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54. Insight into biosynthesis of human urokinase forms

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The molecular identity of urokinase-type plasminogen activators and the interrelationship of different urokinase (UK) forms have been debated for years. At least three distinct forms of urokinase have been characterized: a high molecular mass form consisting of a single peptide chain (SC-UK), a high molecular mass form consisting of two disulfide-linked peptide chains (HUK), and a low molecular mass form (LUK) also consisting of two peptide chains (Günzler et al, 1982a). The complete primary structures of HUK and of LUK have been determined (Steffens et al, 1982; Günzler et al, 1982b). Moreover, the entire cDNA coding for UK has been sequenced (Heyneker et al, 1983). A synopsis of the information available now allows valid conclusions as to the structure of the physiological UK precursor and its conversion into the various UK forms.

Comparison of the amino acid sequences of HUK and LUK reveals that LUK is generated from HUK by limited proteolysis of its A chain, as the A_1 chain of LUK is contained in the A chain of HUK near its C-terminus. The relationship of HUK and LUK with SC-UK is evident from the sequence of cDNA reconstructed from overlapping cDNA fragments coding for UK sequences (Heyneker et al, 1983). The codes for both UK chains are present in a single coherent coding area of 1293 base pairs, as schematized below:

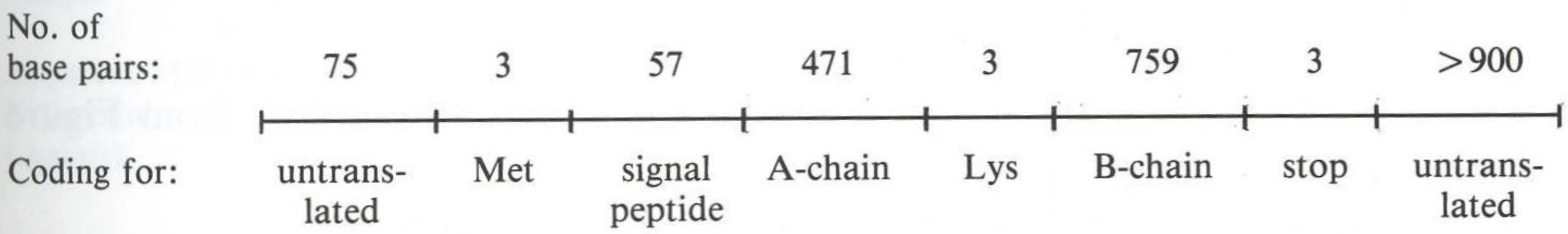


Figure 54.1 shows the amino acid transcript of the entire coding area. It is consistent with the amino acid sequences determined chemically, as far as they are retained in mature UKs. The sites of putative proteolytic processing of the primary expression product (arrows) suggest involvement of trypsin-type activities in most instances. The resulting sizes and N-termini of the most

