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Asymmetry at the molecular level in biology

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Naturally occurring biological molecules are made of homochiral building blocks. Proteins are composed of L-amino acids (and not D-amino acids); nucleic acids such as DNA have D-ribose sugars (and not L-ribose sugars). It is not clear why nature selected a particular chirality. Selection could have occurred by chance or as a consequence of basic physical chemistry. Possible proposals, including the contribution of the parity violating the weak nuclear force, are discussed together with the mechanisms by which this very small contribution might be amplified. Homochirality of the amino acids has consequences for protein structure. Helices are right handed and beta sheets have a left-hand twist. When incorporated into the tertiary structure of a protein these chiralities limit the topologies of connections between helices and sheets. Polypeptides comprised of D-amino acids can be synthesized chemically and have been shown to adopt stable structures that are the mirror image of the naturally occurring L-amino acid polypeptides. Chirality is important in drug design. Three examples are discussed: penicillin; the CD4 antagonistic peptides; and thalidomide. The absolute hand of a biological structure can only be established by X-ray crystallographic methods using the technique of anomalous scattering.

Macromolecular asymmetry

Living organisms show a variety of symmetrical shapes. Such shapes include the fivefold symmetry of starfish, the symmetry shown by flower petals, the regular polyhedra found in the inorganic skeletons of radiolaria, and the bilateral symmetry seen in animals, including man. The bilateral symmetry is the mirror operation that relates our right hand into our left hand. Yet, as discussed elsewhere in this volume, the mirror symmetry is not universal throughout our bodies. Our single heart is on the left side of our body. The mirror symmetry shown at the

macroscopic level is all the more remarkable because the biological molecules that comprise macroscopic structures do not have mirror symmetry.

The basic macromolecules of life are DNA (the genetic material), RNA (the messenger, nucleic acid), proteins (the workhorses) and polysaccharides (the scaffolds). The components of these, the sugars and amino acids, can exist in two enantiomeric forms that are the mirror images of one another – the D and L forms (Figure 1). In living organisms, only one enantiomer is selected: D-sugars and L-amino acids. There are a few exceptions. For example the cell walls of bacteria have a short linking peptide that includes D-amino acids. Pasteur observed that crystals of sodium ammonium tartrate could be separated into left-handed and right-handed forms that he was able to recognize by examining with polarized light. He also recognized that molecules of living organisms had a unique chirality. The molecular asymmetry of natural molecules struck him as profound. In 1860, he commented that 'this was perhaps the only well-marked line of demarcation that can at present be drawn between the chemistry of dead and living matter' (as quoted by D'Arcy Thompson in his book on *Growth and Form*[1]). The distinction still has its weight. How did the selection arise?

Only asymmetry can beget asymmetry. The storage, transmission and expression of genetic information are intricate processes that ensure the maintenance of molecular asymmetry through molecular recognition (Figure 2). During protein synthesis, the hereditary material, DNA, is copied to a template messenger RNA, mRNA, with the enzyme RNA polymerase in a process known as transcription. In higher organisms, transcription takes place in the nucleus. The control of transcription (e.g. which genes are to be expressed and when) is achieved by protein complexes known as transcription factors whose activities are in turn controlled by extra-cellular events such as hormonal stimuli. In higher eukaryotes, the mRNA is further processed to remove introns that are not translated and the message, comprising the exon regions that are to be translated, is then transported from the nucleus to the cytoplasm to initiate protein synthesis. Protein synthesis is accomplished at the ribosome, a large macromolecular assembly composed of an RNA/protein complex. The ribosome-bound mRNA is 'read' by small RNA molecules known as transfer RNAs (tRNAs) that deliver the correct amino acid to the ribosome. The tRNA molecules are specifically charged with the correct L-amino acid by enzymatic processes with enzymes known as t-RNA synthetases. The ribosome then catalyses the formation of the peptide bond between successive amino acids to generate the protein polypeptide chain.

Once living processes had selected a distinct enantiomer, the complex machinery of ribosomal protein synthesis and the stereoselectivity of the tRNA synthetases would ensure that only one enantiomer was perpetuated. Stereoselectivity is carefully controlled but utilization of the other enantiomer is not forbidden. There are some enzymes that will catalyse the transformation of an

