ELECTRONOPTICAL STUDIES OF PROCOLLAGEN FROM THE SKIN OF DERMATOSPARAXIC CALVES

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1. Introduction

Recent evidence indicates that precursors of collagen can be detected in collagen synthesizing tissues [1–3]. The α-chains of this precursor molecule "procollagen" have a higher molecular weight and they also show different chromatographic properties than normal α-chains of collagen. After treatment with pepsin, the chains of the precursor are undistinguishable from normal α-chains of collagen, suggesting that an additional peptide of the precursor was split off by pepsin. The small amount available so far of this precursor material makes further characterization difficult.

A. Lenears et al. [4, 5] described an unusual form of collagen which they isolated from skin of calves with a genetic defect (dermatosparaxis) and which is also claimed to be a procollagen. The connective tissue of these calves has poor mechanical stability [6], and electromicrographs of the skin show no regular bundles of cross-striated collagen fibres but only thin cross-striated filaments surrounded by unstructured material [7]. Salt extractions of this skin contained collagen molecules with α-chains having a molecular weight of about 5–10% higher than α-chains of normal calf skin collagen [5]. In this paper we present evidence that the higher molecular weight of the dermatosparaxic procollagen is due to additional peptides which could be localized to the amino–terminal ends of the α-chains. This is demonstrated by a comparison of segment-long-spacing (SLS) crystallites prepared from normal collagen and dermatosparaxic procollagen. After incubation with proteases these additional peptides are no longer present.

2. Experimental

SLS crystallites were prepared from collagen, which was obtained from the skin of dermatosparaxic calves by extraction with 1 M NaCl [5] by being precipitated by dialysis in dilute acetic acid against 0.4% ATP, pH 2.8, as described earlier [8]. For electron optical studies, the material was stained with phosphotungstic acid (PTA) and uranyl acetate (UA).

Procollagen (1 mg/ml) was incubated with α-chymotrypsin (CDS, Worthington) in 0.05 M $CaCl_2$, 0.05 M Tris buffer pH 7.8 for 24 hr at 18° (collagen/enzyme = 10:1) and with pepsin (3X cryst. Serva, Heidelberg) in 0.5 M citrate buffer, pH 3.1 for 18 hr at 18° (collagen/enzyme = 10:1). The incubation of procollagen (1 mg/ml in 0.4 M sodium chloride) with a procollagen peptidase (0.15 M sodium chloride of extract of normal calf tendon) was carried out at 26° and pH 7.2 [9].

The p-α1 chains and p-α2 chains (see footnote 1) procollagen were obtained by chromatography on CM-

[1] Bellamy and Bornstein [2] called the α1-chain of procollagen which they found in rat calcaria pre-α1. Since it has not been established that the procollagen from skin of dermatosparaxic calves is identical to the procollagen found in organ culture, the chains are labelled p-α1 or p-α2, respectively.

cellulose under denaturing conditions [5]. The desalted chains were dissolved separately in 0.5 M citrate buffer, pH 3.7 and kept for 30 min at 37° for complete denaturation. These two solutions were then combined (ratio of p-α1:p-α2 = 2:1) and reconstitution was carried out at 20° for 4 hr and then at 9° for 12 hr.

3. Results and discussion

The SLS crystallites represent an artificial quaternary structure of collagen in which the molecules are aggregated laterally and parallel to one another, with their ends in register. While the cross striation pattern of the SLS crystallites, after staining with PTA and UA, reflects the distribution of the amino acids along the molecule [10], the length of the SLS corresponds to the length of the collagen molecule. Fig. 1a and 1b show SLS from normal calf skin and from skin of dermatosparaxic calves. The SLS of the latter is longer than that from normal skin, showing an additional piece of about 200 Å at the amino terminal end of the molecule. No additional piece can be seen at the carboxy-terminal end and no difference is noted in the normal cross striation pattern. When procollagen was treated with proteases such as pepsin or chymotrypsin, or with the procollagen peptidase preparation prior to precipitation as SLS, the additional piece could no longer be observed, suggesting that the peptides were cleaved off by the proteases (fig. 1c). As can be seen in the acrylamid electrophoresis p-α1 and p-α2 were converted into normal α1 and α2 [9].