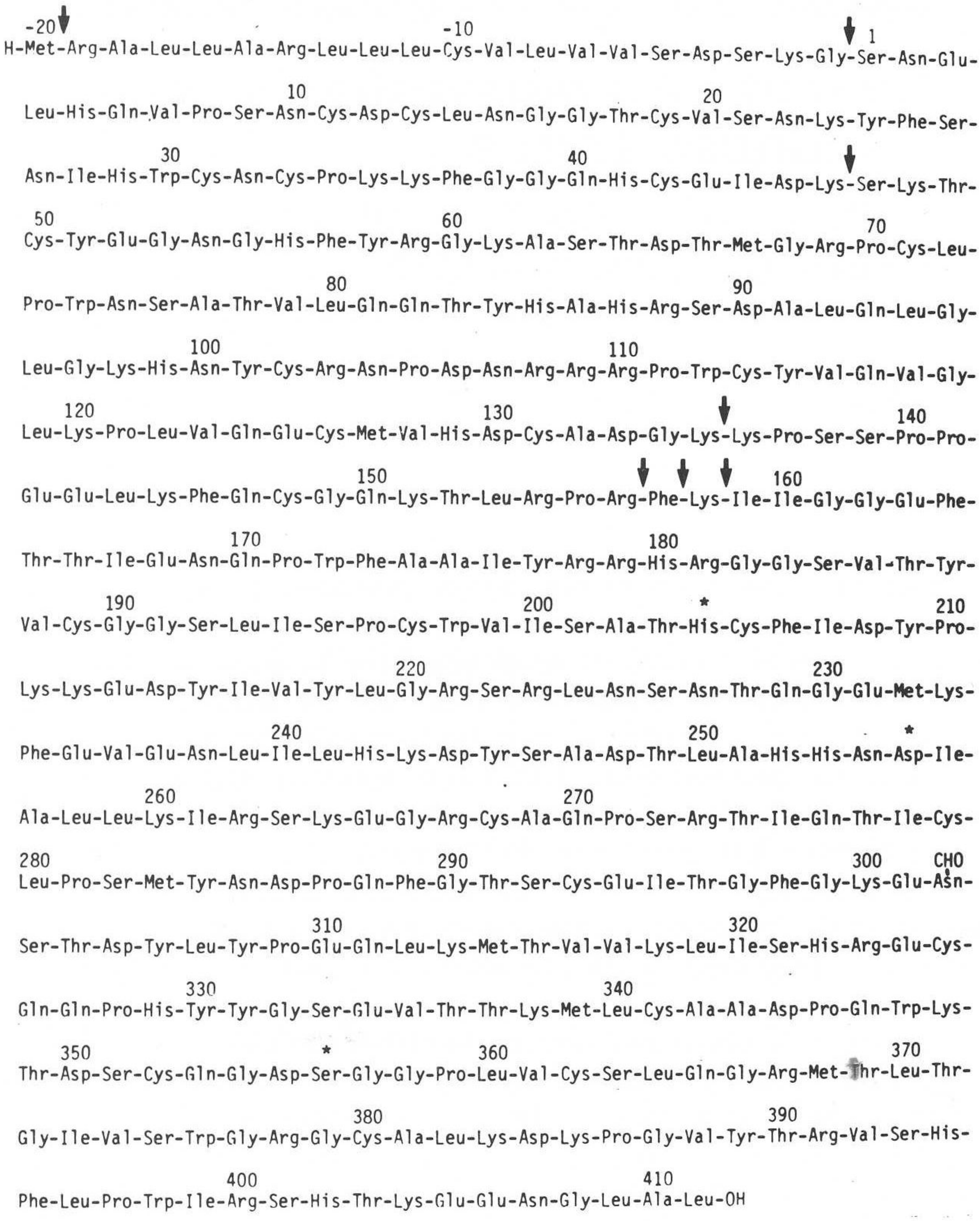