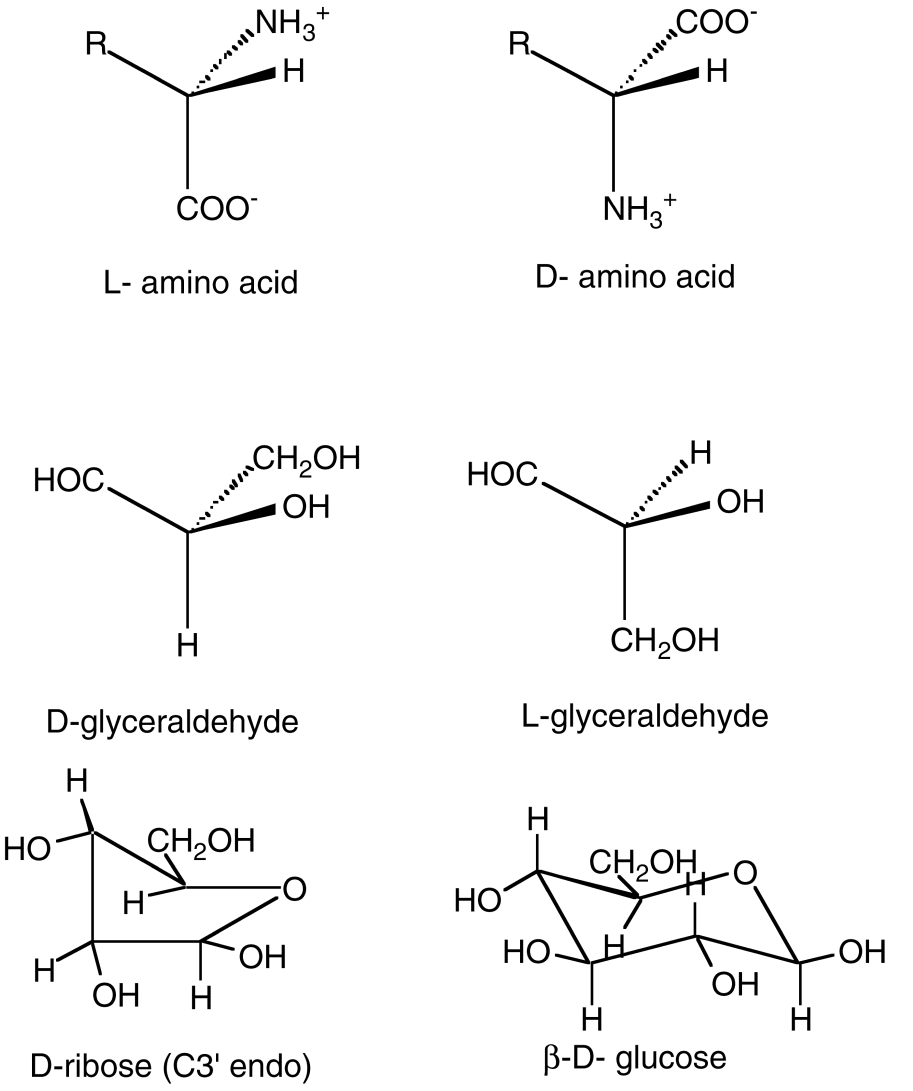

Figure 1. Representations of the L and D amino acids and D and L sugars. In the top diagram the arrangement of the four groups (H, COO^-, R and NH_3^+) around the central Cα atom of an amino acid is shown in the L-configuration (left) and the D-configuration (right). The R group represents one of the 19 chiral amino acid side chains. In the middle diagram the configurations of D- and L-glyceraldehyde are shown. In sugars such as ribose or glucose (bottom diagram) the configuration is established by the configuration at the highest numbered carbon atom with reference to glyceraldehyde.

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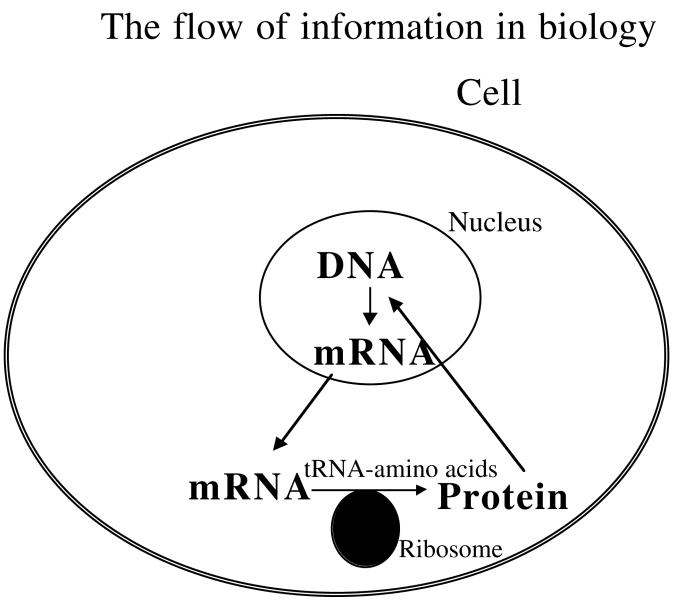

Figure 2. Schematic representation of flow of information from the genes (DNA) to proteins.

L-amino acid to a D-amino acid. For example a DL-racemase exists in mammalian brain leading to the formation of D-serine as a receptor ligand.[2,3] Bacteria have a D-amino acid aminotransferase. The sequence and structure of the bacterial enzyme is similar to the human L-amino acid transferase but the differences in the arrangements of groups at the catalytic sites of these enzymes allow them to specifically recognize an amino acid of one chirality (either a D or L- amino acid). Because of their similarity in sequence and structure, the two enzymes are thought to have arisen by divergent evolution from a common ancestor. Petsko and Ringe comment 'apparently modification of substrate specificity within the context of a given protein fold, even to the extent of reversing the handedness of the substrate, is easier than evolving a completely new catalytic mechanism'.[4]

The possible physical chemical basis for chirality in Nature

The physical-chemical basis for the selection of chiral molecules in naturally occurring macromolecules is not understood but there is an extensive literature that attempts to answer the question (reviewed in Refs 5 and 6). Quantum mechanical calculations based solely on electrostatic energies show equal energies for D and L amino acids since no chirality is implied in the electrostatic energy

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term. Inclusion of the parity-violating weak interaction mediated by the very short range Z^0 bosons gives results that show L-amino acids are stabilized with respect to D-amino acids and D-sugars with respect to L-sugars.[7–9] The parity violating energy difference (PVED) is very small, about 3×10^{-19} eV (10^{-14} Jmol^{-1}) corresponding to an enantiomeric excess for the L-amino acid of only 1 molecule in 10^{17} at room temperature. A small increase in the PVED for amino acids has been shown in later calculations that was dependent on conformational effects related to the orientation of the carboxyl group.[10,11] The PVED term increases with atomic number according to Z^6. This has allowed a very small difference between different enantiomers of a chiral iron complex to be measured by Mossbauer spectra. The result was consistent with that predicted.[12] The smallness of the PVED value for biological molecules comprising only light atoms has prompted chemists to doubt that the parity violating term could give rise to the distinct chirality of molecules observed in Nature.

