# CRYSTALLIZATION OF CYANOGEN BROMIDE PEPTIDES FROM CHICK CARTILAGE COLLAGEN

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## 1. Introduction

The triple helical molecular conformation of collagen has been established with the aid of X-ray fibre diffraction patterns of the native protein and of various synthetic polytripeptide model compounds [1]. However, as fibre patterns provide only very limited data, it has so far been possible to determine only an average structure for the helical regions of the collagen molecule. Diffraction patterns of single crystals, on the other hand, generally provide many more data, and, if these were obtainable for collagen, they might make possible not only a test of the precision of the fibre structure determination, but also an investigation of water binding and specific side-chain interactions, which have been suggested for several amino acid residues [2–4].

Collagen molecules are some 3000 Å long and 15 Å in diameter, and each of their three polypeptide chains contain over 1000 amino acid residues. We have avoided the formidable challenge of trying to crystallize complete molecules of such complexity and extreme asymmetry. However, encouraged by the crystallization of the collagen-like oligopeptide $(Pro\text{-}Pro\text{-}Gly)_{10}$ [5], we have attempted to crystallize peptide fragments derived from α1(II) chains of chick cartilage collagen by cyanogen bromide (CNBr) cleavage at methionyl residues [6]. It has been shown that a 36-residue CNBr peptide of the α1(I) chain of rat skin collagen can be renatured to form a collagen-like trimer [7,8], and we have subjected several CNBr peptides derived from chick cartilage α1(II) chains to similar renaturing conditions and subsequently succeeded in growing crystals. Since the cartilage collagen molecule is comprised of three identical α1(II) chains [9], the crystal structures should correspond to intact sections of the native molecule.

## 2. Materials and methods

CNBr peptides derived from α1(II) chains of chick sternal cartilage collagen were isolated and purified by ion-exchange and molecular sieve chromatography as previously described [6]. Peptides 6, 7, 11, and 12, containing linear sequences of 33, 44, 272, and 84 amino acid residues, respectively, were used for crystallization studies.

For renaturation, solutions of each peptide at a concentration of 35 mg/ml in 0.15 M potassium acetate, pH 4.8, were prepared and allowed to remain at 2°C for at least 4 days [7,8]. The extent of renaturation was not ascertained. Prior to attempts at crystallization, the renatured peptide solutions were diluted slightly to approximately 30 mg/ml by the addition of 5.0 M NaCl to achieve a final NaCl concentration of 0.4–0.5 M in the peptide solutions. Indeed, no crystals were obtained without the addition of this salt. As noted below, ammonium sulfate or 2-methoxy-2,-4-pentane diol (MPD) were also added to the renatured peptide solutions in certain instances.

A wide variety of possible crystallization conditions were surveyed using the technique of slow vapor diffusion [10] with a hanging drop of peptide solution. These experiments were performed using plastic multidish disposo-trays with 24 wells (Linbro Manufacturing Co.). Approximately 1.0–1.5 ml of solution to be assayed for ability to promote crystallization conditions was placed in each well. The ionic strength of the test solutions was varied between 0.5 and 3.0 M NaCl, and the solutions were employed over a variety of pH ranges for a given NaCl concentration. Buffers used for the various pH ranges were as follows: pH 1.6–2.0, 0.15 M potassium chloride; pH 2.1–3.0, 0.1 M potassium hydrogen phthalate-HCl, 0.15 M glycine hydrochloride or dilute acetic acid; pH 3.1–5.8, 0.15 M sodium acetate or potassium acetate; pH 7.5–9.0, 0.15 M ammonium chloride; and pH 9.1–11.0, dilute ammonium hydroxide. The wells containing these solutions were then covered with a siliconized cover glass to which was attached a hanging drop (2.0–5.0 λ) of peptide solution prepared as described above. Evaporation from the chambers was prevented by application of immersion oil between the edges of the depression and the cover glass.

In an alternate crystallization procedure, 20 λ of renatured peptide solution was placed in a perspex cell and covered by a dialysis membrane. The latter was held firmly in place by means of an O-ring fitted in a groove around the cell. Cells of this type were placed in vials containing 3.0–4.0 ml of possible crystallization solutions. The solutions assayed in this manner contained 0.5–3.0 M NaCl and were employed at two pH ranges: pH 6.5–8.2, 0.15 M phosphate buffer; and pH 7.0–9.1, 0.15 M tris hydrochloride. These cells require larger portions of peptide solutions than the depression chambers, but equilibrium conditions are approached more rapidly. They have the advantage that the dialysate can be changed easily allowing a step-by-step approach to crystallization.

All samples for crystallization were stored at 2°C, and the first crystals generally appeared after a three day interval. The crystals were observed by light microscopy.

