CRYSTALLISATION OF ANGIOTENSINAMIDE II

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1. Introduction

The tridimensional structure(s) of the peptidic hormone [1] angiotensin II in a given environment can be approached by several techniques, most of them applied to solutions of the hormone in various solvents or to dry amorphous powders. It would be interesting to confirm and to extend the informations thus ob-

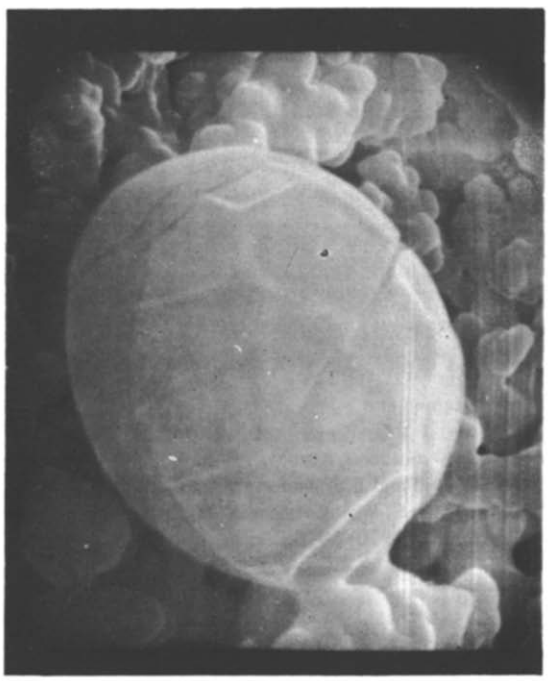

Fig. 1. Angiotensinamide II crystals from trifluoroethanol (× 8500).

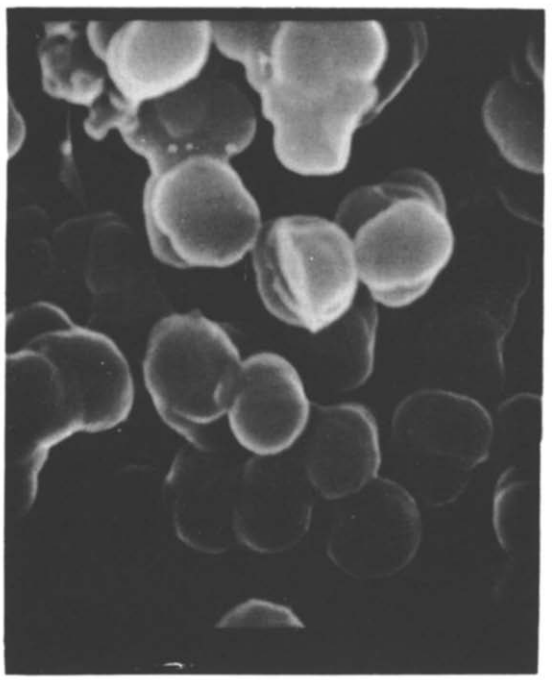

Fig. 2. Angiotensinamide II crystals from alcaline methanol (× 10,000).

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Fig. 3. Angiotensinamide II crystals from ethanol (× 8500).

tained by different techniques such as X-ray or electron diffraction. For this purpose angiotensinamide II crystals have been prepared. The present note describes their preparation and gives preliminary characteristics.

2. Material and methods

Angiotensinamide II has been generously provided by Dr. Riniker of Ciba-Geigy, Basel. 10 mg of pure peptide were dissolved at room temperature in 0.5 ml of trifluoroethanol and trimethylphosphate was added until appearance of a faint turbidity. The mixture was kept alternatively into the ice box and at room temperature. After a few days white crystals were found. These crystals appeared as hexagonal platelets of a size from 1 to 5 μ (fig. 1). A second approach was made with methanol mixed with a small amount of dilute sodium hydroxyde. From an angiotensinamide II solution in this solvent crystalline platelets were also obtained (fig. 2). In pure ethanol, angiotensinamide II is slightly soluble only. A saturated solution made at 40° was slowly cooled and left in the ice box for two weeks. Crystals were formed. Their shape was different from that of the other crystals as shown in fig. 3. Their size was also larger, from 5 to 15 μ.

Table 1
X-ray powder diffraction and electron single crystal diffraction spacings.