Several authors have addressed ways in which the small energy difference might be amplified. Mechanisms, which have experimental support, include asymmetric absorption of a biomolecule onto an inorganic crystal with distinct chirality (reviewed in Ref. 13) or autocatalytic events in which each enantiomer acts as a catalyst for its own production; an excess of one enantiomer in the product suffices to direct any future product to that form.[14] Any mechanism that produces a distinct enantiomer would have to counteract the racemization reaction, which for amino acids occurs with a half-life of $\sim$6000 years. This time period, while long compared with the generation time of living organisms, is relatively short compared with the time during which chemical evolution is thought to have taken place – namely from the formation of the Earth 4.5 billion years ago to the emergence of life around 3.8 billion years ago.

A deterministic possibility has been proposed by Salam[15–17] in an extension of his theory that united the electromagnetic and the weak force into one electroweak force. It was suggested that the PVED might be amplified by a type of Bose condensation phenomenon that gives rise to a second-order phase transition below a certain critical temperature T_c promoting the conversion of the very slightly less stable D-enantiomer to the more stable L-enantiomer. Noting that the calculation of T_c is difficult, partly because of the incompleteness of the standard model of elementary particles, Salam, used analogies to the Barden, Cooper, Schrieffer (BCS) theory of superconductivity. The electro-magnetic interaction was introduced in a gauge invariant way to the BCS Hamiltonian and calculations suggested that, with certain assumptions, the transition temperature could be $\sim$250 K. A number of experiments have reported some indication of a parity violating phase transitions, using a variety of measurements including specific heat measurements by differential scanning calorimetry, temperature dependent magnetic susceptibility, laser Raman spectra of L- and D-alanine, and solid state

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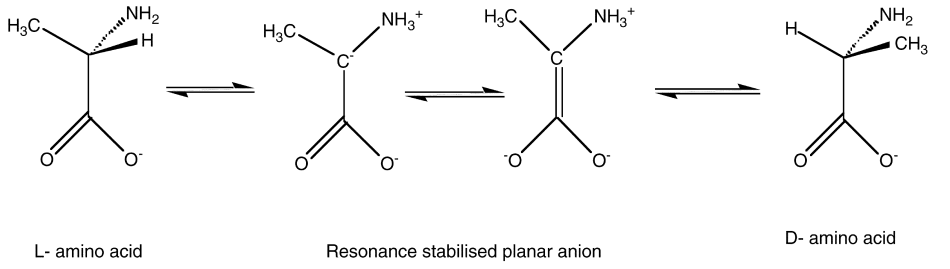

Figure 3. Racemization reaction of L-alanine and D-alanine.

NMR measurements on D and L-alanine crystals.[18–20] More recently some of these the experimental results have been questioned.[21]

Amino acids are known to racemize under basic and acidic conditions and at neutral pH with measurable rates at temperatures above 373 K. The rate of racemization is related to the electronegativity and the size of the amino acid side chain. The rate constant for alanine in aqueous solution at 413 K is 2.0×10^{-6} s^{-1}.[22] The initial step is generally assumed to be loss of a proton from the α-methine carbon, resulting in the formation of a resonance stabilized planar α-carbanion. At alkali pH, the proton could transfer to the amino group, perhaps through a tunnelling mechanism and then transfer back to the Cα with opposite chirality (Figure 3). Calculations of possible transition states for the unimolecular gas phase racemization of alanine have suggested a path in which the hydrogen atom migrates from the α-methine carbon to the amino group. Racemization then occurs when another proton from the amino group migrates back to the chiral carbon centre.[21] In both the aqueous phase and the gas phase there is the expectation of lengthening of the Cα-H bond in the early stages of racemization.

We have examined the possible differences between D- and L- alanine in the solid state by obtaining accurate neutron diffraction data from crystals at temperatures above and below the putative phase transition point (60 K, 240 K, 250 K, 260 K, 295 K and 300 K).[23] Neutron diffraction is a particularly sensitive technique to detect any geometric or structural changes, especially involving the hydrogen atoms. Our work showed no differences in bond lengths or bond angles as a function of temperature for D- or L- alanine. It appears that there is no temperature dependent change in geometry over the temperature range investigated at least in the solid state. In theoretical calculations, it was shown that both the parity violating and parity conserving energy terms were dependent on the torsion angle θ, the angle between the C-H plane with respect to the carboxyl plane.[11] In our crystallographic studies, examination of the temperature and enantiomer dependence of θ showed no significant or systematic variation within the accuracy of our neutron diffraction data.[23] The values of θ vary from 43.4°

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to 45.7° for the different experimental structures of D- and L-alanine. These values are close to the optimum value for the contribution of the parity violating term for neutral L-alanine in the gas phase and solution and slightly higher than optimum values obtained for the zwitterionic form.[11] While it is interesting to note that the conformation is consistent for one in which the PVED term is optimized, the conformation is also dominated by the need to achieve a staggered conformation around the Cα-C bond, as shown by the parity-conserving energies in the above calculations.

