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DIELECTRIC BEHAVIOUR OF DRY SYNTHETIC POLYPEPTIDES

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SUMMARY

Four distinct copolypeptides were synthesised and formed into dry films whose crystal structure was shown by X-ray diffraction to be of the β type. Their dielectric constants were measured in the range 100 kHz to 10 MHz and 25 to 60 °C. It was possible to obtain dielectric constants in excess of 20 at physiological temperatures. The relationship of these results to the understanding of the transport of ions across cell membranes is discussed.

INTRODUCTION

If an ion is to pass through a membrane two conditions must be fulfilled. (a) A pore must exist or the membrane material must yield to the passage of the ion. (b) The electrostatic energy of the ion must change by not more than a few kT, as the ion moves from the aqueous phase to the interior of the membrane.

An approximation to the hydration energy of an ion immersed in a medium of dielectric constant, ε, can be obtained by using the Born formula [1]

$$E = -\frac{e^2}{8\pi\varepsilon_0 r}\left(1-\frac{1}{\varepsilon}\right) \tag{1}$$

where r is the effective radius of the ion and the other symbols have their usual meanings. For the alkali metal ions 1 yields a hydration energy of a few electron volts. The figures derived from 1 putting r equal to the Pauling radius are always a little too large as compared with the results obtained by Noyes [2] using thermodynamic data. These discrepancies can be removed if proper allowance is made for the dielectric saturation of water [3] but 1 is still useful as a simple indication of the type of behaviour one can expect. Now ε for water at physiological temperatures is about 80 whereas ε for saturated hydrocarbons (corresponding to phospholipid tails) is about 2.1. Thus, it is clear that the increase of energy of an alkali metal ion as it moves from water to the centre of a cell membrane is approximately one electron volt or rather more. Thus the translation of such an ion through the lipid material is a very improbable event. This prediction is confirmed by the fact that undoped artificial lipid membranes are generally found to be very poor conductors of ions.

These conditions indicate that the regions immediately surrounding ion pores in cell membranes must consist of some material which is far more polarisable than are saturated hydrocarbons. One of us has proposed [4] that these regions probably consist of protein and that the required polarisability is provided by the high dipole moments (3.7 Debye units [5]) of the peptide bonds. This point of view is supported by the numerous experiments [6, 7] which have been carried out using artificial membranes and the polypeptide antibiotic gramicidin A, which material facilitates the passage of alkali metal ions through these membranes. Nevertheless, there is very little direct information available concerning the polarisability of protein material. The majority of naturally occurring proteins which are readily available in pure form only retain their proper structure in the presence of a substantial quantity of water of crystallisation. Work carried out in this laboratory [8] shows that attempts to study the dielectric behaviour of proteins in this hydrated condition are masked by the behaviour of the water of crystallisation.

It was thus decided to attempt the synthesis of appropriate polypeptide chains which would, under suitable circumstances, form continuous crystalline structures in a completely dry state and thus provide direct evidence about the polarisability of protein structures.

Simple geometrical considerations show that the peptide bonds are fairly rigidly held in place in the α structure. The anti-parallel β structure however allows the peptide bonds more room for motion, though even here restraints on their motions exist. These arise from the existence of the C-N hydrogen bonds between adjacent polypeptide chains and also from the fact that the rotation of alternate peptide bonds in opposite directions (a requirement for polarisation) also involves some movement of the R groups attached to the α carbons. We thus decided to select a polypeptide structure known to crystallize in the anti-parallel β structure and then to synthesise several variants of this polypeptide. In each case we grew dry films of the material of the order 20 μm thick and studied its dielectric properties. X-ray studies on each material were also carried out to confirm that the β structure was retained, to explore the variation of inter-chain distance with variations of R groups and to establish the orientation of the direction of the polypeptide chains in relation to the plane of the film.

We decided to use the basic structure of silk as the starting material on which variations could be made. The basic repeating unit in silk is Gly-Ala-Gly-Ala-Gly-Ser. The silks produced by various organisms differ in that, from time to time, the sequence given above is broken by the appearance of other amino acid residues, the particular residues and their particular points of occurrence characterising the silk produced by the particular organism. However, these additional residues are sufficiently infrequent so that the basic 6-fold sequence predominates. This statement is especially true of the silk produced by, *Bombyx mori*, the common chinese silk worm. It is known [9] that this material, if dissolved in dichloracetic acid, will recrystallize in the β form. The dielectric behaviour of recrystallized material has already been studied by Magoshi [10] and we were able to repeat and confirm his results.

