Disassembly and Reassembly of Skeletin Filaments*

TORGNY STIGBRAND, ANDERS ERIKSSON and LARS-ERIC THORNELL

Department of Physiological Chemistry and Institutes of Forensic Medicine and Anatomy, University of Umeå, S-901 87 Umeå, Sweden

Filamentous cell organelles, with an intermediate diameter as compared with microtubules and microfilaments, have in the last years been recognized as an ubiquitous structure for eukaryotic cells. Unlike the myofibrillar filaments, these intermediate-sized filaments are not depolymerized in high salt solutions. This property of the intermediate(-sized) filaments has permitted, in certain cell types, the selective extraction of other cell organelles, leaving a residue mainly composed of intermediate filaments. Such a highly concentrated preparation of intermediate filaments *in situ* has been shown to be capable of maintaining the three-dimensional appearence of the cells, thus acting as a cytoskeleton. This finding has inspired the term *skeletin filaments* for the intermediate filaments.[1]

The relative insolubility of the skeletin filaments hindered initially the characterization of the protein constituting the filaments. However, with the introduction of detergents and agents which split hydrogen bonds, it has been possible to identify a 55 000 dalton protein with four predominating amino acids (glu, asp, ala, leu) as the main constituent. This molecular weight and amino acid composition seems to be typical of intermediate filament proteins prepared from several different tissues (*e.g.* neuronal, glial, muscular and fibroblastic origin), with the exception of the epidermal tonofilament protein α-keratin.[2]

We have now further investigated the solubility of skeletin filaments by exposing cells to acidic and basic solutions. The cells used in the present study were the Purkinje fibres of the cow's heart, one of the most filament-rich cell types of all. The study of solubility was followed by parallel light microscopy, SDS polyacrylamide gel electrophoresis and rocket immunoelectrophoresis.

It was shown that only small amounts of skeletin could be dissolved at physiological pH values. The solubility was greater in both acidic and basic solutions. This was parallelled by light microscope observations when the cell bundles showed increasing transparency and were flattened within 10 to 20 seconds from exposure to 1 M acetic acid (Fig. 1). In about 5 min only fragmented cell ghosts remained. Similar results were obtained with buffered citric acid, pH 2.6. SDS-PAGE of the centrifuged extractant revealed a highly purified preparation of skeletin. When the pH of the extract was adjusted towards 7, a floccular precipitate was instantly formed. Electron microscopy of negatively stained preparations of such precipitates demonstrated irregular networks of short branching filaments with a diameter of approximately 8 nm (Fig 2).

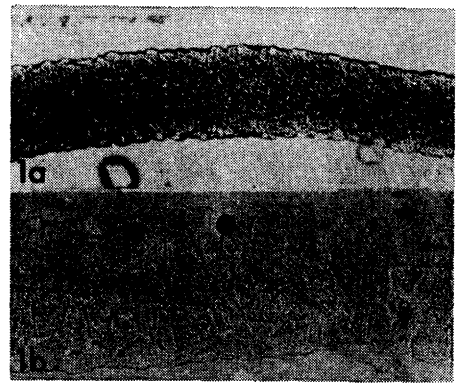

Fig. 1a. Untreated Purkinje fibre column. The protrusions represent individual cells; *1b.* The same Purkinje fibre column after 10 s in 1 M acetic acid. The cell column is flattened and markedly more transparent (magnification × 40).

These results further strengthen the previously proposed cytoskeletal function, by the demonstration of a total collapse of the three-dimensional arrangement of the cells when the skeletin filaments are specifically removed. The results also suggest a nearly complete polymerization of skeletin filaments at a physio-

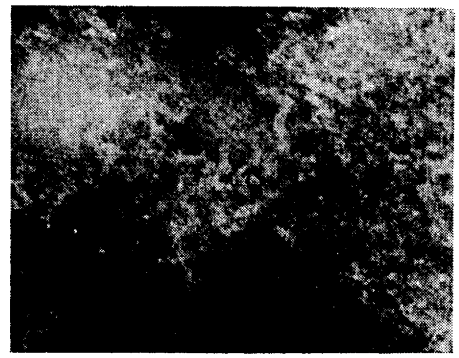

Fig. 2. Reconstituted filaments formed by precipitation of extract obtained with 1 M acetic acid. Short fragments of irregularly arranged filaments are seen (magnification × 200 000).

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logical pH, which obviously would be required for the performance of a cytoskeletal function.

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Partial Reaction of Thymine 7-Hydroxylase*

ELISABETH HOLME, GÖRAN LINDSTEDT and SVEN LINDSTEDT

Department of Clinical Chemistry, University of Gothenburg, Sahlgren's Hospital, S-413 45 Gothenburg, Sweden

Thymine 7-hydroxylase (EC 1.14.11.6) from *Neurospora crassa* catalyzes the sequential oxygenation of thymine to 5-carboxyuracil *via* 5-hydroxymethyluracil and 5-formyluracil. In each step 2-oxoglutarate is oxidatively decarboxylated to carbon dioxide and succinate.[1] A reaction mechanism has been proposed in which 2-oxoglutarate reacts with oxygen to produce monopersuccinic acid as the reactive intermediate which acts as hydroxylating agent on the other substrate.[2] This mechanism is supported by reports on uncoupling of the 2-oxoglutarate decarboxylating activity from hydroxylation in reactions catalyzed by thymine 7-hydroxylase[3] and proline hydroxylase.[4,5] In preparations of thymine 7-hydroxylase, we have observed a 2-oxoglutarate decarboxylating activity, which depends on pyrimidines that are noncompetitive inhibitors of the thymine hydroxylase activity.[6]

In the presence of these inhibitors the formation of $^{14}CO_2$ from 2-oxo [1-^{14}C] glutarate is linear with enzyme concentration and linear with time for 10 min. The pyrimidines which are active in promoting the reaction are listed in Table 1. The same cofactors are required as for the thymine hydroxylase activity (Table 2) but there is an absolute requirement for ascorbate. Possibly, ascorbate protects the enzyme from

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Table 1. 2-Oxoglutarate decarboxylating activity in the presence of various pyrimidines. For incubating conditions and specific activity of enzyme in the complete systems see Experimental.

Pyrimidine added	Specific activity μmol min^{-1} g^{-1}	Relative activity %
Thymine	74	100
5-Aminouracil	4.9	6
5-Hydroxyuracil	2.3	3
5-Mercaptouracil	2.1	3
Uracil	0.7	1
None	0.1	0.1

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