A further possibility for the origins of chirality in nature envisages an extraterrestrial origin. Could the seeds for homochirality have been planted by the heavy bombardment of the early Earth by meteorites? Such proposals are attractive because they allow a longer time scale and different extreme conditions for the development of homochirality than could have been encountered on earth.[24] For example, magnetochiral anisotropy – an effect linking chirality and magnetism – can give rise to a small enantiomeric excess in a photochemical reaction driven by unpolarized light in a parallel magnetic field.[25] Field strengths of the order of 7.5 T were required, which are much stronger than the Earth's magnetic field ($\sim 10^{-4}$ T) but less than the stray field of neutron stars ($\sim 10^{8}$ T on the surface). The carbonaceous Murchison meteorite that impacted in Australia in 1969 has provided a rich source of material.[26] Several natural and non-natural amino acids were found and there was a slight preponderance of L-amino acids over D-amino acids (e.g. L-alanine was 3% in excess of D-alanine). Interestingly, there was an even higher preponderance (up to 15%) of the L- form of several α-methyl substituted amino acids such as Cα-methyl valine and Cα-methyl alloisoleucine. The Cα-methyl amino acid derivatives do not undergo racemization because they lack the weakly acidic Cα-hydrogen. Hence, once formed they are stable over long time periods. In recent work it has been shown that carboxy activated Cα-methylated short peptides can react with natural amino acids and favour the incorporation of their particular enantiomer over the other.[27] A chiroselective peptide of natural amino acids has been shown to be capable of efficiently amplifying homochiral products from a racemic mixture of peptide fragments through an autocatalytic cycle.[28] Nearly 70% stereo chemical excess was observed after 100 min of reaction and the system exhibited some stereochemical editing in which incorporation of a D-amino acid in a predominantly L-amino acid peptide still resulted in a catalytically formed peptide with unique L-amino acid stereochemistry.

The α-methyl substituted amino acids, such as those observed in the Murchison meteorite, have been shown to promote the stereo-selective synthesis of threose and erythrose from glycoaldehyde, suggesting that the bombardment by meteorites could have provided a continuous and unique chiral influence for prebiotic sugar synthesis.[29] The possibility that ribose nucleic acids preceded

protein synthesis has been probed through measurements that showed that surface-bound RNA composed of D-ribose sugars becomes selective for amino acylation by only L-amino acids at the 2' ribose position, perhaps suggesting that, once a self-replication polynucleic acid had formed, composed of D-ribose enantiomers, the polynucleotide could enhance the selection of L amino acids.[30] Thus there are several mechanisms by which, once certain preferences for a particular chirality had been established, the enantiomeric excess could be amplified. The mechanisms leading to enantiomeric excess, whether terrestrial or extraterrestrial, remain to be established. The enhancement of the PVED energy contribution under extreme conditions seems to be a likely origin for such excess. As D'Arcy Thompson commented: 'Nature keeps some secrets longer than others'.[1]

The higher order structural consequences of molecular chirality

DNA adopts a double helical structure first elucidated by Watson and Crick in 1953. In this structure, each strand of DNA wraps around the other so that the 5'-3' phosphate-deoxyribose backbone is exposed to the solvent and counter ions and the four bases, adenine, guanine, thymine and cytosine, are buried in the interior (Figure 4). There are specific hydrogen bonds between the purine, adenine, and the pyrimidine, thymine, and between the purine, guanine, and the pyrimidine, cytosine. The specific hydrogen bonding arrangements between a purine with a pyrimidine ensures that the long polymer has a constant diameter. As described by Watson and Crick, the helix is right handed – the turn is clockwise as one moves along the helix. With this particular geometry the right-handedness of the helix is determined by the D configuration of the deoxyribose sugars. The geometry of the phospho-ribose backbone is governed by the conformation and configuration of the sugars. This classic form of DNA can be bent or supercoiled – it has significant flexibility while maintaining the characteristic Watson–Crick hydrogen bonds between the bases and the right-handed twist. As often in Nature nothing is absolute. In 1979, Rich and colleagues discovered a new form of DNA called Z–DNA. This conformation is preferred by sequences that have alternating cytosine and guanine bases and where the bases are methylated. Here the repeating unit is a dinucleotide and the helix is left-handed. The physiological function of this form of DNA is not fully understood but Z-DNA is present in chromosomes and may play a role in alleviating torsional strain induced by the RNA polymerase reaction during transcription and provide sites for specific protein recognition.

Protein molecules are polymers of amino acids in which each amino acid is linked to its neighbour by a peptide bond (Figure 5). The chirality of the L-amino acids that comprise the polypeptide chain has significant consequences for the structures that can be adopted by a polypeptide. There are two degrees of freedom

