

chemical explanation for the finding that the 5HT stored in platelets is freely exchangeable with exogenous 5HT³².

The platelets of rabbits probably take up more 5HT during their life span than those of guinea-pigs. Thus, in mature rabbit platelets, most of the organelles seem to be virtually saturated with 5HT, whereas in guinea-pigs and possibly in man this is not the case, because the majority of these organelles do not show osmiophily on electron microscopical examination.

Formation of high molecular weight aggregates with nucleotides and bivalent metals in specific subcellular organelles seems to be a general principle for the storage of biogenic amines. Thus, evidence for an aggregation of catecholamines and ATP has also recently been obtained in chromaffin granules of bovine adrenal medulla³⁵.

Zusammenfassung. Blutplättchen verschiedener Spezies speichern 5-Hydroxytryptamin zusammen mit Nukleotiden (hauptsächlich Adenosin- und Guanosin-5'-

Triphosphat) und bivalenten Kationen (Ca und Mg) in spezifischen, subzellulären Organellen, welche elektronenoptisch eine starke Osmiophilie aufweisen. Speicherstellen für 5HT scheinen bereits in den Megakaryozyten vorhanden zu sein, da in diesen Zellen nach Zufuhr von exogenem 5HT osmiophile Organellen erscheinen, die denjenigen der Plättchen entsprechen. Die Aufnahme von biogenen Monoaminen durch isolierte Speicherorganellen hängt von der chemischen Struktur der Amine und der Temperatur ab; es bestehen keine Anhaltspunkte für einen aktiven 5HT-Transport.

Analytische Ultrazentrifugation des Inhalts von isolierten Speicherorganellen von Kaninchen-Blutplättchen sowie von artifiziellen Lösungen ergibt, dass 5HT wahrscheinlich in Form hochmolekularer, gemischter Aggregate mit Nukleotiden und bivalenten Kationen akkumuliert. Die Bildung solcher Aggregate erklärt die osmotische Stabilität sowie möglicherweise andere biologische Eigenschaften der Speicherorganellen vor allem bezüglich Aufnahme von 5HT und dessen Freisetzung durch gewisse Substanzen, z. B. Tyramin.

SPECIALIA

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Oriented Overgrowth (Epitaxy) of a Polyamide Model Biopolymer on Non-Identical Polyamides

With the epitaxy of polyethylene on polyoxymethylene as well as on Nylon⁶ (poly- ϵ -caprolactam) the first epitaxial association of non-identical macromolecular compounds was described^{1,2}. These previous results suggested the idea that epitaxy of the natural polyamides, the proteins, and other natural macromolecular compounds might be of potential significance in biological ultrastructures and in the molecular mechanisms of biological processes^{1,2}. In view of these thoughts epitaxy of non-identical polyamides seems to be a point of particular interest.

As non-identical polyamides the polyamide model biopolymer poly- γ -benzyl-L-glutamate (PBLG) as a deposit and a drawn double oriented film of Nylon 6 as a substrate were chosen. Both polyamides proved to be especially suitable for studying epitaxy, since PBLG is soluble in solvents in which Nylon 6 is insoluble. Under biological aspects it is remarkable that the molecules of PBLG-crystals oriented on alkali halides are reported to be in α -helix conformation³.

The PBLG obtained from Dr. SCHUCHARDT, Munich, had an indicated molecular weight of 200,000–400,000. In the Nylon film the hydrogen-bonded (001) sheets were oriented approximately parallel to the plane of the film.

Initial attempts to obtain oriented crystals of PBLG on the surface of N 6-films using xylene or tetrachloroethylene as solvents were unsuccessful. In contrast thereto and in accordance with previous experience³ crystallization from mesitylene gave the desired result. Following the experimental method by which epitaxy of PBLG on

alkali halides had been obtained³, 6 cm³ of a highly concentrated solution (1.7×10^{-3} g PBLG/ml) were placed in a test tube immersed in a thermal oil bath heated to 110 °C for crystallization. A film of Nylon 6 was suspended in the crystallizing medium for 10 min. Upon removal, the substrate film carrying the overgrowth of PBLG was dried at ambient temperature.

The PBLG was oriented on the film surface in the form of long-needle-like objects, often bent in a characteristic manner, the long needle axis being preferably inclined about $60 \pm 2^\circ$ to the drawing direction of the film, i.e. to the axis of the Nylon 6 macromolecules. These needles were observed only in one position from 'bottom left to top right' in accordance with the symmetry of the hydrogen-bonded (001) sheets of the surface of the substrate film (Figures a and b). Beside these needles others were formed with the long axis parallel or perpendicular to the drawing direction of the substrate film. The average thickness of the needles was about 2000 to 3000 Å, in contrast to PBLG crystals oriented on alkali halides, which have a thickness of about 600 Å at the utmost³.

¹ J. WILLEMS, *Naturwissenschaften* 50, 92 (1963). – J. WILLEMS, British Patent 1,062,707 (1965).

² J. WILLEMS, *Experientia* 23, 409 (1967).

³ S. H. CARR, A. G. WALTON and E. BAER, *Biopolymers* 6, 469 (1968).

A satisfactory explanation of this epitaxy can be given on the assumption that the PBLG crystals are associated with the surface of the substrate by the same contact plane as in the epitactic overgrowth on the alkali halides.

