ELECTRON MICROSCOPIC METHOD OF ORDERING THE CYANOGEN BROMIDE PEPTIDES OF THE $\alpha 1$ CHAIN FROM ACID SOLUBLE CALFSKIN COLLAGEN

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Kühn and Tkocz [1,2] have recently shown that under certain conditions, isolated $\alpha 1$ chains from acid-soluble calf- and ratskin collagen form the triple-helical structure of collagen molecules. The properties of these $(\alpha 1)_3$ molecules are similar to those of native collagen molecules $[(\alpha 1)_2(\alpha 2)]$. On precipitation with ATP they show long spacing segments (SLS) with the same cross-striation pattern as the segments from native molecules.

Stark and Kühn [3] furthermore demonstrated that fragments of $\alpha 1$ chains are also able to build up triple-helices with the chains in register, as in native collagen molecules. The $\alpha 1$ fragments were obtained from molecular fragments prepared by collagenase digestion of acid-soluble calfskin collagen. Even the $\alpha 1$ chain from the smallest of these fragments, 780 Å in length, forms triple-helices which can be precipitated with ATP to yield SLS identical to those obtained from the native 780 Å fragments. These SLS appear in the electron microscope as fragments of SLS from untreated collagen, thus making it possible to correlate the molecular fragments with the whole molecule.

Using the reaction with cyanogen bromide, Bornstein et al. [4-6] split the $\alpha 1$ chain of acid-soluble ratskin collagen into eight peptides, which corresponded to the seven methionine residues of this chain. The largest of these BrCN peptides have approximately the same length as the $\alpha 1$ chain of the 780 Å-long collagenase fragment and should, therefore, be able to form triple-helix molecules. By precipitating SLS from these molecules and comparing their cross-striation pattern with that of SLS from untreated collagen, it should be possible to correlate the BrCN-peptides with the whole $\alpha 1$ chain.

In our experiments we used $\alpha 1$ chains of acid-soluble calfskin collagen, which also contain seven methionines, since we found these to have a greater tendency to renature than those from ratskin collagen.

The $\alpha 1$ component isolated by CM-cellulose chromatography according to the method of Piez et al. [7] was subjected to BrCN-cleavage under the conditions given by Bornstein et al. [6]. The separation of the peptide mixture on CM-cellulose performed at pH 3.6 according to the method of Butler et al. [5] is shown in figure 1. Fractions b, d and e were non-dialysable high molecular weight peptides. Formation of triple-helix molecules was achieved in the following manner: The individual fractions (0.5 - 1 mg/ml) were heated in 0.225 M citrate buffer for 30 minutes at a temperature of 38°C in order to destroy any preformed structures. The solution was cooled stepwise until a definite increase in negative specific optical rotation occurred (in five hours from $[\alpha]_{405} = -360°$ to about $-500°$). The renaturation temperatures thus obtained were

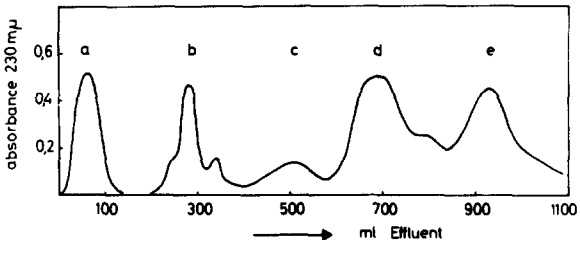

Fig. 1. Chromatography of the products of BrCN cleavage of $\alpha 1$ from acid-soluble calfskin collagen on CM-cellulose at pH 3.6. Linear gradient from 0.02 M sodium citrate – 0.04 M NaCl (pH 3.6) to 0.02 M sodium citrate – 0.14 M NaCl (pH 3.6) over a volume of 1600 ml.

