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LETTERS TO THE EDITOR

Crystalline B-Granules: Rhombic Dodecaheders (a = 7.4 nm ?) * **

Sir,

Recent research in collaboration with Dr. S. Boseck[1] revealed double evidence for the existence of crystalline secretory granules in B-cells of the grass snake (Natrix n. natrix L.). These granules have the shape of rhombic dodecaheders — like cubic pig insulin as described by Schlichtkrull (1961) — with unit cell dimensions (in one interpretation of data, a = 7.4 nm) remarkably ressembling those of cubic pig insulin (a = 7.6 nm; Harding, Hodgkin, Kennedy, O'Connor, and Weitzmann, 1966).

Grass snake pancreas was chosen as tissue source since its B-cells are particularly rich in crystal-like granules. Tissue was processed according to standard techniques (Glutaraldehyde, osmium tetroxide; acetone; Durcupan ACM Fluka).

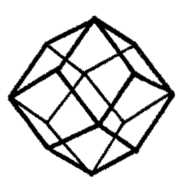

Fig. 1. (see text below)

The shape of crystalline secretory granules (rhombic dodecaheders, Fig. 1) was determined by three-dimensional reconstruction from serial sections. Section thickness — critical in this approach — was checked by interference color in the reflected light. Additional support for the mentioned shape was drawn from the observation that all profiles of random cross-sections through crystalline granules (trigon, rectangle, rhomb, pentagon, hexagon, octagon) could be easily explained by the dodecahedric form.

Investigation of the periodic structure formed the second part of our work. Series of electron micrographs were taken at unchanged magnification on roll-film. Enlargement of every film length was calibrated by a grating replica (E.F. Fullam, Schenectady). Image analysis was performed by optical diffraction (Boseck and Lange, 1970) with respect to periodicities and angles between them. Because of relatively thick sections (about 50 nm, corresponding to 7 unit cells stacked up within one section) periodicities will be revealed by electron microscopy only if lattice planes run virtually perpendicularly through the section: periodicities recorded thus represent true plane distances altered only by preparatory influences (e.g., by compression during sectioning).

Sixty-four measurements of periodicities are depicted in the frequency distribution (Fig. 2) in semilogarithmic scale. Right angles between periodicities (ranging from 74° to 106° because of compression) occurred in the expected frequency. The results of our measurements (Fig. 2) can be interpreted in two ways differing in the assumptions to be made.

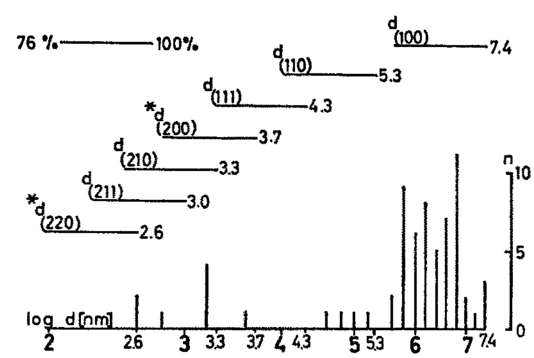

Fig. 2. (see text below)

One interpretation (the simplest one possible) is demonstrated in Fig. 2. It is assumed that the crystalline array is directly visible on the electron micrographs (explicit representation). The maximum value of 7.4 nm is taken as $d_{(100)} = a$, and the following six largest distances for cubic lattice planes are computed from it. The most outstanding group of data (from 5.6 to 7.4 nm) obviously represents the spectrum of $d_{(100)}$ values produced by different degrees of section compression. The range of compression can thus be

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1 Pathologisches Institut der Universität, Lehrstuhl II, D-6300 Gießen, and Max Planck-Institut fürPsychiatrie, D-8000 München.

assumed to reach from 0% to 24%. Every plane distance therefore is represented in Fig. 2 as a horizontal bar showing its 100% and 76% dimensions. The frequency distribution would fit well with the assumption of cubic system based on the form of the crystals. Fortunately, the maximal compression in our material was not so strong as to make disappear the gap (from 5.2 to 5.6 nm) separating $d_{(100)}$ and $d_{(110)}$ values. Two bars ($d_{(200)}$ and $d_{(220)}$, Fig. 2) are marked with asterisks (*) because values falling into their range probably represent second order reflections of periodicities of $d_{(100)}$ and $d_{(110)}$.

Alternative interpretation. Assumption is made that the cubic crystalline array is not directly visible on the electron micrographs (implicit representation). Certain electron microscopical properties of the unit cell content could lead to a lattice image related to the crystalline array but not identical with it. According to this interpretation we could state that there are two periodicities of 7.4 nm and of 5.3 nm (ratio $\sqrt{2}$). The exact relation between these values and the dimensions of the underlying cubic lattice would remain to be determined.

Presented data justify the conclusion that the crystal-like B-granules are really crystalline. Unfortunately chemical and crystallographic studies on snake insulin are not available.

References

Boseck, S., Lange, R.: Ausschöpfung des Informationsgehaltes von elektronenmikroskopischen Aufnahmen biologischer Objekte mit Hilfe des Abbeschen Beugungsapparates, gezeigt am Beispiel kristallartiger Strukturen. Zschr. wiss. Mikrosk. **70**, 65–79 (1970).

Harding, M.M., Hodgkin, D.C., Kennedy, A.F., O'Connor, A., Weitzmann, P.D.J.: The crystal structure of insulin. II. An investigation of rhombohedral zinc insulin crystals and a report of other crystalline forms. J. mol. Biol. **16**, 212–226 (1966).

Schlichtkrull, J.: Insulin-Kristalle. Chemische und biologische Studien über Insulin-Kristalle und Zink-Insulin-Suspensionen. Dissertation. Kopenhagen 1961.

Prof. R.H. Lange
Abt. Elektronenmikroskop
Anatomisches Institut d. Univ.
Friedrichstraße 24
D-6300 Gießen