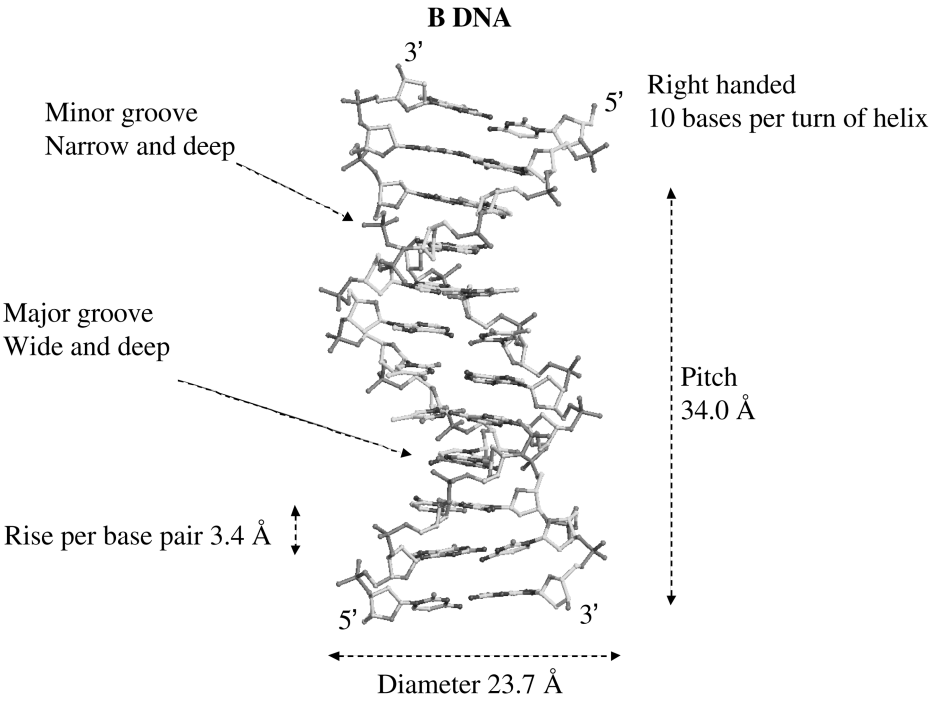

Figure 4. The structure of DNA. The double helix has a right-hand twist.

for each amino acid in a polypeptide chain. These are defined as the torsion angle ϕ (dihedral angle defined by C_i–N_{i+1}–$C\alpha_{i+1}$–C_{i+1} and the angle ψ (dihedral angle defined by N_{i+1}–$C\alpha_{i+1}$–C_{i+1}–N_{i+2}) (Figure 5). The peptide bond has a partial double bond character and rotation about this bond is restricted. As shown by G. N. Ramachandran and colleagues in 1963, not all ϕ,ψ angles are possible. Many result in steric clashes between non-bonded atoms. The two major allowed regions (Figure 5) correspond to the regular structures elucidated for polypeptides by Pauling and Corey, namely the α-helix and the β sheet, when these angles are repeated for many adjacent amino acids.

The α-helix (Figure 6) ($\phi \sim -50°$, $\psi \sim -60°$) has 3.6 residues per turn and the main chain carbonyl oxygen of the *i*th residue is hydrogen bonded to the main chain NH group four residues along ($i + 4$). The helix is right handed. This is a consequence of the chirality of the L-amino acids of the polypeptide chain. A left-handed helix would lead to steric clashes between adjacent side chains of the amino acids. The second major regular conformation is the β sheet in which the polypeptide chain is in the extended conformation ($\phi \sim -130°$, $\psi \sim +125°$). The sheet is formed by two or more adjacent polypeptide chains that may be may parallel or antiparallel with specific hydrogen bonding between the main chain

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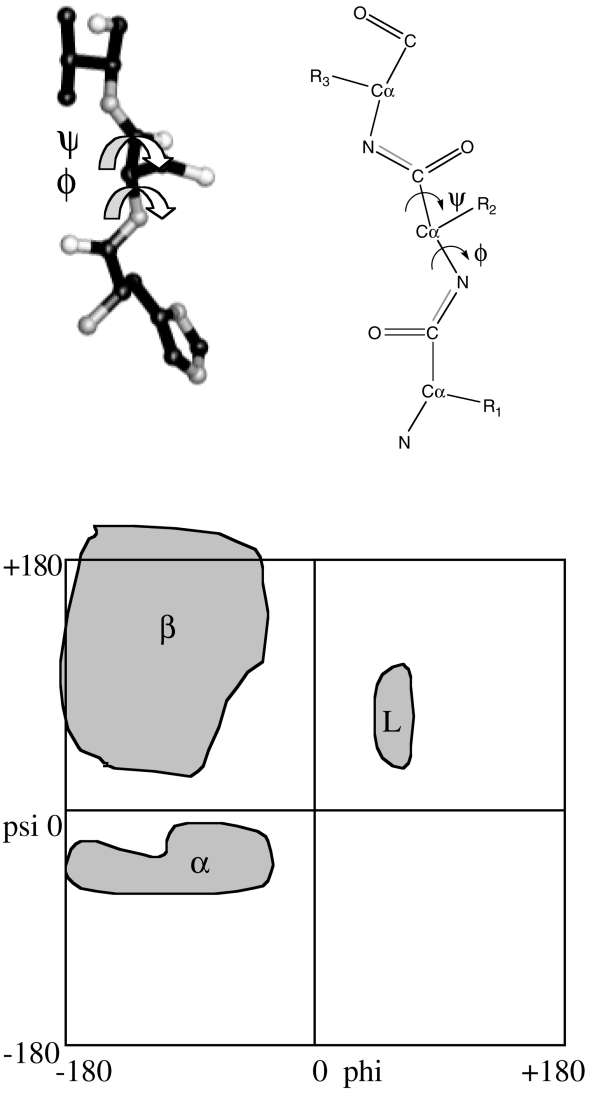

Figure 5. The peptide bond and the Ramachandran plot. In the top left diagram the structure of a short three amino acid peptide is shown. Carbon atoms are black, nitrogen grey and oxygen light grey. The sequence is histidine, serine, valine. In the top right diagram the three amino acid peptide is presented in chemical representation with the side chains represented by R1, R2 and R3. For each amino acid there are two possible rotations about the N-Cα bond (ϕ) and the Cα-C bond (ψ), as indicated. Rotations about these bonds generate different conformations of the polypeptide chain. As shown by Ramachandran and colleagues in 1963, not all combinations of ϕ and ψ are allowed. The Ramachandran plot is shown in the lower diagram. Allowed regions are shaded in grey.

