MOLECULAR LIQUID CRYSTALLINE ORGANISATION OF COLLAGEN IN PHYSIOLOGICAL CONDITIONS

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Polarized light and electron transmission microscopies have shown that in many biological tissues, for example compact bone, tendon, skin or cornea, the three-dimensional arrangements of the collagen fibrillar networks follow the same spatial distribution as those described in certain liquid crystals. How are such complex structures established *in vivo* ?

A previous study using polarized light microscopy has demonstrated that in concentrated acidsoluble collagen solutions, monomers self-assemble into nematic, precholesteric and cholesteric liquid crystals (Giraud-Guille M. M. (1992). J. Mol. Biol., 224, 861-873). Here we are studying the ability of procollagen, the soluble precursor of the collagen molecule, to self-assemble into ordered phases.

Type I procollagen is purified from chick embryo tendons, and the final solution is concentrated between 5 and 30 mg/ml. Polarized light microscopy of viscous drops of procollagen in physiological buffer conditions reveals that molecules spontaneously evolve into nematic phases, precholesteric banded structures and precholesteric cords. Rotary shadowing electron microscopy and electrophoresis testify that procollagen molecules are not processed into collagen.

Procollagen concentration is likely to be several tens of mg/ml *in vivo* as in our *in vitro* preparations.These results suggest that the three-dimensional architecture of collagen fibrillar network is governed by liquid crystalline assembly of procollagen molecules, along with other components of the extracellular matrix, at a stage prior to the assembly of collagen fibrils.

TUNING BILAYER TWIST USING CHIRAL COUNTERIOUS

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From seashells to individual cells and molecules, chirality is expressed atevery level of biological structures. In self-assembled structures, it mayemerge co-operatively at a large scale from chirality at a molecular scale:amphiphilic molecules can form a variety of aggregates that express molecular chirality at asupramolecular scale of order micrometers (Schnur J.M. (1993) Science 262,1669-1676). However, control over the expression of chirality at thesupramolocular level is difficult to achieve (Spector M.S. et al (1998) Langmuir 14, 3493-3500): mixing ofopposite handed enantiomers alone, for instance, rarely permits acontinuous variation of twist because of separation of the constituents(Rowan A.E. and Nolte R.J.M. (1998) Ang. Chem. Int. Ed. 37, 63-68). Here we report on experiments and theory of thedevelopment and continuous variation of twist in approximately micron sizestructures (Oda R. et al (1998) Nature in press). We observeself-assembled twisted ribbons consisting of geminisurfactant bilayers, in which the degree of twist or pitch can becontinuously tuned by the introduction of chiral counter-ions in variousproportions . The formation of these ribbons occurred to us in the contextof a study of the gelling properties of gemini surfactants having chiral counterions such as 16-2-16 L-tartrate (Oda R. et al (1998) Ang. Chem. Int. Ed. 37, 2689-2691). These amphiphiles havethe ability to form gels in both water and some organic solvents by creatingextended networks of multilamellar twisted ribbons. Another novel feature of this system lies in thefact that the chirality does not belong to the amphiphile itself, but toits counterion. This allowed us to add excess chirality in the form ofsodium tartrate salts, the presence ofwhich causes an increase in the twist of the ribbons. The twisted ribbonsthat we observe bear several original features. (i) geometrically, theirsaddle-like curvature differs from the cylindrical curvature reported forhelical ribbons. (ii) also in contrast with the tubule-forming systems, we observe no healing of the edges toform larger chiral aggregates. The twisted ribbons may in some cases bethermodynamically stable, as is predicted by our theoretical model. (iii)More importantly, we demonstrate that the chirality of the microstructures can be *continuously varied* by mixing of counterionsenantiomers

ULTRA STRUCTURE OF LIPID -DNA COMPLEXES: INFLUENCE ON THEIR STABILITY ANDEFFICACY FOR IN VIVO GENE TRANSFER

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The design of plasmid DNA-cationic lipid complexes or lipoplexes (Lx) mustovercome several obstacles, which exist from Lx administration to theproduction of a therapeutic molecule in targeted cells. Because plasmid DNAis a large and highly biodegradable molecule, it must be protected and condensed in the delivery particles while outside oftargeted cells, and then it must obey to a reversible DNA condensationprocess. Consequently, the structure of Lx appears to be crucial for theirefficacy. We have designed a formulation making up possible the formation of negatively charged and stable Lx. Wehave demonstrated that the stability of Lx is greatly influenced by theirstructure. These Lx particle have shown a specific ultra structure asobserved by means of cryo-EM and small angle X-rays scattering (SAXS). We have observed a distinct concentric ringlike pattern appearing as spherulite structure with striated shells whenusing plasmid DNA. SAXS analysis revealed repetitive order, suggestingordered lamellar structure. This same structure was also observed by cryo EM and SAXS when using linear dsDNA,ssDNA or oligonucleotides. Also we were able to show the DNA condensedmolecule inside the Lx particles. With phage DNA we obtain particles insidewhich the DNA is hexagonally packed. In light of the numerous observations made on DNA packing in a naturalsetting or by various organic or inorganic condensing agents, our datacorroborate the notion that a parallel between natural and synthetic DNAcompaction can be drawn.

Multilamellar complexes as formulated here are of great interest as theyreveal a near neutrality or a negative net charge, high homogeneity, lowmean size, and stability. Hence, this optimised formulation appears to besuited for Lx preparation for systemic administration. A pharmacokinetic study confirmed our findings.

HIGH RESOLUTION MAPPING OF TOTAL CALCIUM IN OSTEOBLASTS BY ELECTRON ENERGY LOSS SPECTROSCOPY AND ELECTRON SPECTROSCOPIC IMAGING

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Subcellular localization of total calcium requires tissue processing that preserves the chemical composition of the samples and a highly sensitive micronalytical technique.

In this study rat foetal bone samples and cultured rat osteoblasts were quick-frozen against a liquid nitrogen cooled gold block, freeze-dried, exposed to OsO_4 vapours and directly included in Spurr resin. Ultrathin sections (40-50 nm) are quickly collected on uncoated grids and analysed unstained.

Osteoblasts ultrastructure was well preserved: the cells had clearly defined plasma and nuclear membranes with nuclear pores, dense mitochondria, numerous ribosomes and clearly visible reticulum.

Electron spectroscopic imaging (ESI) and Electron energy loss spectroscopy (EELS) allowed obtaining high-resolution calcium images using the Ca-$L_{2,3}$ ionisation edge (344 eV). In biological samples the Ca-$L_{2,3}$ signal is superimposed to the carbon edge and may induce artefacts. Therefore background was subtracted using a three images method, allowing obtaining high-resolution calcium images. Calcium maps were confirmed by spectra collected on defined areas of the images and the shape of the net Ca-$L_{2,3}$ edges was compared to the characteristic Ca-$L_{2,3}$ edge obtained from bone mineral and/or intramitochondrial granules.

Clear calcium maps were obtained, showing intramitochondrial calcium stores and calcium accumulation within the endoplasmic reticulum and the Golgi apparatus.

These procedures allow obtaining intracellular total calcium maps, will provide new informations about total calcium localization in bone cells and may be used to examine the distribution of other elements.