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APPENDIX 2

Possible Molecular Models for Gramicidin S and their Relationship to Present Ideas of Protein Structure

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The knowledge that has accumulated on the precise structure of amino acids and simple peptides makes it possible to build a number of models which may represent the detailed arrangement of the atoms in gramicidin S. These models must be judged in relation to the evidence described that the molecule behaves as a decapeptide in which two pentapeptide halves are related by a twofold axis of symmetry, and that the atoms tend to be concentrated in layers 4.8 Å apart in a direction at right angles to this twofold axis. In addition, the results of the studies made by Abbott & Ambrose (1953) of the infrared spectra of gramicidin S crystals have to be considered.

Abbott & Ambrose (1953) showed that, in the two main series of crystals examined, hexagonal *N*-acetylgramicidin S and orthorhombic gramicidin S hydrochloride, the infrared dichroism of the crystals is strong and is consistent with an orientation of the CO and NH bonds not far from parallel with the *c* axis, i.e. normal to the strongly reflecting planes of spacing 4.8 Å. Further, they favour a folded α -chain configuration on the evidence that the frequency of the C=O stretching mode in gramicidin S is 1646 cm.⁻¹, similar to that of α - and not β -keratin, evidence which is supported by observations on the dichroism of the absorption bands in the 4500–5000 cm.⁻¹ region. These observations suggest that we should concentrate our model-building on chain configurations which have been put forward for α -folded proteins, particularly those related to the Pauling, Corey & Branson (1951) α -helix and similar helices. But since there is some doubt about the reliability of conclusions based on small shifts in the frequencies of spectral lines

(Ehrlich & Sutherland, 1953), we have thought it best to include also models based on extended β -chain configurations, while accepting the evidence of Abbott & Ambrose (1953) on the general orientation of the CO and NH groups in our crystals. In our model-building we have not considered the possibility put forward by Gavrilov and others (Silaev, Trefilova & Ioanisiani, 1951; Ioanisiani, Gavrilov & Plekhan, 1954; Akimova & Gavrilov, 1954; Reznichenko, 1954) that the peptide structure of gramicidin S may be modified chemically by internal condensation between certain carbonyl and imino groups. None of our data at present are sufficiently precise to exclude such a condensation, but it would be incompatible with most of the models we have so far built.

Within the framework of a simple peptide molecule the following models appear to us most worthy of serious consideration.

α_{11} *Ribbon*. Abbott & Ambrose (1953) have put forward a decapeptide model for gramicidin S based on the Huggins (1943) α_{11} ribbon configuration. Their particular version has not a strict twofold axis of symmetry; it is possible, however, to build a model on this configuration which has the required symmetry. It is illustrated in Fig. 1 and is composed of two four-residue α_{11} ribbons joined at the two ends by the remaining two residues. The rupture of the hydrogen-bonding system at the two proline residues caused by the eclipse of the NH groups allows freer rotation at these two points, and the two halves of the ring can be so oriented that they are related exactly by a twofold axis of symmetry passing through the centre of the ring.

The configuration starting at the α -carbon of a proline residue and working round the ring is as follows:

CR *cis* to NC_δ
CO *cis* to CC_β } proline residue

CR *cis* to NH
CO *cis* to CR } valine residue

CR *cis* to NH
CO *cis* to CR } ornithine residue

CH *cis* to NH
CO *cis* to CH } leucine residue

Intermediate between

CR *trans* and CH *cis* to NH
CR *cis* and CH *trans* to CO } phenylalanine residue

This molecular model is sterically satisfactory and will fit into the crystal with the CO and NH bonds approximately parallel with the *c* axis. But it is difficult to see how it could give rise to the observed strong 4.8 Å reflexions, as the repeat distance between residues along the chains is 2.75 Å (Donohue, 1953).

α -Folded chain models based on the α -helix. If moves of the type used in constructing the α -helix

of Pauling *et al.* (1951) are made, the five residues of gramicidin S link most naturally into a single pentapeptide ring, with all CO groups projecting from one side of the ring and all NH groups from the other (Fig. 2). Rings of this kind may be piled one above the other in the crystal lattice to form a completely hydrogen-bonded column with atoms in layers about 4.8 Å apart and CO and NH bonds nearly parallel with *c*.

In this otherwise attractive structure the pentapeptide molecules within one column cannot be exactly related by twofold axes of symmetry. It would be possible in the hexagonal crystals to relate two-thirds of the columns in pairs by symmetry axes, leaving the remaining third to be disordered, alternately pointing up and down; or one might perhaps suppose that pentapeptide rings of opposite sense were related within the columns by approximate but not exact twofold axes. We are doubtful whether at the present stage of our investigation we could exclude this type of solution on X-ray evidence alone. But it does appear to be excluded by other evidence mentioned above, and particularly by the reported synthesis of the peptide.

Fig. 1. A decapeptide model for gramicidin S based on the Huggins α_{11} ribbon configuration, viewed down the two-fold axis.

Fig. 2. Pentapeptide models for gramicidin S based on the moves in: (a) a left-handed α -helix; (b) a right-handed α -helix.

An alternative possibility within the framework of the α -helix configuration is to form a true cyclic decapeptide by breaking the two pentapeptide rings between the α -carbon of the D-phenylalanine and the CO of the proline residue and rejoining to form a horseshoe-shaped molecule. One join is made easily, the other only with distortion of the hydrogen-bonded system, and again the molecule has at best only a pseudo-twofold axis of symmetry. This molecule has packing advantages similar to those of the pentapeptide units, advantages which are not shown by other types of model we have tried to construct in the α -helix configuration; in other respects it is an awkward object.