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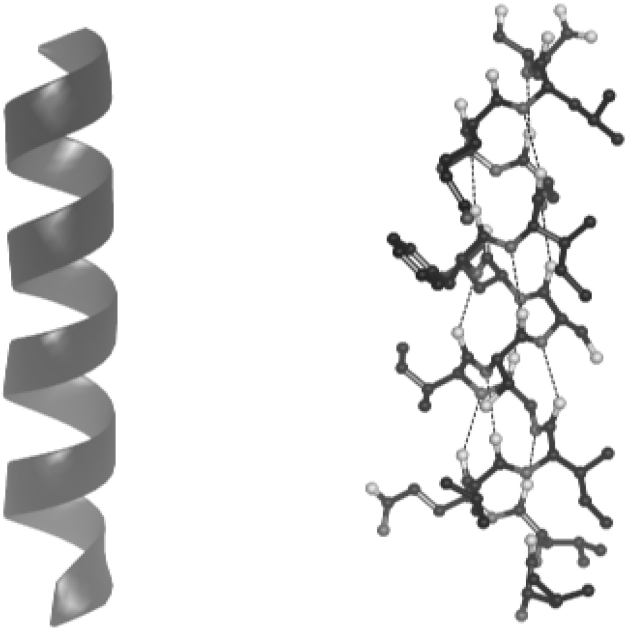

Figure 6. The alpha helix. In the left diagram the right-handed α-helix is shown as a spiral. In the right diagram all atoms are shown with the same atom colouring as in Figure 5. There are hydrogen bonds (shown dotted) between the NH groups and the C = O groups of every fourth amino acid. The conformation of each amino acid corresponds to the area marked α in the Ramachandran plot (Figure 5).

NH and C = O groups (Figure 7). The β strands have a pronounced left-handed twist when viewed along the sheet perpendicular to the strands. As seen in the Ramachandran plot, the mean position of the large area of available energy does not lie on the precise twofold diagonal but is offset, corresponding to an overall twist of the sheet.[31]

Thus, the chirality of the amino acids imposes a chirality of the secondary structural elements of protein molecules resulting in right-handed α-helices and left-handed twisted β sheets. Protein molecules adopt complex structures composed of either all α-helix or all β-sheet or a mixture with regions of secondary structures linked by regions with less regular conformations. One such example is the protease from the Human Immunodeficiency Virus (HIV) (Figure 8). The protein is composed of two subunits arranged with twofold symmetry. Each subunit is mostly β-sheet and there is one right-handed α-helix. The enzyme is a target for anti-HIV drugs, which have been successful in the clinic.[32] Part of the success came in finding non-peptidic mimics of the natural polypeptide substrate,

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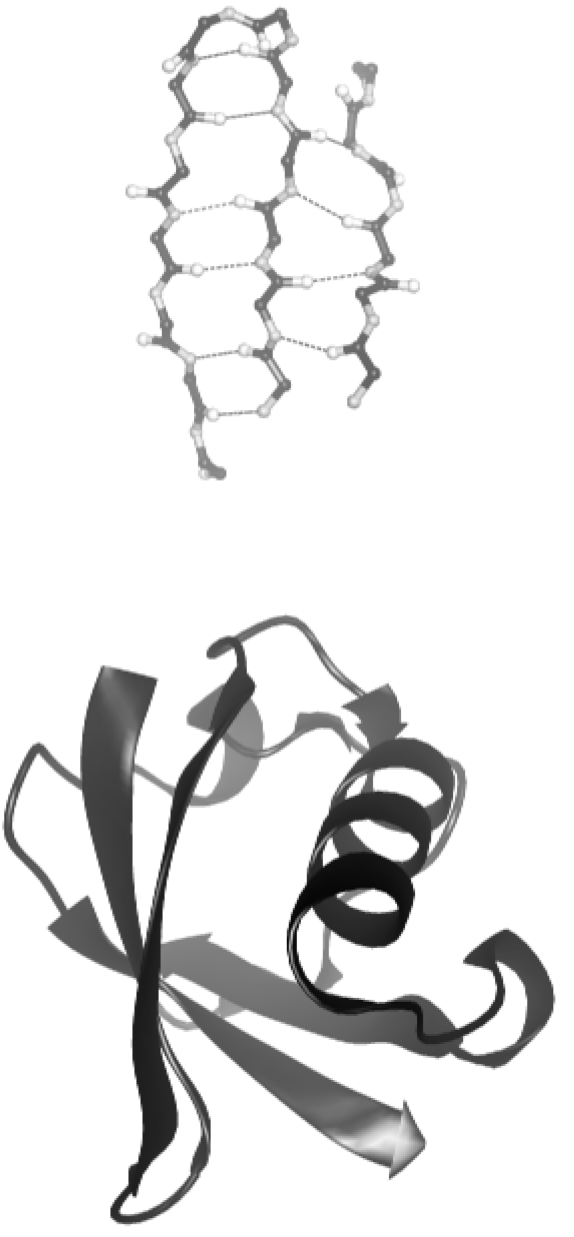

Figure 7. The beta sheet. In the top diagram three strands of polypeptide are shown in their extended conformation (corresponding to the area marked β in the Ramachandran plot (Figure 5)). Hydrogen bonds between peptide groups are shown dotted. The two strands on the left are antiparallel and the two strands on the right are parallel. Side chains have been omitted for clarity. The lower diagram shows the assembly of a beta sheet in a tertiary protein structure. The sheet has a left-handed twist when viewed edge on.