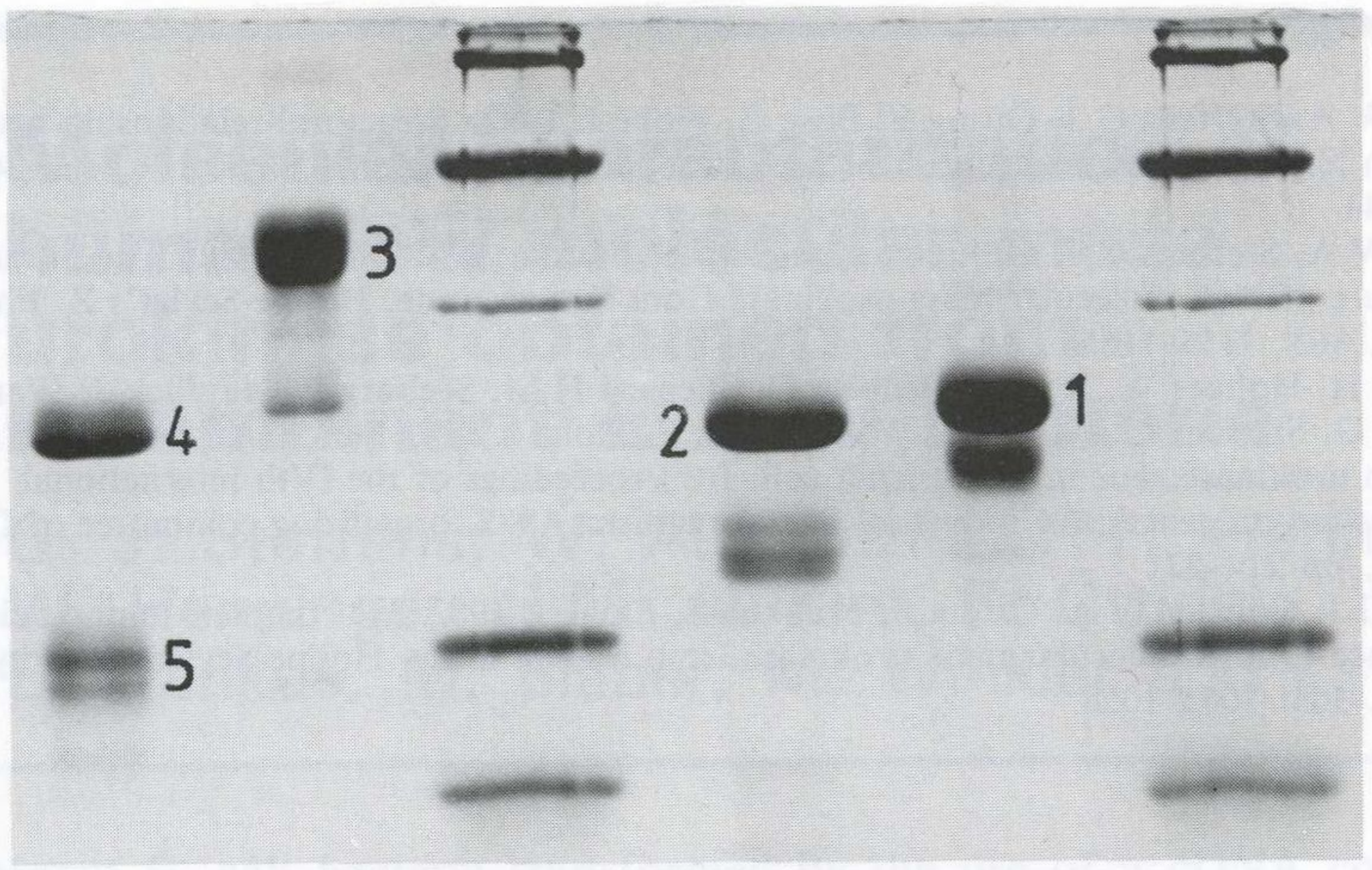


