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Hydroxyapatite Crystals Orientation during a Pathological Calcification of Human Tendons

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The structural relationship between collagen fibers and apatite deposits in mineralized tissues has been widely investigated by electron microscopy and X-ray diffraction techniques [1]. For these studies oriented samples, such as osteons and turkey leg tendon at different degrees of calcification, have been particularly useful [2–4]. However many features of the problem are still obscure. The availability of oriented samples containing collagen fibers at low degrees of calcification is an essential condition to obtain information about the sites of the initial deposition of the apatite crystals in the collagen fibers structure using diffraction techniques. Tendon affected by diffuse idiopatic skeletal hyperostosis, which is a disease characterized by calcification and ossification of tendons and ligaments [5], is a suitable tissue because calcium hydroxyapatite is laid down within the tissue to different degrees along its length and to different degrees with the development of the disease.

We have examined fragments of human tendons which showed radiographic evidence of this pathological calcification at the sites of attachment to olecranon by small and high angle X-ray diffraction techniques in order to investigate the inorganic deposits and their interaction with collagen fibers.

X-ray diffractometric analysis carried out on powdered mineralized samples permitted the recognition of the mineral deposit present in the tissue as hydroxyapatite. This inorganic phase exhibits a good degree of crystallinity and cell parameters $a = 9.40(1)$ Å, $c = 6.89(1)$ Å very close to those of bone apatite.

High angle X-ray diffraction analysis carried out by means of a flat camera on fragments dissected from several zones of the tendons revealed a different orientation of the apatite crystals as a function of the degree of calcification of the tissue. The apatite crystals appear to be preferentially oriented with their *c*-axis parallel to the tendon axis in the zones at a low degree of calcification. This orientation reduces progressively as the degree of calcification increases and no preferential orientation can be observed in the fully calcified zones. The X-ray reflections characteristic of collagen molecular structure appear superimposed on those of the inorganic phase and show that collagen fibers orientation is always coincident with that of the *c*-axis of the hydroxyapatite crystals.

The small angle X-ray diffraction pattern recorded on fragments at a low degree of calcification shows the characteristic reflections of collagen fibrillar structure which appear oriented parallel to the tendon axis. No diffraction reflection due to collagen can be observed in the small angle X-ray diffraction pattern obtained from fully calcified fragments.

These results show a clear relationship between apatite crystals deposition and collagen fibers distribution and orientation. In fact at a low degree of calcification apatite crystals are aligned parallel to the collagen fibers of the tendon whereas at a high degree of calcification no orientation of collagen fibers as well as of apatite crystals could be revealed. This agrees with the morphological observations which show that at a high degree of calcification the collagen fibers lose their characteristic orientation parallel to the tendon axis and assume an isotropic distribution in a structure closely resembling that characteristic of cancellous bone. Furthermore it must be noted that the collagen molecular packing does not seem to be affected by the mineralization process, at least during the initial stage of the deposition.

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Cu(II) Adriamycin Complexes. Identification and Interaction with DNA

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Adriamycin (Adr) is an anthracycline antibiotic widely used in the treatment of various human cancers. Once in the cell, adriamycin localizes in the nucleus and is believed to act by inhibiting both DNA replication and RNA transcription [1]. Since metal

TABLE I. Stability Constants and Spectral Data of Complexes I and II.

	log β	Spectral data											
I	16.7	Ab λ(nm)		304			400			512			
		ϵ		8300			2400			6700			
		CD λ(nm)	230 260	300	350	380		430	490		540		
		$\Delta\epsilon$	−26.5 −8.8	−18	+4	+5.5		+1.7	−6		+8.5		
II	12.2	Ab λ(nm)			316			420			540		
		ϵ			9400			3000sh			6000		
		CD λ(nm)	260	305							570	605	
		$\Delta\epsilon$	+16.4	+2							+4.5	+6.2	

ions are present in all biological processes involving the nucleic acid and since DNA seems to be the target for adriamycin action, the occurrence of metal-adriamycin complexes inside the cell may play a crucial role in chemotherapeutic action.

Several recent observations have focused attention on the interaction of adriamycin and Cu(II) in the absence and in the presence of DNA [2–5]. In this communication we report the results of a detailed potentiometric and spectroscopic investigation which was undertaken to characterize accurately Cu(II)–adriamycin complexes, their stability constants and their effect on DNA.

The addition of Cu(II) to Adr at 1:1 molar ratio yields a first complex (I) at pH 5.8 and a second one (II) at pH 7.2. Using the results of potentiometric titrations these complexes can be formulated as $Cu(AdH)_2$(I) and Cu(Ad)(II). AdH and Ad stand for Adr in which the 1,4-dihydroxianthraquinone moiety is half deprotonated and fully deprotonated respectively. Absorption, CD data and the stability constants of both complexes are reported in Table I. The visible CD spectrum of complex I is of the couplet type indicating stacking of Adr due to the presence of Cu(II). Resonance Raman spectroscopy measurements indicate that coordination takes place through quinone and phenolate oxygens as shown by the shifting of the corresponding CO stretching Raman bands (carbonyl and phenolic).

When DNA is added to Adr at pH 7.4 (HEPES 0.05 *M*) precipitation of a DNA·Adr complex occurs, with a molar ratio of one nucleotide per Adr, if the Adr concentration is higher than 100 μM. When DNA is added to complexes I or II a Cu·Adr·ADN species precipitates. In this case, however, an Adr concentration lower than 20 μM has to be reached to prevent precipitation.

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Fe(III)·Bleomycin–DNA system. Evidence of Fe(III) to DNA Coordination

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Bleomycin (BLM) are a family of glycopeptide antibiotics clinically prescribed for the treatment of selected neoplastic diseases [1]. This drug, which both chelates metal ions and binds to DNA, induces a degradation of DNA in a reaction that has been shown to depend, *in vitro*, on the presence of Fe(II) and molecular oxygen [2]. At the end of the reaction Fe(III)·BLM and degraded DNA are obtained.

In this communication we report evidence suggesting that in the BLM·Fe(III)–DNA system, Fe(III) is directly bound to DNA.

In Fe(III)·BLM complex four nitrogens, from the secondary amine, the pyrimidine ring, a peptide bond, and the histidine imidazole coordinate to Fe(III) as the basal planar donor; at pH 7 the two axial positions (hereafter labelled A and B) are occupied by the α amino nitrogen and probably an oxygen atom of a glucide, respectively [3]; the complex is then in a low spin form. At pH 4 the α amino nitrogen is no longer bound in A (being presumably superseded by a water molecule) and the metal is therefore in the high spin form.

The following experiments suggest that position A can be occupied by different types of ligands and particularly by DNA.