EXPERIMENTAL PROCEDURE

We employed the Merrifield [11, 12] technique to grow the polypeptide chains. As this technique involves twelve changes of reagent in the reaction vessel for each

residue attached, we constructed an electronic control circuit which made it possible to programme both the sequence of reactions necessary for the attachment of one residue and also to programme the sequence of residues. We have reported in detail on the design and operation of this device elsewhere [13]. The starting materials were BOC-amino acids (Sigma Ltd. London), and the procedure was generally as described in ref. 12 with the following minor additions and variations. To test that the synthesis was proceeding as programmed, portions of the product were extracted at four stages (usually 3, 7, 11, 15 for a final 15 residue peptide), hydrolysed and the product examined using a Locarte amino acid analyser. In each case the procedure was standardised by a control of known composition which was hydrolysed and passed through the analyser immediately before the specimen. The standard deviation from exact stoichiometry was 1.04% which probably represents the limit of accuracy of the instrument. The largest number of residues of one kind in any one final product was 8 and thus one might suppose that, in the worst possible case, not more than about one completed polymer in 12 is defective. We believe that the real situation is very much better than this as otherwise we would expect larger percentage errors in the less frequently occurring residues. In any case a small proportion of defective polymers is unlikely to have a serious effect on the type of measurements described in this paper.

The resin-polypeptide product was washed in ethanol and glass distilled water before the polypeptide was cleaved from the resin by passing hydrogen bromide through a suspension in trifluoracetic acid. The peptide was separated from the resin by filtration and evaporated to dryness, subsequently being purified by repeated washing in aqueous methanol followed by evaporation of the solvent. The product was subsequently dissolved in dichloracetic acid (100 mg peptide per ml acid) and a portion of the solution floated on the surface of mercury, the solution being contained in a PTFE ring (10 mm inside diameter). The solvent was removed by careful treatment under gradually reduced pressure over a period of 3 days before final dehydration at 10^{-3} Torr for a minimum of 24 h. Dehydration was continued until it was impossible to detect any further change in physical properties.

Dielectric measurements were carried out using a Wayne Kerr Universal R. F. Bridge type B602 with a source and detector SR 268 within the frequency range 100 kHz to 10 MHz. The samples were supported on mercury in a small dish which forms the lower electrode, the upper electrode consisting of a small pool of mercury placed on the upper side of the specimen. The sample and electrodes were contained in a perspex cell through which oxygen-free dry nitrogen was flushed in order to prevent rehydration of the specimen. The dish containing the mercury was supported on a hotplate controlled by a Eurotherm PID/SCR power controller which allowed the specimen to be maintained at temperatures between 20 and 80 °C. A thermocouple located in the mercury lower electrode monitored the temperature which was recorded on a Bryans 27 000 chart recorder. Electrode areas were ascertained by photographing the upper electrode together with a scale and enlarging the photographs. Specimen thickness was measured using a Mercer mechanical thickness gauge immediately after measurements were completed. Typical specimens were about 20 μm thick.

In order to check the reliability of this method of measurement the dielectric behaviour of films of a non polar polymer were studied. Hexacosane (Sigma) was dissolved in a mixture of benzene and *n*-decane and floated on mercury. The films were vacuum dried and measurements were made on them generally as for polypep-

tides. The mean of the results for three films gave a value of dielectric constant of 2.08 which compares well with an expected value of 2.07.

X-ray diffraction studies were made on the films using a Philips PW 1009 X-ray generator with a fine focus molybdenum target together with a flat bed camera. Films were mounted in the beam both with the plane of the film perpendicular to the beam and along the beam. Exposures were taken for 16 h at 30 kV, 25 mA. Indexing of the diffraction patterns indicated that the material was largely crystalline but with some amorphous material present. The peptides were in the β conformation with the chains running in the plane of the specimen and thus normal to the applied electric field. The four materials studied and the interplanar distances are given below.