In order to determine whether the prolongation at the amino-terminal end of the procollagen molecule is due to covalently linked peptide, the p-α1 and p-α2 chains of procollagen were separated on CM-cellulose under denaturing conditions. The native structure of the procollagen molecule was reconstituted from the isolated p-α1 and p-α2 chains and SLS were precipitated as outlined above. This preparation also showed the additional piece at the amino-terminal end as one can see in fig. 1d. This rules out the possibility that non-collageneous proteins of the NaCl extract of dermatosparaxic skin were incorporated during the formation of the SLS and caused the prolongation at the amino-terminal end. The prolongation observed in the SLS preparations of procollagen was found to be about 7%

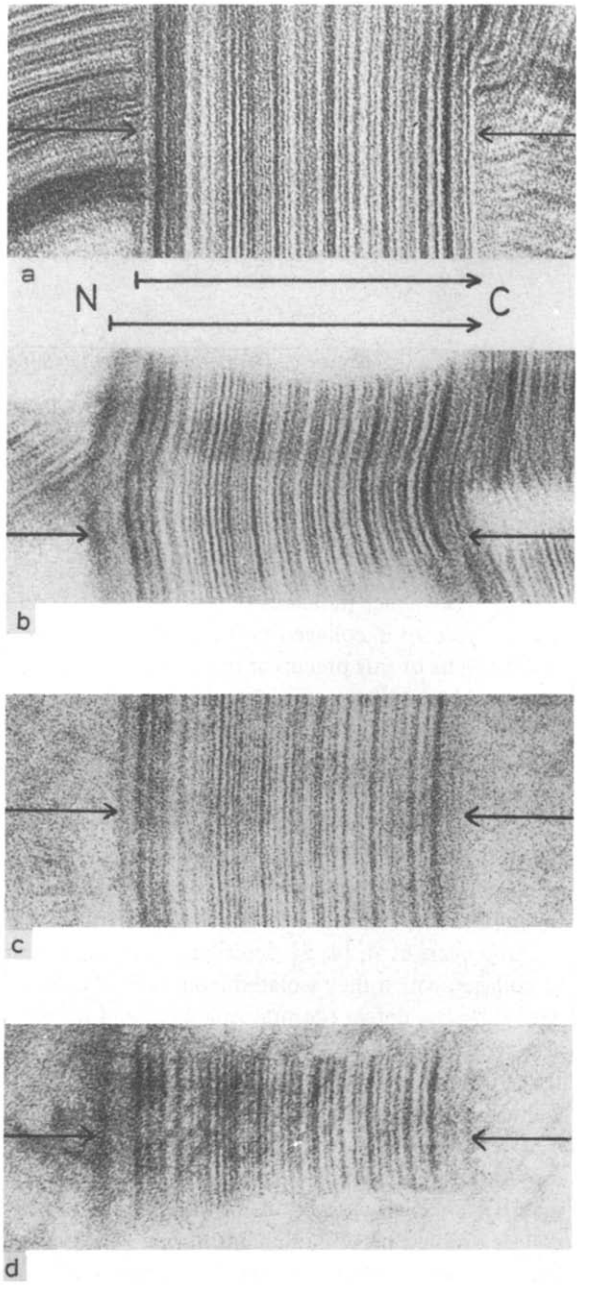

Fig. 1. Segment-long-spacing crystallites from: (a) normal calf skin collagen (No. 1548/68), (b) procollagen of skin of dermatosparaxic calf (No. 234/71), (c) procollagen pretreated with chymotrypsin (No. 237/71), (d) procollagen reconstructed from p-α1 and p-α2 (No. 355/71).

and is therefore in good agreement with the 5–10% higher molecular weight determined for the α-chains of procollagen by Lenaers et al. [5].

While fibrils with the cross striation pattern of the native type were obtained from calf skin collagen by dialysis of acid solution against buffers of neutral pH, no fibrils could be detected when the same procedure was applied to procollagen. When neutral salt solutions of procollagen were heated up to 37°, the formation of fibrils was also impaired [5]. This indicates that the additional peptides at the amino-terminal end impede or prevent the precipitation of fibrils. There are two possible explanations: (1) steric hindrance, or (2) hindrance by an excess of negative charges on the additional peptide [5]. This finding supports the observation that skin from dermatosparaxic calves contains only a few fibrils of the native type in addition to large amounts of poorly structured material.

Lapiere et al. [9] found an enzyme (procollagen peptidase) in various connective tissues of normal calves which cleaves off the additional peptides of procollagen. They could not find this activity in the connective tissue of dermatosparaxic calves. This leads to the conclusion that an enzyme defect is responsible for the accumulation of the procollagen since the conversion of procollagen into collagen is impaired. It is likely, therefore, that this type of a procollagen is identical with that described by Bellamy et al. [2] and Müller et al. [3].

Two possible functions for such additional peptides have been discussed so far: the coordination of the assembly of the three α-chains into the triple-helical molecule [11], and the transport of the native molecule from the cell to the site of fibre formation [1]. According to our results, an additional function should be taken into account, namely the control of fibril formation. Since the peptides are found at the amino-terminal end of the molecule, synthesis of the α-chains of collagen starts with these peptides characteristic for procollagen. This soluble precollagen is secreted into the extracellular space where the peptides are cleaved off and thereafter fibril formation can occur.

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