## 3. Results

*Peptide 6.* Clusters of small needle-like crystals (maximum length, approximately 0.05 mm) were observed in the peptide solutions following exposure by vapor diffusion to solutions at pH values between 1.7 and 3.2 and NaCl concentrations from 0.5 to 1.4 M. Neither factor appeared to be critical, although the crystals grew more readily near the midpoint of the indicated pH and NaCl concentration ranges. Addition of ammonium sulfate 15% of saturation in the peptide solution and up to 50% of saturation in the well solution did not appear to affect crystallization. Addition of MPD at a concentration of 10% in the peptide solution and from 10 to 40% in the well solution appeared to inhibit crystallization, and no crystals were formed over these ranges of pH and NaCl concentration. Similar clusters of very small needle-like crystals of renatured peptide 6 were obtained in two other instances: in vapor diffusion experiments when the solution in the well ranged in pH from 8.0 to 10.7 at an NaCl concentration of 1.5 M; and during dialysis experiments when the dialysate was in the pH range of 7.2 to 8.2 and the NaCl concentration was in the range of 1.5–2.5 M.

*Peptide 7.* Fairly large tabular or plate-like crystals up to 0.7 mm in length were obtained in vapor diffusion experiments with the well solution in the pH ranges of 2.6–3.0 and 7.5–9.0 at an NaCl concentration of 0.5 M (fig.1). Although crystals of renatured

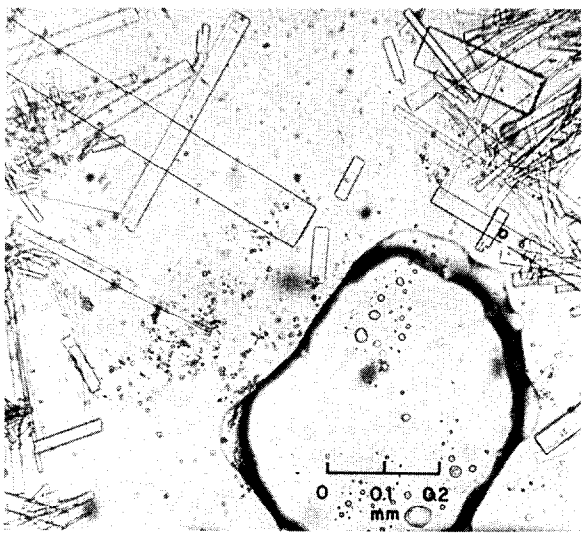


Fig.1. Crystals of renatured peptide 7 derived from chick sternal cartilage α1(II) collagen chains. The crystals were grown at pH 8.0 (0.15 M ammonium chloride, 0.5 M NaCl, 35% ammonium sulfate).

peptide 7 were generally observed under these conditions, the largest crystals were obtained when ammonium sulfate was added at 15% and 35–65% of saturation in the peptide drop and well solution, respectively.

Somewhat smaller crystals of renatured peptide 7 were also observed in dialysis experiments using solutions in the pH range of 7.6 - 9.1 and an NaCl concentration of 1.8 M.

*Peptide 11.* Very small needle-like crystals were grown by vapor diffusion with the well solution at pH 2.6 and 0.7 M NaCl. Using the dialysis method, slightly larger crystals (maximum length, 0.5 mm) were obtained with the dialysate at pH 7.6 and 1.8 M NaCl.

*Peptide 12.* Very small needle-like crystals were grown by vapor diffusion with the well solutions at pH 2.0–2.6 and 0.7 M NaCl as well as at pH 7.5–10.7 and 0.9–1.8 M NaCl.

## 4. Discussion

We have demonstrated that a number of renatured CNBr peptide fragments derived from collagen α1(II) chains can be induced to precipitate in crystalline form under a wide variety of conditions. Although the renatured peptides were not examined with respect to helical content and perfection of helix, the observation that each renatured peptide could be induced to form crystals strongly suggests that virtually complete refolding with a structure very similar to the native configuration had been achieved.

Our main interest in growing crystals of these renatured collagen peptides is, of course, the eventual determination of structure by X-ray crystallography. In this regard, the relatively large crystals observed for renatured peptide 7 show considerable promise provided they can be induced to grow somewhat thicker and remain stable at room temperature during the collection of X-ray diffraction data. Alternatively, it may be possible to collect diffraction data at suitably lower temperatures.

Apart from the question of crystallizability, peptides 6 and 7 are both attractive candidates for X-ray structure analysis in that they have relatively low molecular weights, approximately 10 000 and 12 000 respectively per trimer. Peptides 11 and 12 with trimer molecular weights of 75 000 and 24 000 respectively, would certainly prove to be much more difficult structures to analyse. They do, however, have an additional interest in that each of these peptides contain several of the many residues of galactosylhydroxylysine and glucosylgalactosylhydroxylysine normally associated with collagen α1(II) chains (E.J. Miller, unpublished observations). Furthermore, in the case of peptide 11, there existis the possibility of achieving a staggered fibril-type assembly [11].

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