protein.

Also in the urea-soluble protein fraction a 45 000 $M_r$ band is present (see fig.1b). Since it is known that, as a rule, actin occurs both in a water-soluble (G-actin) and a water-insoluble form (F-actin), attempts were made to verify the nature of the 45 000 $M_r$ band in the urea-soluble fraction. Two-dimensional electrophoresis with isoelectric focusing in the first direction reveals that both the water-soluble and the urea-soluble fraction contains a 45 000 $M_r$ polypeptide with identical isoelectric point (fig.2(a,b)). For comparison in fig.2(c) a two-dimensional electropherogram of hamster skeletal muscle protein is also shown.

SDS–polyacrylamide gel electrophoresis of the water- and urea-soluble proteins from different parts of the hair follicle (bulb, elongation zone, sheath and shaft) (see fig.3) demonstrates that G-actin is more or less evenly distributed. On the other hand, F-actin appears to be mainly located in the elongation zone. The actin nature of the 45 000 $M_r$ band in different water-soluble and water-insoluble fractions was demonstrated by two-dimensional electrophoresis. Moreover, in a comparative isofocusing electrophoresis experiment we demonstrated that skeletal (α) actin migrates ahead of hair follicle actin (not shown).

## 4. Discussion

In hair follicle cells, as in most eukaryotic cells, actin occurs in two different molecular forms, one being soluble in water, the other comprised within highly polymerized water-insoluble complexes. As judged by isoelectric focusing hair follicle actin can be distinguished from that found in muscle cells. Whether the insoluble actin constitutes the 7 nm filaments, or together with other protein cofactors participates in the formation of stress fibers or other filamentous structures in the hair follicle, has to be established. Another intriguing question is related to the presence of water-soluble G-actin which seems to be rather abundant with respect to the insoluble form. In other biological systems the soluble pool ensures the dynamic properties of the cytoskeleton, in particular involved in cellular shape and movement.

The actin distribution in cultured skin fibroblasts of patients suffering from carcinoma of the colon and rectum seems to be associated with inherited perturbation of the motility proteins in particular actin [7,8]. These results and our method allowing in vitro culture of hair follicle cells [9,10] may provide a useful tool for further studies on motility proteins in human epithelial cell lesions potentially involved in carcinogenesis. Many human neoplastic processes, among which adenomatosis of the colon and rectum, have an epithelial origin.

Epithelial culture systems useful for these studies have been rarely available. The 'feeder-cell' technique for cultivation of skin keratinocytes was developed in [11]. However, this system has the disadvantage that two types of living cells are present in the same cultures, hampering discrimination of various biochemical parameters of the cells. Our human hair follicle cell culture system mentioned above might be more satisfactory due to its exclusive epithelial nature.

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