β -Chain models. By far the most obvious type of model to build for gramicidin S is one based on the

extended β -form of chain. Two chains, each composed of four residues, may be set antiparallel to one another and linked at the ends by means of the proline residues to form a decapeptide ring. The chain configuration may be made to conform closely with that of the β pleated sheet of Pauling & Corey (1951). Four hydrogen bonds are formed between the two antiparallel chains, while the remaining CO and NH groups are available for hydrogen bonding between molecules. Several variants are possible, of which one is shown in Fig. 3.

This model is of a decapeptide with a twofold axis of symmetry. In the crystal, molecules could be arranged with their lengths at right angles to c and approximately 4.8 Å apart, the CO and NH bonds projecting parallel with c . There are certain

Fig. 3. A decapeptide model for gramicidin S based on the β -pleated sheet of Pauling & Corey (1951) viewed (a) down the twofold axis, and (b) at right angles to the twofold axis.

problems of intermolecular hydrogen bonding between molecules of this type in the hexagonal crystals which may be surmounted by the intervention of water or ethanol molecules. Otherwise the only immediate serious argument against this model is the optical evidence in favour of an α -type configuration.

Mixed α - β model. A last model which may be mentioned can be built by following partly α , partly β moves (Fig. 4). These may be described as follows:

(a) valine to ornithine, α -type move (the NH bonds point in the same direction, and perpendicular to the plane of the ring); (b) ornithine to leucine, β -type move (the NH bonds point in opposite directions); (c) leucine to phenylalanine, α -type move. This model can be formed either as a pentapeptide or as a decapeptide, and appears to be stereochemically satisfactory in either form. In packing it is very similar to the α -helix models described above, but has a true twofold axis of symmetry. Also the formation of the decapeptide need involve no strain.

We have, in addition, considered a number of other models, one, for example, based on *cis* orientation of the CO and NH groups, but none of these seem so satisfactory from a crystallographic

point of view as the last two varieties mentioned here. Of these two, the model based on the β configuration seems on our evidence alone to be by far the more likely.

If gramicidin S has a structure of this type a great deal of reinterpretation of recent data on peptide-chain configuration in proteins will be necessary. Infrared studies have indicated a strong correlation between the modified frequency of certain C=O and N-H modes of vibration observed for different ' α -type' proteins. The characteristics of the X-ray photographs of these proteins have in turn been correlated with the type of diffraction pattern given by an α -helical chain configuration. We are led by our observations on gramicidin S to consider either that the frequencies of the infrared vibration spectra cannot be as exactly correlated with chain configuration as has been supposed, or that the particular mode of chain folding indicated for gramicidin S may occur in some ' α ' folded proteins. There are observations on at least one protein, insulin (Ambrose & Elliott, 1951), which underline the same conflict and indeed support both these solutions. Insulin is transformed very readily and reversibly into fibrous insulin (Koltun, Waugh & Bear, 1954). It is difficult to imagine that the change involves a

Fig. 4. A mixed α - β decapeptide model for gramicidin S.

major alteration of chain configuration and yet shifts occur in the frequencies of the CO and NH bonds similar to those expected for the change from α - to β -type chain configurations. Further, chain-folding of the type indicated by the model based on the β configuration may well occur in at least part of the insulin molecule (Hodgkin & Oughton, 1956). Unlike the α -helix, this type of folding would permit very easy formation of S-S links between cysteyl residues placed five residues apart in a polypeptide chain, exactly the interval found by Sanger, Smith & Kitai (1954) in the A chain of insulin.

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The Ionization of Acidic Metmyoglobin

IONIC-STRENGTH CHARGE EFFECTS

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We have shown in a previous publication [George & Hanania, 1952, subsequently referred to as paper (1)] that measurements of the equilibrium concentrations of acidic and alkaline metmyoglobin (MetMb), under conditions of very low ionic strength, I , yield a constant value for the ionization constant only within a narrow range of pH from about 8.6 to 9.3. In more alkaline solutions progressively higher pK values were obtained, and the trend in more acidic solutions was towards lower pK values.

From determinations of the mean pK values between pH 8.6 and 9.3 at very low ionic strengths, the limiting slope of a plot of pK against $\sqrt{I}$ showed that the ionization, which is believed to be that of a water molecule co-ordinated to the haemin iron,

is accompanied by the change from -2 to -3 in the effective charge on the iron. The effective charge determined in this way has been called the ionic-strength charge Z (cf. Barnard, 1944). However, the formal charge on the ferric iron atom is not -2

but $+1$, since two of its three positive charges can be regarded as neutralized by negative charges on two pyrrolic nitrogen atoms of the porphyrin ring. This difference is not altogether unexpected, since the ionization occurs about two pH units on the alkaline side of the isoelectric point of myoglobin, and the net negative charge on the molecule, which the acid-base titration curve suggests is about 10 (George & Irvine, 1955), may be considered to influence the reactions of the haemin iron.

Our studies of the formation of metmyoglobin fluoride (George & Hanania, 1954*a-c*) substantiated this idea, for in the pH range 6.5–7.5, which spans the isoelectric point, the change in Z was found to be approximately equal to the corresponding change in formal charge, $+1$ to 0 , according to the equation

That is, somewhere between pH 7.5 and 8.6 the ionization of acidic groups on the molecule alters the value of Z for MetMb from $+1$ to -2 . This in turn suggests that the variation of pK' with pH, at low ionic strengths and outside the range of pH 8.6–9.4, might well be due to changes in Z brought about by the ionization of these groups. We present below some detailed measurements made to test this hypothesis.

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