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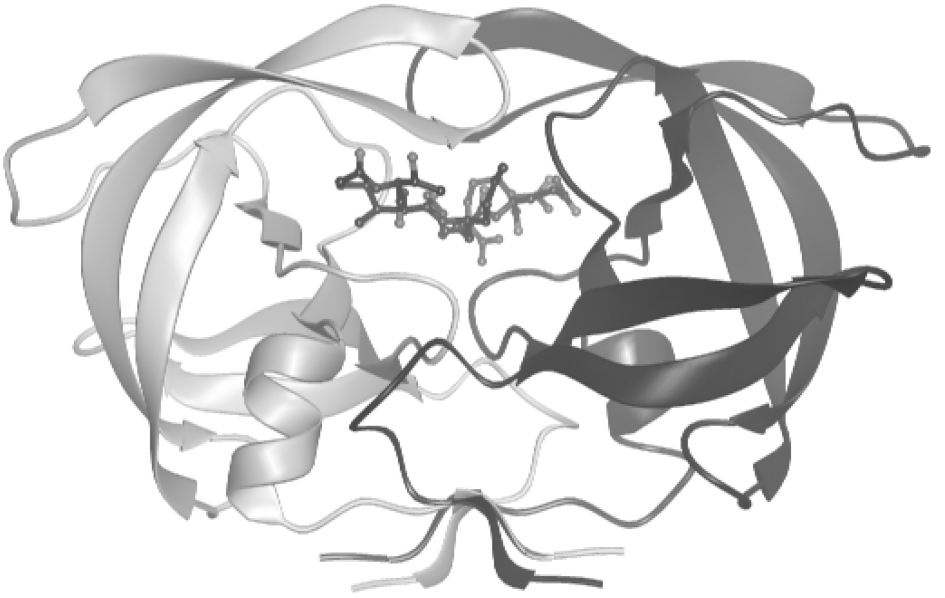

Figure 8. The structure of HIV protease. The protein is a dimer. One subunit is shown in light grey and the other in dark grey. The twofold axis of symmetry runs in a vertical direction. The left-handed sheet and the right-handed α-helices are apparent. A potent inhibitor is shown bound at the catalytic site. (Coordinates from Ref. 34.)

which bound in a conformation that mimicked the transition state of the catalysed reaction. The HIV protease is a small protein composed of only 99 amino acids. It is possible to synthesize it in the laboratory using chemical methods. The synthetic approach was extended to the synthesis of the same polypeptide but composed of D-amino acids.[33] The L and D-HIV proteases showed reciprocal chiral specificity on peptide substrates and their specific inhibitors, indicating that the chemically synthesized D- and L-enzymes were mirror images of each other. The D-amino acid enzyme folded to a stable structure and analysis by X-ray crystallography showed that it had the identical conformation to the naturally occurring L-amino acid enzyme but was the mirror image. The helix was left-handed and the sheet had a right-hand twist.[34] This example suggests that there is no inherent energetic stability in polypeptides composed of L-amino acids compared with those composed of D-amino acids. Polypeptides composed of a mixture of D and L amino acids would be inherently unstable in that they would be unable to adopt the regular secondary structures. Where D-amino acids do occur in nature, such as in bacterial cells walls or bacterial products, they only occur in short peptides or cyclic peptides.

Chirality and drugs

Understanding chirality is important for drug design. In order to target a particular biological receptor, the specificity and chirality of the recognition site must be correct. The fortuitous discovery of penicillin demonstrates the importance of chirality in drug design. The bacterial cell wall is composed of a polysaccharide component that is cross-linked by a short peptide. This peptide link contains D-alanine amino acids and is assembled by enzymatic reactions and not by the ribosome. The cross-linked cell wall macromolecule forms a stable shell that protects the bacteria from extreme conditions. Penicillin is such an effective antibiotic because it targets the synthesis of this large macromolecule that is specific for bacteria and not for the human host. Penicillin inhibits the cross-linking transpeptidase by mimicking the D-Ala-D-Ala dipeptide. It reacts with the enzyme to form a covalent adduct that is not released (Figure 9).

A further example shows that D-amino acids can be turned to advantage in drug design. Experimental allergic encephalomyelitis is an acute inflammatory autoimmune disease of the central nervous system and, elicited in rodents, is a model for multiple sclerosis. The immune reaction involves a protein CD4 from helper T-cells. Therapeutic effects can be achieved by using a peptide that mimics the recognition surface of the CD4 protein that is recognized by immunoglobulins. When such peptides are composed of L-amino acids, they are rapidly degraded *in vivo* by naturally occurring proteases but when composed of D-amino acids the peptide is resistant to degradation giving the possibility for therapeutic use.[35]

A less happy example is thalidomide (Figure 9). Thalidomide is a racemic compound that exists as an S(−) and R(+) enantiomer, which interconverts under physiological conditions. The S(−) form potently inhibits the release of the inflammatory signalling molecule tumour necrosis factor while the R(+) form seems to act as a sedative, probably mediated by sleep receptors. Both forms are metabolized by the cell. In the R(+) form, thalidomide was prescribed as a sedative and as an antiemetic for morning sickness in pregnant women, with devastating side effects. The teratogenic properties of thalidomide probably arose from interconversions to the S(−) form and the malformations were probably a result of the drug's interference with the growth of blood vessels. The anti-growth properties of thalidomide are now emerging as a possible treatment for cancer and inflammatory diseases (reviewed in Ref. 36).