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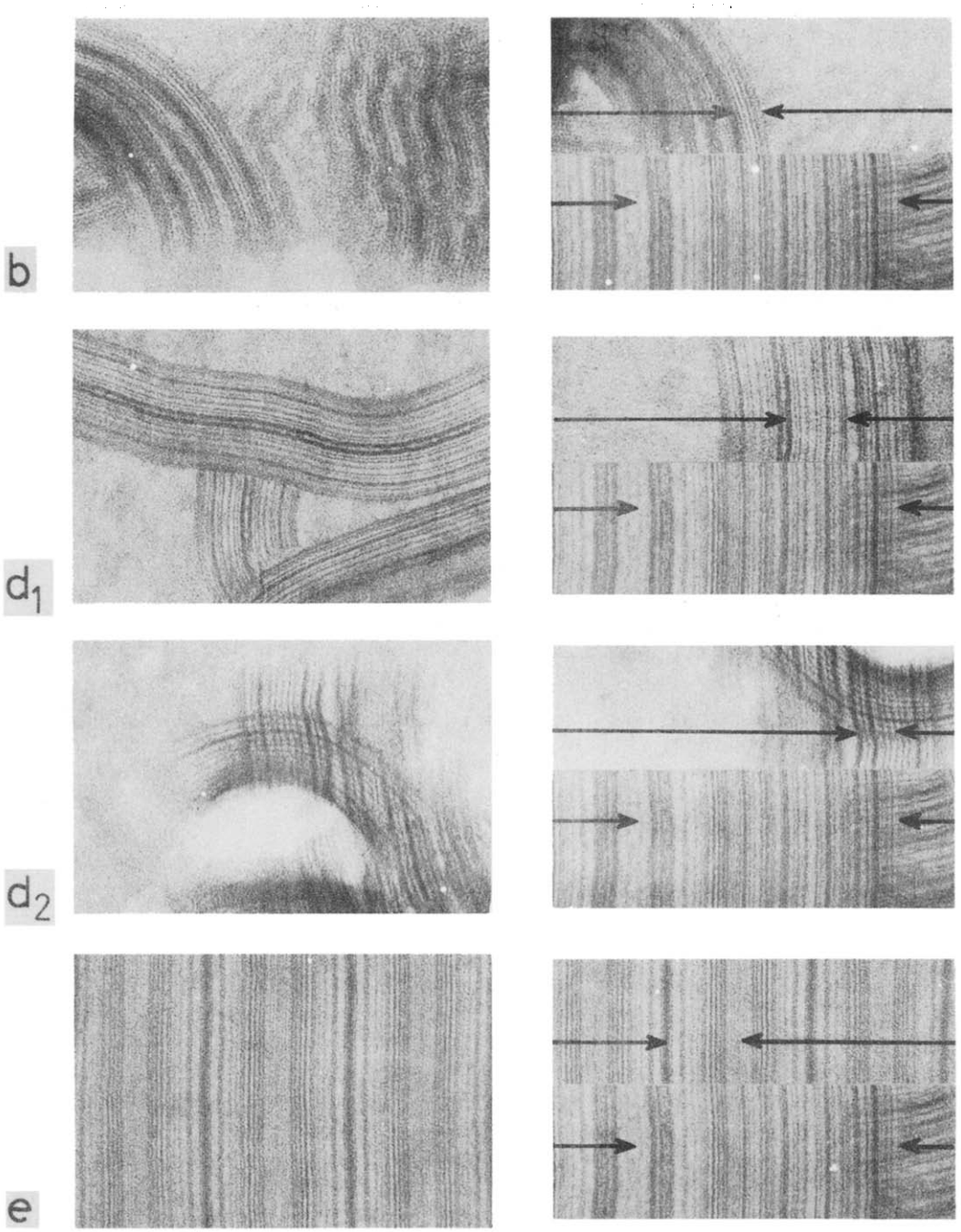

Fig. 2. Left hand side: Long spacing segments obtained from the non-dialysable BrCN peptides after renaturation in citrate buffer pH 3.7. The segments tend to end-to-end aggregation on one end (d_1 and d_2) or on both ends (b and e). For preparation and staining of the segments see text. Right hand side: Correlation of the BrCN peptide segments with a segment obtained from native collagen molecules, 2900 Å in length. The arrows indicate the ends of individual segments.

10° for fraction b, 15° and 12° for fractions d and e, respectively. The solutions were held at these temperatures for three days and then for an additional 24 hours at temperatures 3° lower. SLS were precipitated by dialysis against 0.05% acetic acid and finally against 0.4% ATP solution (pH 2.8). For electron microscopic examination the samples were stained on the grid with phosphotungstic acid and uranyl acetate. Clearer segments were obtained when the solution was treated with pepsin (substrate : enzyme = 10 : 1 at renaturation temperature) before dialysing against acetic acid in order to remove the non-renatured constituents. A shortening of the segments caused by pepsin treatment was not observed.

Fig. 2 shows the segments obtained from the cyanogen bromide peptides and their correlation with a segment from untreated collagen. Fractions b and e each show one type of segment, whereas fraction d shows two indicating the presence of two different peptides in this fraction. They can be separated from each other by rechromatography on CM-cellulose at pH 4.8. On rechromatography fractions b and e showed, in addition to smaller peaks, one main peak from which the same segments could be obtained as from the original fractions.

The four undialysable BrCN peptides make up about 85% of the total $\alpha 1$ chain. Since $\alpha 1$ chains contain seven methionines, further peptides of much smaller size should be found as was shown by Bornstein et al. with ratskin collagen [6]. The electron microscopic correlation suggests that these peptides originate from the 350 Å long region of the A-end which does not correspond to any of the identified peptides. With these findings we are now able to localize four of the seven methionine residues of the $\alpha 1$-chain.

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References

[1] K. Kühn, C. Tkocz, B. Zimmermann and G. Beier, Nature 208 (1966) 685.
[2] C. Tkocz and K. Kühn, in preparation.
[3] M. Stark and K. Kühn, Europ. J. Biochem., submitted for publication.
[4] P. Bornstein and K. A. Piez, Science 148 (1965) 1353.
[5] W. T. Butler, K. A. Piez and P. Bornstein, Biochemistry 6 (1967) 3771.
[6] P. Bornstein and K. A. Piez, Biochemistry 5 (1966) 3460.
[7] K. A. Peiz, E. A. Eigner and M. S. Lewis, Biochemistry 2 (1963) 58.