IMPACT 23 …ON BIOCHEMISTRY: Analysis of DNA by X-ray diffraction

Bragg's law (eqn 15B.1a in Topic 15B, $n\lambda = 2d \sin \theta$) helps to explain the features of one of the most seminal X-ray images of all, the characteristic X-shaped pattern obtained by Rosalind Franklin and Maurice Wilkins from strands of DNA (Fig. 1). This image was used by James Watson and Francis Crick in their construction of the double-helix model of DNA.

To interpret this image by using Bragg's law it is necessary to be aware that it was obtained by using a fibre consisting of many DNA molecules oriented with their axes parallel to the fibre axis, with X-rays incident from a perpendicular direction. All the molecules in the fibre are parallel (or nearly so) but are otherwise randomly distributed; as a result, the diffraction pattern exhibits the periodic structure parallel to the fibre axis superimposed on a general background of scattering from the distribution of molecules.

There are two principal features in Fig. 1: the strong 'meridional' scattering upward and downward by the fibre and the X-shaped distribution at smaller scattering angles. Because scattering through large angles occurs for closely spaced features (from Bragg's law, if d is small, then θ must be large to preserve the equality), it can be inferred that the meridional scattering is due to closely spaced components, and that the inner X-shaped pattern arises from features with a longer periodicity. Because the meridional pattern occurs at a distance of about 10 times that of the innermost spots of the X-pattern, the large-scale structure is about 10 times bigger than the small-scale structure. From the geometry of the instrument, the wavelength of the radiation, and Bragg's law, it can be inferred that the periodicity of the small-scale feature is 340 pm whereas that of the large-scale feature is 3400 pm (that is, 3.4 nm).

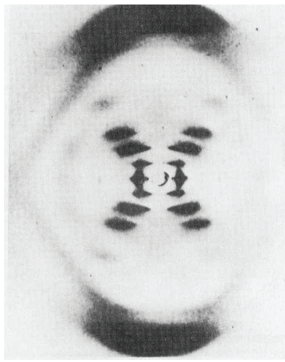

Figure 1 The X-ray diffraction pattern obtained from a fibre of B-DNA. The black dots are the reflections, the points of maximum constructive interference, that are used to determine the structure of the molecule. (Adapted from an illustration that appears in J.P. Glusker and K.N. Trueblood, *Crystal structure analysis: A primer*. Oxford University Press (1972).)

To see that the cross is characteristic of a helix, refer to Fig. 2. Each turn of the helix defines two planes, one orientated at an angle α to the horizontal and the other at $-\alpha$. As a result, to a first approximation, a helix can be thought of as consisting of an array of planes at an angle α together with an array of planes at an angle $-\alpha$ with a separation within each set determined by the pitch of the helix. Thus, a DNA molecule is like two arrays of planes, each set corresponding to those treated in the derivation of Bragg's

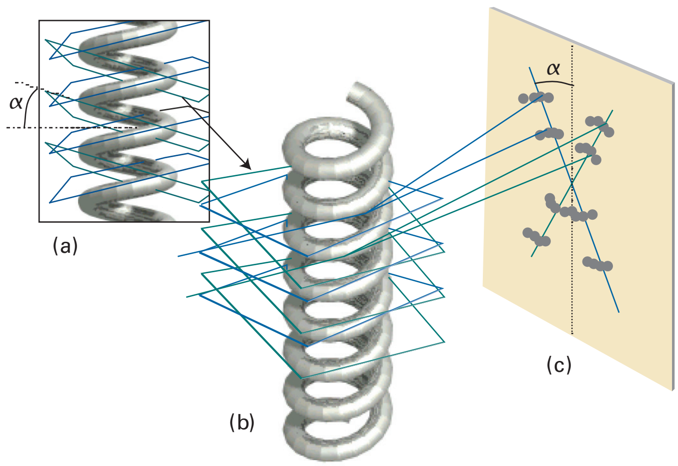

Figure 2 The origin of the X pattern characteristic of diffraction by a helix. (a) A helix can be thought of as consisting of an array of planes at an angle α together with an array of planes at an angle $-\alpha$. (b) The diffraction spots from one set of planes appear at an angle α to the vertical, giving one leg of the X, and those of the other set appear at an angle $-\alpha$, giving rise to the other leg of the X. The lower half of the X appears because the helix has up-down symmetry in this arrangement. (c) The sequence of spots outward along a leg of the X corresponds to first-, second-, … order diffraction ($n = 1, 2, \ldots$).

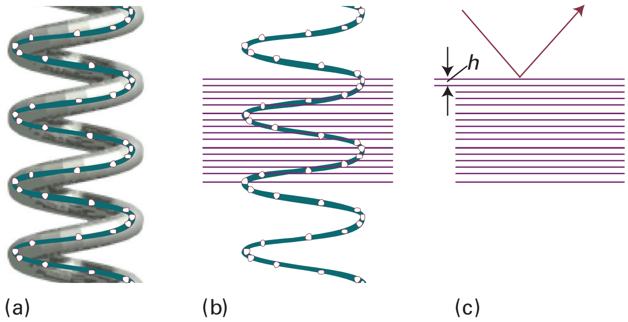

Figure 3 The effect of the internal structure of the helix on the X-ray diffraction pattern. (a) The residues of the macromolecule are represented by points. (b) Parallel planes passing through the residues are perpendicular to the axis of the molecule. (c) The planes give rise to strong diffraction with an angle that permits the determination of the layer spacing h from $\lambda = 2h \sin\theta$.

law, with a perpendicular separation $d = p\cos\alpha$, where p is the pitch of the helix, each canted at the angle $\pm\alpha$ to the horizontal. The diffraction spots from one set of planes therefore occur at an angle α to the vertical, giving one leg of the X, and those of the other set occur at an angle $-\alpha$, giving rise to the other leg of the X. The experimental arrangement has up–down symmetry, so the diffraction pattern repeats to produce the lower half of the X. The sequence of spots outward along a leg corresponds to first-, second-, … order diffraction ($n = 1, 2, \ldots$ in Bragg's law). Therefore from the X-ray pattern, it is seen at once that the molecule is helical and it is possible to measure the angle α directly as 40°. Finally, with the angle α and the pitch p determined, the radius r of the helix can be determined from $\tan\alpha = p/4r$, from which it follows that $r = (3.4\,\text{nm})/(4\tan 40°) = 1.0\,\text{nm}$.

To derive the relation between the helix and the cross-like pattern the detailed structure of the helix, that it is a periodic array of nucleotide bases, not a smooth wire, has been ignored. In Fig. 3 the bases are represented by points, and it is seen that there is an additional periodicity of separation h, forming planes that are perpendicular to the axis to the molecule (and the fibre). These planes give rise to the strong meridional diffraction with an angle that allows the layer spacing to be determined from Bragg's law, in the form $\lambda = 2h\sin\theta$, as $h = 340\,\text{pm}$.