Determination of absolute configuration

Our knowledge of structures of biological macromolecules comes from X-ray diffraction studies of macromolecules in the crystalline state. A crystal is composed of a basic parallelepiped-shaped block that can be repeated indefinitely

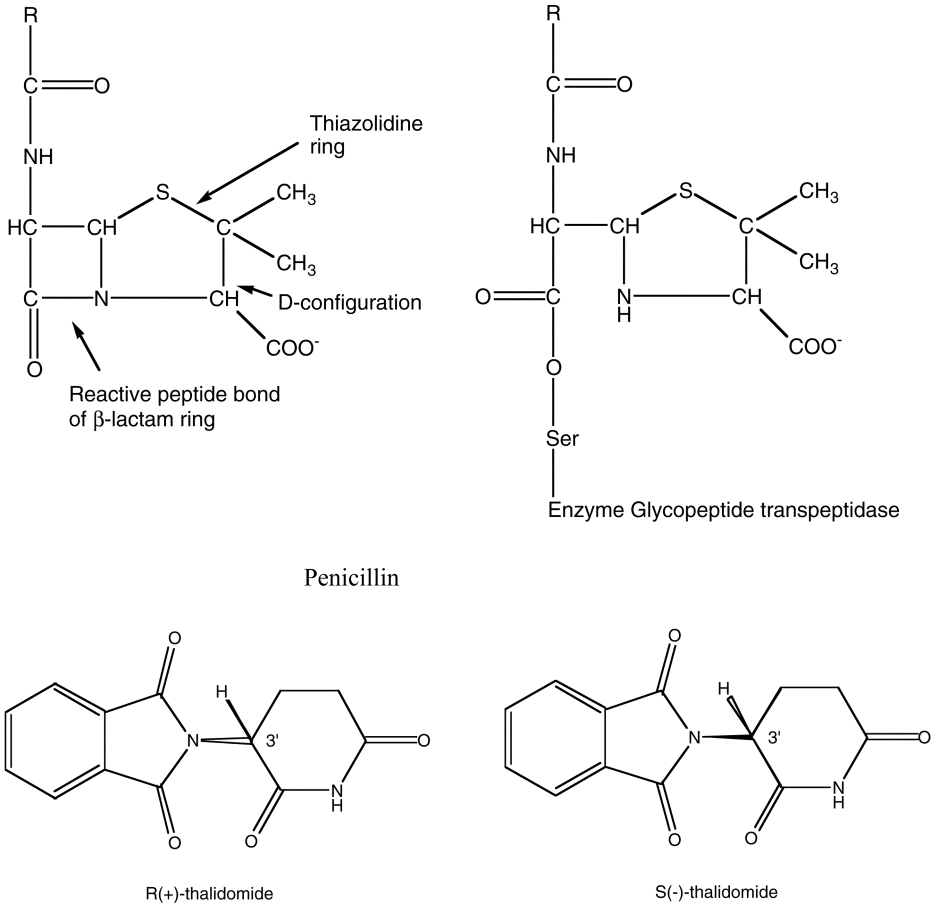

Figure 9. Chirality is important for drug design: Penicillin and Thalidomide (for further details see text).

in three dimensions. The condition that the crystal must be able to repeat indefinitely in three dimensions imposes limits on the symmetry elements that can be incorporated into the crystal lattice. Crystals can contain rotational symmetry elements based on 1 (the identity), 2-, 3-, 4- and 6-fold rotational symmetry. No other rotations are possible. Crystals may contain combinations of rotational symmetry elements and may contain mirror planes, glide planes and rotations combined with translations. At the end of the 19th century, Schoenflies, Barlow and Federov showed independently that the combination of allowed symmetry elements led to the finite number of 230 space groups. For crystals of biological

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macromolecules the number of space groups is restricted to 65, for macromolecules composed of L-amino acids or D sugars cannot crystallize in space groups that have mirror planes or glide planes.

X-ray diffraction theory shows that an X-ray diffraction pattern has a centre of inversion even though the crystal is composed of molecules that do not have a centre of inversion and are themselves asymmetric, such as the L-amino acids. In the early days of crystallography, the choice of hand for a molecule whose image had been reconstructed from an X-ray diffraction pattern was arbitrary. It was impossible to distinguish the absolute configuration, although once a choice had been made for one molecule, the choice for related molecules could be made consistent with known chemistry and rotation of plane polarized light. Following the work of Bjivoet in 1949, it was recognized that the ambiguity could be resolved by using anomalous scattering observed when the wavelength of the incident radiation is close to the natural absorption edge (K, L or M edge) of an element in the crystal.

The interaction of an electromagnetic wave with electrons gives rise to oscillations. The oscillating (i.e. accelerating) charges act as new sources and give rise to the scattered wave. Where there is more than one group of charges, the intensity of the scattered beam is modified by interference of the scattered waves from the different groups with each other. When the incident X-rays have an energy that is far from the absorption edge (K, L or M shell) of the atoms in the crystal, the atomic electrons scatter as free electrons. The atomic scattering factor is a real number (f_0). When the incident X-ray has an energy that is close to an absorption edge of an atom in the crystal, the atomic electrons re-radiate with a different phase than the free electron model. The scattering is modulated by f' (dispersive component) and by f'' (absorption component) that is $\pi/2$ in advance of the scattered wave. The so-called anomalous component breaks the centre of inversion of the diffraction pattern so that the intensity of a reflection with indices (h,k,l) is not equal to the intensity of a reflection with indices (-h,-k,-l). The inequality in intensities of the related reflections (h,k,l) and (-h,-k,-l) can be used to deduce phase information and to solve structures and it results in being able to establish the absolute configuration of a biological macromolecule. The first protein structure to be solved, myoglobin by John Kendrew and colleagues in 1959, did not incorporate anomalous scattering and amino acids were built in their L-configuration from knowledge of the absolute hand from small molecule crystal structures. The second protein, lysozyme solved in 1965, did utilize anomalous scattering and it was rewarding to find that the hand had been correctly assigned to yield L-amino acids and right-handed helices.[37] Today, anomalous scattering using wavelength-tuned X-rays from synchrotron sources is one of the most widely used methods for determination of macromolecular crystal structures and the application to the very small ($\sim 10\ \mu$m)

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and weakly diffracting crystals has been made possible by third generation synchrotron sources.

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