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The far infrared spectra of proteins and enzymes in the solid state

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Abstract—The far i.r. spectra of powdered collagen and collagen complexed with glyoxal, and related proteins and enzymes are found to be detailed and extremely intense over the whole of the far i.r. region of the electromagnetic spectrum.

The study of the absorption of far i.r. electromagnetic radiation by proteins and enzymes may produce information which could be useful in many fields of investigation. For example, the hypothesis [1] of giant dipole vibrations at frequencies near the GHz region can be examined directly with radiation in the microwave/far i.r. range. If such a general property is corroborated experimentally then support would be given to the theory that the efficiency of enzymes as catalysts is based on a general physical property rather than on a specific array of mechanisms. The property may be a metastable excited condition with a very high dipole moment as suggested by FRÖHLICH [1]. In this case physical experiments should aim at measuring polarization and vibrational properties during activity of the enzymes. An ideal system for such work would be the stable oriented purple membrane from *Halobacterium halobium* [2], and such far i.r. experiments are under way.

The existence of low frequency modes in proteins has been demonstrated experimentally with the laser Raman technique but not in the range down to 1 cm^{-1}, as reported in this paper. We aim to complement these data by studying the enzymes as powdered disks in the region 30 GHz–6 THz (1–300 cm^{-1}) by Fourier transform interferometry [3]. As far as we know this is the first attempt of its kind.

In Fig. 1 we illustrate the far i.r. power absorption of collagen as a powdered disk, and of collagen complexed with methylglyoxal [4]. At 379 K the noise level is high, but it is possible that the extra peaks indicate a complex underlying spectral structure in these systems; a structure which is obscured at lower temperatures by hydrogen bonding in the secondary and tertiary structure. Some features are common to all the specimens of Figs. 1–3. At frequencies from

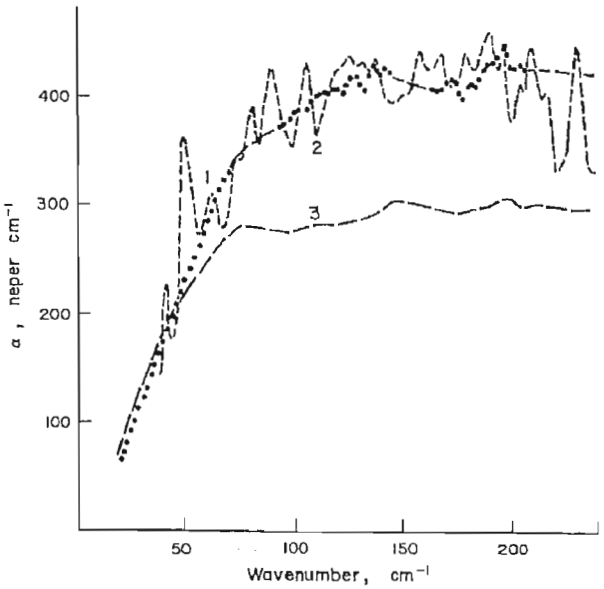

Fig. 1. Far i.r. power absorption of bovine Achilles tendon collagen and collagen complexed with methylglyoxal. The samples were in the form of powdered disks. (1) Collagen at 296 K; (2) collagen at 379 K; (3) methylglyoxal–collagen complex.

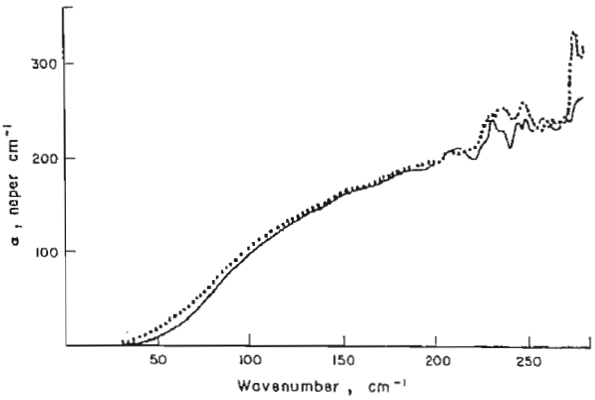

Fig. 2. Power absorption of egg white albumen · · · 296 K; —— 110 K.

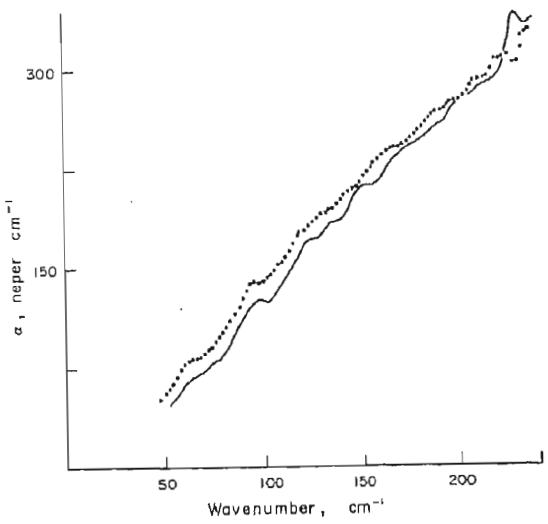

Fig. 3. Power absorption of beef liver catalase · · · 296 K; —— 110 K.

1 cm^{-1} to about 100 cm^{-1} the power absorption coefficient (in neper cm^{-1}) rises very rapidly from a very low value (below 10 cm^{-1}) to an intense plateau region. This behaviour is roughly reminiscent of the liquid water spectrum in this region. We surmise that much of the structure observed [5] in the far Raman is broadened by hydrogen-bonding.

Recent molecular dynamics simulations [6] of the interior of pancreatic trypsin inhibitor have evidenced the existence of large fluctuations in the structure and energy components of the protein. These last for as long as 15 ps and may be associated with bond-stretching, bond bending, and non-bonded interactions. This means that evidence for such fluctuations could be found at frequencies of inverse ps, i.e. those of the far i.r. A growing body of information [7] is available in this frequency range from laser Raman scattering. Far i.r. work would complement the work of, for example, TWARDOWSKI [8] on the solid isoenzymes I and II of acid phosphatase from rat liver. Both have strong bands in the 10–300 cm^{-1} region due mainly to protein-prosthetic-heterosaccharide complexes. The low frequency bands below 300 cm^{-1} observed in the native lyophilized isoenzymes are obtainable from the sialic acid groups of the glycoproteins. These bands disappear after treatment with neuraminidaze enzyme, leaving broad bands due to the protein motion. It therefore seems possible that the far i.r. region may be useful:

(a) conventionally, for structural assignments;

(b) to investigate the (2–15) ps fluctuations predicted by the molecular dynamics simulations of Karplus *et al.* [6];

(c) to evaluate electronic and protonic conduction mechanisms in exhaustively dried and moist protein powders at frequencies high than those available in microwave Hall effect measurements [9].

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