Fig. 54.1 Amino acid transcript of the cDNA coding for urokinase. Arrows indicate putative cleavage sites of the primary expression product. Asterisks mark amino acid residues constituting the catalytic center. Positive position numbers start with the amino terminus of the A chain as determined in isolated HUK.

important urokinase species SC-UK, HUK and LUK are evident from Figure 54.2.

From these data the following conclusions can be drawn:

1. UK like other serine proteases is biosynthesized as a single chain protein.
2. Newly synthesized UK starts with an N-terminal hydrophobic amino acid sequence (Fig. 54.1; positions −19 through −1) never seen in the isolated enzyme and most probably representing a signal peptide to be expected for a secreted protein. It appears to be obligatorily eliminated during excretion.
3. In SC-UK, the A chain (positions 1–157) represents the N-terminal and the B chain (positions 159–411) and C-terminal part of the sequence.



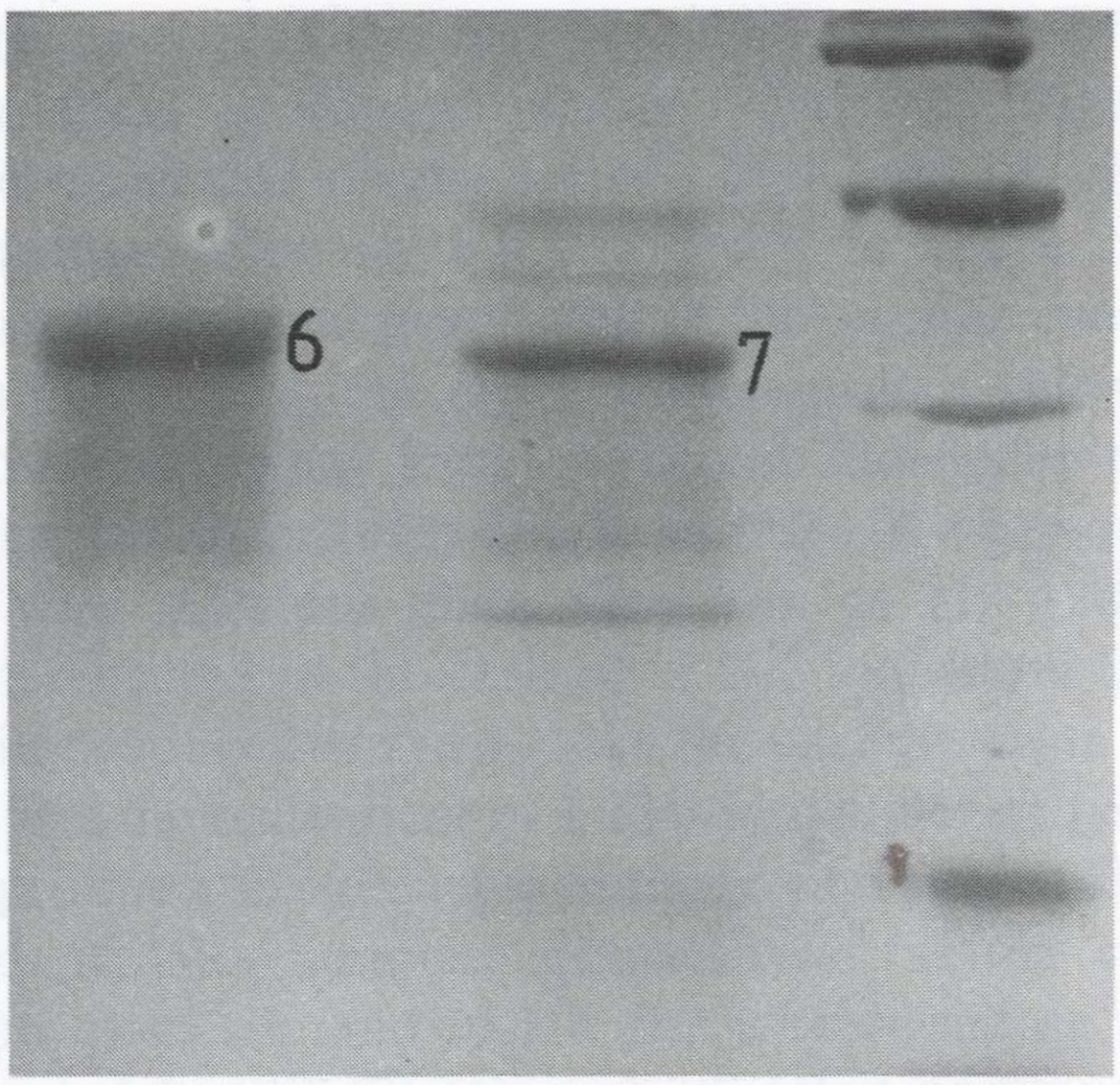


Fig. 54.2 SDS PAG electrophoresis of LUK, HUK and SC-UK in native (nat.) and reduced (red.) state. LUK and HUK were commercial products obtained from Ares and Hypolab, respectively; SC-UK was isolated from a recombinant E. coli strain described by Heyneker et al (1983). The numbered protein bands shown are further characterized as follows:

Band	1	2	3	4	5	6	7
Sample	nat. LUK	red. LUK	nat. HUK	red. HUK	red. HUK	nat. SC-UK	red. SC-UK
Apparent MW (kda)	32	31	49	31	19	48	48
N-Termini	Lys,Ile	Ile	Ser,Ile	Ile	Ser	Ser	Ser
Chain(s)	A_1–B	B	A–B	B	A	AB	AB

4. Limited proteolysis of a lysyl-isoleucine bond (positions 158–159) and elimination of lysine 158 yields fully activated HUK from SC-UK.
5. A_1 chain of LUK is generated from the A chain by a kind of tryptic cleavage of the 'growth factor domain' (positions 1–46) and the 'kringle domain' (positions 47–135) and loss of the C-terminal Phe 157.

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