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Magnetic alignment of collagen during self-assembly

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Magnetically induced birefringence is used to monitor the thermally induced self-assembly of collagen fibrils from a solution of molecules. The magnetic torque alone can, at best, only orient the fibrils into planes normal to the field direction. Nevertheless, the gels formed have a high degree of uniaxial alignment, probably due to the additional ordering effects of surface interactions. Thus magnetic orientation is potentially useful in the study of fibrillogenesis and in the production of highly oriented collagen gels.

Collagen has a number of applications, e.g. it is widely used clinically (Chvapil, 1979) and as an experimental substrate for cellular growth studies (Elsdale & Bard, 1972). Consequently highly oriented collagen gels have a variety of potential uses. Also the study of re-assembly *in vitro* from pure collagen molecules with orientation-sensitive techniques would be significantly assisted if orientation could be induced to accompany fibril formation without the risk of chemical modification, as is present with electric fields. Magnetic fields are not known to be destructive, and magnetic orientation has been successful with a number of fibrous biological structures (Ribeiro *et al.*, 1981; Torbet *et al.*, 1981; Torbet & Maret, 1981; Yagi & Offer, 1981; Vassilev *et al.*, 1982; Freyssinet *et al.*, 1983). In addition, magnetic birefringence has given new information about the processes involved in fibrin assembly (Freyssinet *et al.*, 1983). Therefore, as tendon is, as has been known for a long time (Cotton-Feytis & Faure-Fremiet, 1942; Arnold *et al.*, 1958), magnetically anisotropic, it was decided to examine the self-assembly of collagen fibrils from soluble monomers in a strong magnetic field.

Materials and methods

Sample preparation

Lathyritic rat skin collagen (0.6mg/ml) in 32mM-potassium phosphate buffer, pH7.04, was prepared as described in Bernengo *et al.* (1983), and was stored at −20°C except for a short period in melting ice during transit, after which it was separated into small portions and re-stored.

Birefringence measurements

The samples were contained in quartz cells with a 1mm or 2mm optical path-length and a width of 1cm. These were placed in a temperature-stabilized (±0.1°C) sample-holder within a Bitter-type magnet (maximum field ~13T) that had a small radial optical bore. The magnetically induced birefringence Δn ($\lambda = 632.8$nm) was measured by using a combined photoelastic modulation and compensation technique (Maret & Weill, 1983). $\Delta n = n_{\parallel} - n_{\perp}$, where $n_{\parallel}$ and $n_{\perp}$ are respectively the refractive indices parallel to and perpendicular to the field. Stock samples of frozen soluble collagen were melted at 4°C, placed in the sample chamber preheated to 27.5°C, and then the field was switched on and measurements were begun.

Magnetic orientation

The diamagnetic anisotropy, $\Delta\chi$, of a particle having an axis of rotational symmetry is equal to the difference in the value of the diamagnetization parallel to and perpendicular to the axis of symmetry. Diamagnetism is always negative, but $\Delta\chi$ can also be positive. In a magnetic field these particles experience a torque, so that their symmetry axis moves towards the field direction when $\Delta\chi$ is positive and towards the normal to the field when $\Delta\chi$ is negative. Probably most molecules are diamagnetically anisotropic, but in a constant magnetic field, H, the orienting energy, $\Delta\chi H_2/2$, is usually much smaller than kT, the thermal energy of disorienting Brownian motion. However, diamagnetic anisotropy is to a good approximation an additive property when the particles assemble into rotationally symmetric rigid polymer with their

symmetry axes parallel. Thus the diamagnetic anisotropy of this polymer composed of N monomers is approximately $N\Delta\chi$, and for large N it is possible that $N\Delta\chi H^2/2$ is sufficiently larger than kT to allow a high degree of orientation, as has, for example, been observed with fibrin (Torbet *et al.*, 1981; Freyssinet *et al.*, 1983). If there is an angle, θ, between the symmetry axes of the monomer and polymer, the diamagnetic anisotropy of the latter is decreased by a factor $1.5\cos^2\theta - 0.5$.

The structural origins of diamagnetic anisotropy in proteins has been discussed in detail by Worcester (1978), and is summarized below with reference to collagen. Peptide bonds and aromatic residues are the main potential sources of anisotropy. As there are relatively few aromatic groups in collagen, the anisotropy must be dominated by the peptide contribution. The diamagnetic anisotropy of a peptide bond, $\Delta\chi_p$, is taken to be $-14.6\times10^{-24}\,J\cdot T^{-2}$ (Worcester, 1978), but it could easily be about 50% less (Pauling, 1979; Torbet & Maret, 1981). A collagen molecule is approximated as a straight rigid rod in which θ is the angle between the normal to all N peptide planes and the long axis. Thus, taking $\theta = 45°$, the approximate value in the triple helix, the upper limit for the diamagnetic anisotropy of a single molecule, given by $N\Delta\chi_p$ $(1.5\cos^2\theta - 0.5)$, is approx. $-1\times10^{-25}\,J\cdot T^{-2}$.