A	$(Gly\text{-}Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser)_n$	0.98 nm
B	$(Gly\text{-}Val\text{-}Gly\text{-}Val\text{-}Gly\text{-}Ser)_n$	1.01 nm
C	$(Gly\text{-}Leu\text{-}Gly\text{-}Leu\text{-}Gly\text{-}Ser)_n$	1.02 nm
D	$(Gly\text{-}Ala)_n$	0.97 nm

In each case individual chains consisted of 15 molecules so that in the case of A, B and C above, there were 2.5 repeats per chain. In each case at least 3 independent specimens were studied and reproducibility was good.

Real and imaginary components of dielectric constant were measured and the real parts are shown in Figs. 1 and 2 for fixed temperature and varying frequency and vice versa respectively. The imaginary parts were at least two orders of magnitude less than the real parts in the frequency range studied. Above 60 °C irreversible damage appeared to be done to the films and it was impossible to obtain repeatable results if films were heated above this temperature.

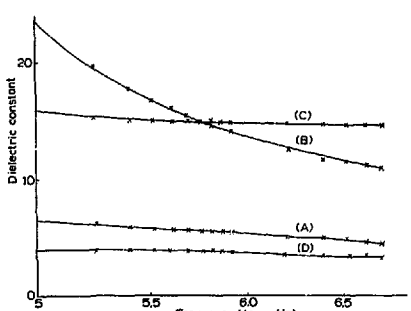

Fig. 1. Dielectric constant of polypeptide films as a function of frequency. (A)-$(Gly\text{-}Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser)_n$; (B)-$(Gly\text{-}Val\text{-}Gly\text{-}Val\text{-}Gly\text{-}Ser)_n$; (C)-$(Gly\text{-}Leu\text{-}Gly\text{-}Leu\text{-}Gly\text{-}Ser)_n$; (D)-$(Gly\text{-}Ala)_n$.

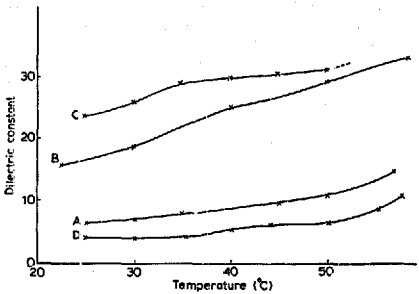

Fig. 2. Dielectric constant of polypeptide films as a function of temperature. (A)-Gly-Ala-Gly-Ala-Gly-Ser)$_n$; (B)-(Gly-Val-Gly-Val-Gly-Ser)$_n$; (C)-(Gly-Leu-Gly-Leu-Gly-Ser)$_n$; (D)-(Gly-Ala)$_n$.

DISCUSSION

The interesting feature of these results is the fact that it was possible to obtain dielectric constants in these materials of well in excess of 20 at physiological temperatures. Now saturated hydrocarbons usually have dielectric constants of about 2.1 and this is thus the kind of value which we would expect the Ala, Val and Leu side groups to contribute. The serine groups possess a dipole moment and might be expected to enhance the overall dielectric constant. However even the Gly-Ala co-polymers have a dielectric constant much greater than 2.1 and it is thus reasonable to deduce that we are observing a substantial contribution from the dipole moments associated with the peptide bonds. It was pointed out in the introduction that the β structure involved two main inhibitions to the movement of the peptide bond dipoles. One of these is the

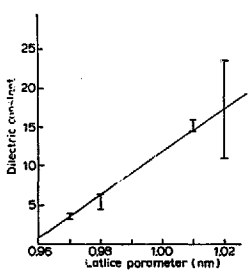

Fig. 3. Real part of dielectric constant as a function of lattice parameter. The bars show the variation corresponding to the frequency range 100 kHz to 10 MHz.

effect of the interchain hydrogen bonds. These are unlikely to change much as between one combination of residues and another. The other inhibitory effect derives from the fact that the peptide bonds cannot be displaced from their equilibrium orientation without some movement of the associated side groups. Thus a more loosely packed structure involving larger interlayer spacing exemplified by polymers B and C above would be expected to have a higher dielectric constant than polymer A which corresponds to natural silk. Fig. 3 shows the relationship between lattice parameter and dielectric constant and indicates how a more open structure leads to a a larger value of the latter quantity.

These results support the view that relatively high levels of polarisability resulting from the movement of the dipoles associated with peptide bonds may be a feature of those proteins which mediate the translation of small cations across membranes. We hope to explore these ideas further using the synthetic techniques here described combined with the use of artificial membranes.

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