Short Communications and Preliminary Notes

Proton-deuteron exchange in proteins dissolved in heavy water

It has recently been shown by HVIDT AND LINDERSTRØM-LANG[1,2] that when insulin is dissolved in D_2O at pH 3 all the protons attached to nitrogen or oxygen atoms in the protein exchange with deuterons within 48 hours at 38° C. About 65% of these protons exchange very rapidly and the rest more slowly. LINDERSTRØM-LANG[2] suggests that the latter are situated in the peptide backbone, in that part of the peptide chain which is stabilized by S – S linkages. Simple peptides, insulin A-chain, and oxidised ribonuclease, all of which may be assumed to be in the form of a single peptide chain, show complete and rapid exchange[2].

Infra-red absorption spectra of proteins dissolved in D_2O, taken at wavelengths near 6 μ also give information on hydrogen-deuterium exchange[3,4]. For serum albumin LENORMANT AND BLOUT[3] find that a certain proportion of the peptide backbone protons do not exchange at room temperature at pH 6, but do exchange when the solution is heated to 100° C or brought to pH 10. PARKER[7] has published the spectrum of keratin in the 3 μ region after exposure to an atmosphere of D_2O for 14 days. The keratin has become partially deuterated, leaving a relatively sharp absorption band near 3300 cm^{-1} which shows a higher dichroic ratio than that of the undeuterated material.

If a dry protein film is formed from D_2O solution without any exposure to atmospheric water vapour, the infra-red spectrum through the region 3700 cm^{-1} to 2000 cm^{-1} should give a reliable semi-quantitative picture of the $H \rightleftharpoons D$ exchange. This result will refer to the $H \rightleftharpoons D$ exchange in solution provided there is no denaturation and further exchange on drying. If it can be assumed that there is no redistribution of deuterons in the protein during drying, the spectrum can potentially give information about which protons are free to exchange in solutions at different temperatures and pH values.

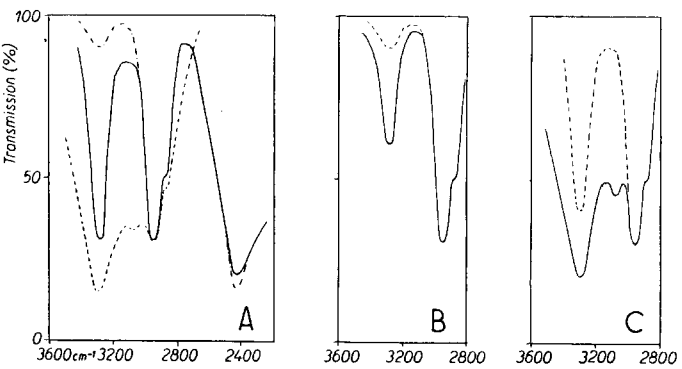

Fig. 1. A. Dried insulin film. – – – – from H_2O solution at pH 4; ——— from D_2O solution at pH 4 after 48 h at 20° C; ·-·-·-· from D_2O solution at pH 4 after 48 h at 38°. B. Bovine serum albumin film from D_2O solution at pH 7. ——— after 48 h at 38° C; – – – – after a further 2 minutes at 100° C. C. Seal myoglobin. ——— dry crystal after exposure to D_2O vapour for 4 days; – – – – dried film from D_2O solution after 48 h at 38° C.

The spectra in Fig. 1 were taken on a Perkin Elmer 12C spectrometer fitted with a calcium fluoride prism, a reflecting microscope and a lead telluride detector. A rocking mirror arrangement with electronic ratio recording was also included to give a double beam through the microscope. These modifications will be described in detail in a subsequent publication. The range 3700 cm^{-1} to 3000 cm^{-1} corresponds to N–H and O–H stretching vibrations, the range 3100 cm^{-1} to 2800 cm^{-1} to C–H stretching vibrations, and the region around 2400 cm^{-1} to D-stretching[5,6].

The dried protein films were prepared in the following way: a small volume of protein solution in D_2O (0.1 ml) at a concentration of 2%, prepared with minimum exposure to atmospheric water vapour, was sealed in a phial with a long drawn out neck of flattened cross-section (Fig. 2). After 48 hours at 20° C or 38° C the phial was tipped so that a small drop of the solution was transferred to a point between two re-entrant bulges in the neck. The body of the phial was then immersed in a bath at —70° C, the neck remaining at 20° C until the drop had dried to a film. While the body of the phial was still at —70° C the neck was sealed off and a spectrum subsequently taken on the dry film in the sealed tube.

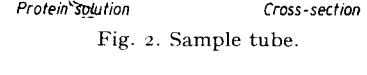

Fig. 2. Sample tube.

For the spectra of myoglobin crystals a small drop from a suspension of crystals in saturated ammonium sulphate (in H_2O) was deposited in the neck of the phial while the body contained a relatively large volume of D_2O, saturated with NaCl to bring its vapour pressure to approximately that of the crystal suspension. After 4 days at 38° C the crystals were dried by the method described above.

Insulin shows virtually complete exchange after 48 hours at 38° C. The small absorption band remaining in the N–H and O–H stretching region could be due to dilution of the D_2O with atmospheric H_2O during the preparation of the solution and addition of DCl to bring the pH to 4. After 48 hours, or longer, at 20° C a pronounced band remains at 3290 cm^{-1}. HVIDT AND LINDERSTRØM-LANG's[1,2] results for insulin also show incomplete exchange under these conditions. 3290 cm^{-1} is the main H-stretching frequency associated with the peptide bond[6], and these results are therefore consistent with the view that the less readily exchangeable protons are situated in the peptide backbone. For bovine serum albumin (Armour) after 48 hours at 38° C, or 4 days at 20° C, there is considerable but not complete exchange, and the 3290 cm^{-1} band may be further reduced if the droplet in the neck of the phial is heated to 100° C for 2 minutes before drying. The different results for insulin at 20° C and 38° C must be due to a different exchange in solution, rather than during drying, since in both cases the dry films were prepared at 20° C. Dry films formed from solutions of serum albumin which have been kept at 20° C or 38° C may be readily redissolved.

In myoglobin crystals, exposed to D_2O vapour, a band remains at 3290 cm^{-1}, and also a much less intense band at 3070 cm^{-1}. These bands show no dichroism either in the dry crystals or before drying, although there is X-ray diffraction evidence for an overall alignment of the peptide chains in seal myoglobin in the crystal form and orientation at which the spectra were taken[8]. This throws some doubt on the interpretation of the 3290 cm^{-1} band as being due to non-exchanging protons in peptide bonds. Possibly the crystals of the present sample were not identical with those on which the X-ray diffraction measurements were made.

A weak absorption band at 3070 cm^{-1} is characteristic of proteins and peptides, but has not been satisfactorily interpreted[6]. It could be due to interaction between peptide chains in the crystalline state, or to salt linkages of the type $N^+-H\cdots O^-$. In dry films prepared from myoglobin in D_2O solution the band at 3070 cm^{-1} disappears, and there is a further reduction in the absorption at 3290 cm^{-1}.

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Pathways of glucose utilization in the mammary gland of the rat

We have shown previously[1] that levels of activity of enzymes of the hexose monophosphate oxidative pathway of glucose metabolism in the rat mammary gland vary considerably according to the physiological state of activity of the gland. It was found that levels of activity of both glucose 6-phosphate (G 6-P) dehydrogenase and 6-phosphogluconate (6-PG) dehydrogenase increase rapidly from the end of pregnancy to the end of lactation, these increases being approximately 60 fold for G 6-P dehydrogenase and 20 fold for 6-PG dehydrogenase, and then fall to