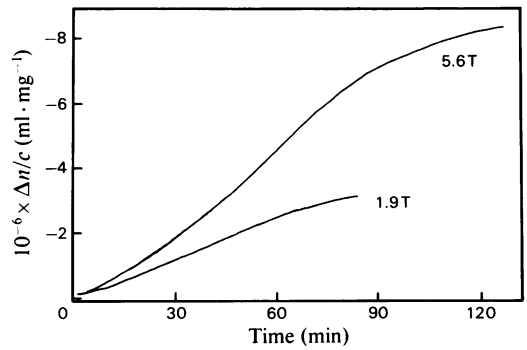

Fig. 1. *Magnetically induced birefringence normalized to concentration $\Delta n/c$ ($c = 0.6$ mg/ml), as lathyritic rat skin collagen re-assembles at 27.5°C in a constant magnetic field as indicated*

The optical path-length was 1 mm. The birefringence is negative because collagen, which has a positive optical polarizability in the direction of the fibre axis, is orienting perpendicular to the field. The difference in signal between 1.9 T and 5.6 T is only about 50%, although the orienting torque is proportional to the square of the field for diamagnetically anisotropic particles. This indicates that 5.6 T is above the critical field for maximum orientation in these conditions.

Results and discussion

In Fig. 1 the heat-induced re-assembly of neutral type I collagen in a constant magnetic field is monitored with birefringence. This technique, which has been used previously to study fibrin formation (Freyssinet *et al.*, 1983), focuses on large ordered aggregates, as monomers and small oligomers orient poorly and consequently contribute little to the signal. The curves resemble the characteristic sigmoidal shape given by turbidity measurements (Comper & Veis, 1977*a*,*b*; Piez, 1982). Although some samples did give rise to a lag phase, none is evident in Fig. 1, probably because aggregates were present from the outset as no centrifugation step was performed before use (Bernengo *et al.*, 1983).

Under the influence of the magnetic force alone collagen fibrils, as they have a negative diamagnetic anisotropy (Worcester, 1978; and the Materials and methods section), would lie perpendicular to the field at maximum magnetic orientation but otherwise lack preferred directionality. However, as Fig. 2 shows, the orientation is not only normal to the field but is nearly uniaxial, which is probably a felicitous consequence of surface interactions. This conclusion is supported by the finding that on increasing the sample thickness the induced birefringence decreased.

The degree of orientation in a magnetic field, H, of a particle with a diamagnetic anisotropy, $\Delta\chi$, is a function of the ratio $\Delta\chi H^2/2kT$, which must exceed 6 for better than 75% maximum magnetic orientation when $\Delta\chi$ is negative (Torbet, 1983). As this ratio is only approx. 5×10^{-3} for a single collagen molecule at room temperature in the highest magnetic fields available, i.e. approx. 20 T, significant magnetic orientation of non-interacting molecules is impossible. Therefore the high degree of orientation observed (Fig. 2) must be due to the summation of the molecular diamagnetic anisotropies (see the Materials and methods section).

The results presented here indicate that magnetic orientation could be usefully applied to the study of the later stages of fibrillogenesis. The most rewarding application is likely to be in conjunction with techniques such as diffraction (X-ray or neutron) or spectroscopy, which give more-detailed structural information when dealing with oriented samples. Similarly, the structure of the resulting oriented gel, which is the form often found *in situ*, can be more effectively investigated. As a significant degree of orientation can be obtained in only 1.9 T (Fig. 1) and improvement can be expected by varying the conditions of assembly (e.g. increasing the concentration, sub-

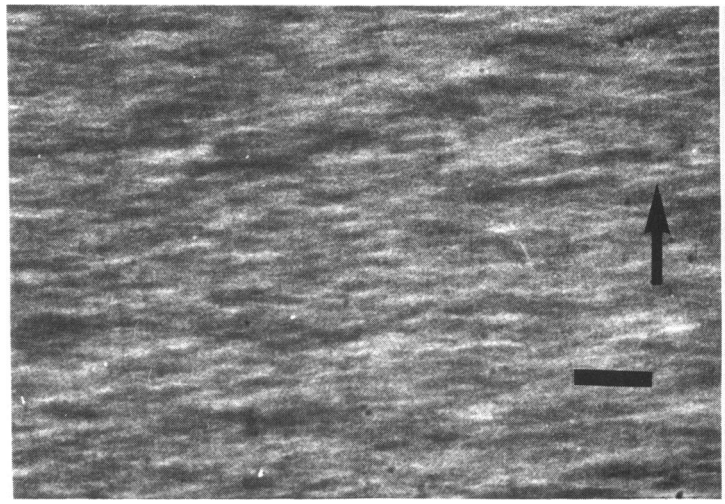

Fig. 2. *Optical micrograph taken between crossed polarizers of the samples measured in Fig. 1 in 5.6T* The arrow indicates the field direction and bar is equivalent to 100 μm.

jecting the gel to cycles of assembly and partial disassembly by heating and cooling), there is probably no real need to resort to unusually strong fields in order to produce highly oriented gels of a few cm^2 in area. Finally, these results are of some general significance because they show that it is possible to obtain near-parallel alignment of a stiff polymer in a simple way even when orientation is perpendicular to the magnetic field.

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