X-rays		Electrons
d_0Å	Intensity	d_0Å ± 3%
10.20	very strong	6.36
5.10	very weak	5.72
4.67	strong	5.20
3.66	weak	4.57
3.36	weak	4.50
3.32	weak	4.02
2.93	weak	3.18
2.79	weak	2.93
2.52	weak	2.73
		2.67
		2.49
		1.96

Examination of the crystals was carried out with a scanning electronic microscope JSM-U-3 from Jeol (Japan). The crystals were dried under high vacuum, placed on a brass holder and coated with a film of carbon and then of gold. The crystalline powder obtained from alcaline methanol and from trifluoroethanol was analyzed by X-ray diffraction in the laboratory of Dr. Monteilhet. The crystals were packed into a quartz capillary. The capillary to film distance was 125 mm. The camera was of the Guinier type and Cu $k_{\alpha 1}$ radiation was used. The exposure time was 30 hr. The Debye-Scherer pattern obtained showed sharp lines. The spacings are given in table 1. The size of the crystals yet available discouraged single crystal analysis by X-ray diffraction. However electron diffraction is possible and was performed with a Philips electron microscope EM 300 operated at 8 kV. Crystals were placed on a carbon coated copper grid (distance to camera 286 mm) and directly examined. Typical electron diffraction patterns are given in fig. 4.

3. Results

Figs. 1–3 indicated that angiotensinamide II has

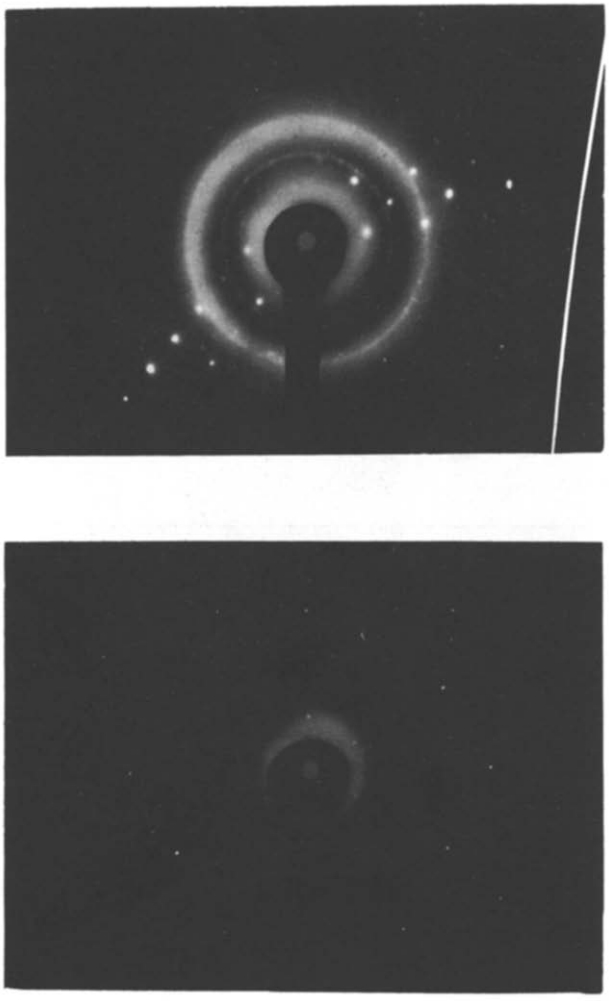

Fig. 4. Electron diffraction patterns of angiotensinamide II crystals obtained from alcaline methanol.

been obtained in crystalline form. The crystals are deliquescent and their edges have been smoothed during their handling and the process of coating. That the crystals represented angiotensinamide indeed was ascertained by a variety of techniques, circular dichroism and infra-red absorption spectra and by their specific biological activity on the whole rate [2]. The crystalline nature of the material obtained was further demonstrated by the X-ray and electron diffraction patterns. They corresponded to peptidic material as expected. The data of the X-ray diffraction pattern could be indexed to several crystalline systems. Thus, the sharp and intense reflection at 4.67 Å might represent the plane 100 of an orthogonal system. On the other hand the strong reflection at 10.20 Å might represent the plane 100 of a hexagonal system. More details are expected in the future and especially from the electron diffraction patterns which are under investigation. Suffice it to say that in the latter, the reflection at 10.20 Å has not been found. The first system could be best fit by a β structure, whereas the second would correspond more nicely to helical structures. Comparing the data collected for other peptides of known structure with circular dichroism spectra of angiotensinamide II solutions in trifluoroethanol and in alcaline methanol, as well as with infra-red spectra of angiotensinamide II powders prepared from the same solvents, indicated the probable predominance of an antiparallel β conformation. If this comparison holds, the orthogonal system for the crystalline hormone is the most likely. In this case the unit cell parameters would be: a = 4.67 Å, b = 6.96 Å and c = 10.20 Å.

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References

[1] I.H.Page and F.M.Bumpus, Physiol. Rev. 41 (1961) 331.
[2] W.S.Peart, Biochem. J. 59 (1955) 300.
[3] E.M.Bradbury, L.Brown, A.R.Downie, A.Elliott, R.D.B. Frazer, W.E.Handby and T.T.R.MacDonald, J. Mol. Biol. 2 (1960) 276.
[4] J.C.Andries and A.G.Walton, J. Mol. Biol. 56 (1971) 515.
[5] B.K.Vainshtein and L.J.Tatarinova, in: Conformation of Biopolymers, Vol. 2, ed. G.N.Ramachandran (Academic Press, New York, 1967) p. 569.