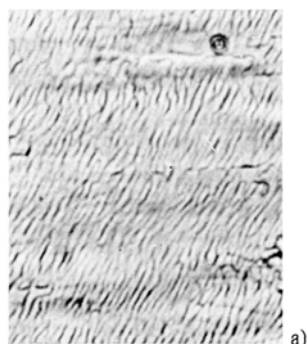
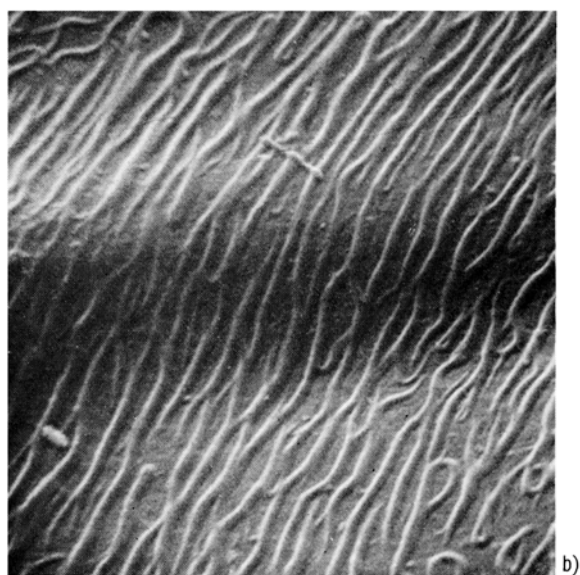


Fig. 1. a) Light micrograph ($\times 1000$) and b) scanning electron micrograph ($\times 5000$) of the oriented overgrowth of the polyamide model biopolymer poly- γ -benzyl-L-glutamate on the polyamide Nylon 6.



This problem will be discussed when the results of an electron microscopy and diffraction investigation are available.

The results of cursory attempts with PBLG as a deposit on other Nylon types such as Nylon 6,6, Nylon 6,10, Nylon 8 and Nylon 11 under the same experimental conditions, point to a pronounced specific character of this epitaxy.

The arrangement of the macromolecules of the overgrowth with respect to the known arrangement of the macromolecules of the surface of the substrate can be found by electron diffraction. From this arrangement can be inferred the molecular details of the association, such as correspondence of charge distribution in substrate and deposit molecules in the contact plane as well as the kind of intermolecular forces between the associated molecules.

Thus epitaxy between non-identical polyamides opens a new line of study at the molecular level of the law of association of such polyamides, especially of biopolymers of this type.

Zusammenfassung. Die orientierte Verwachsung zwischen zwei nicht identischen Polyamiden wird als neuartiger Typ der Epitaxie am Beispiel der Aufwachsung des biopolymeren Polyamids Poly-L-Glutaminsäure- γ -benzylester auf Poly- ϵ -caprolactam beschrieben. Der Verwachsungstyp bietet einen neuen Weg zur Untersuchung der Voraussetzungen für die Assoziation nicht identischer hochmolekularer Polyamide.

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⁴ The author thanks Dr. R. HOLM, Farbenfabriken Bayer AG, Leverkusen, for the scanning electron micrograph.

A New Reagent for the Cleavage of the Tertiary Butyloxycarbonyl Protecting Group

The tertiary butyloxycarbonyl (BOC) protecting group¹ is now widely used in peptide chemistry, especially in the solid phase peptide synthesis². Mostly, the splitting off is effected with 1N HCl/acetic acid, 4N HCl/dioxane or trifluoroacetic acid/methylene chloride (1:1).

The use of mercaptoethanol in conjunction with trifluoroacetic acid/methylene chloride has been proposed by WESTALL and ROBINSON³, but in a footnote to their paper, the authors advised another more stable reducing agent.

This is the reason why we decided to publish our results obtained with mercapto ethane sulfonic acid (Mesna®)⁴. This reagent, dissolved in glacial acetic acid, splits off very rapidly and selectively the BOC group without damaging the benzyl ester (O Bzl) bonds, or the benzyl-oxycarbonyl (Z) group.

A 50% solution of Mesna (3.45M) was used in our first experiments: the BOC amino acid (3 μ moles) was dissolved in 0.1 ml of the reagent and left for 2–5 or 30 min. After these periods, concentrated NaOH was added to stop the reaction.

High voltage electrophoresis was then effected in a pyridine/acetic acid/water buffer (pH 6.2). This experiment was made with BOC Gly, BOC Ala, BOC Val, α BOC ϵ Z Lys, α BOC γ O Bzl Glu and BOC Trp.

After 2 min, none of the BOC amino acid could be detected. The only products were Gly, Ala, Val, ϵ Z Lys, γ Bzl Glu and Trp.

Even after 1 h of reaction, we did not detect any compound migrating at the same position as free lysine

¹ F. C. MCKAY and N. F. ALBERTSON, J. Am. chem. Soc. 79, 686 (1957).

² R. B. MERRIFIELD, J. Am. chem. Soc. 85, 2149 (1963); Biochemistry 3, 1385 (1964).

³ F. C. WESTALL and A. B. ROBINSON, J. org. Chem. 35, 2842 (1970).

⁴ Mesna® is the trade mark of the sodium salt of the mercapto ethane sulfonic acid marketed by UCB as a mucolytic agent; it is stable and non-toxic. In this paper, we used Mesna® as mercapto ethane sulfonic acid. The free acid is obtained from the sodium salt by ion exchange with a Dowex 50H⁺ resin and lyophilisation of the aqueous solution.