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Hydration of globular proteins: U. V. Studies

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Biofisica. — *Hydration of globular proteins: U. V. Studies.* Nota di LUCIANO GIANNINI e ENRICO GRATTON (*), presentata (**) dal Corrisp. G. CARERI.

RIASSUNTO. — La nostra tecnica di misura permette la misura simultanea dell'idratazione e della densità ottica sullo stesso campione. Applicando questa tecnica su films di lisozima siamo stati capaci di seguire le variazioni spettrali causate dall'idratazione sulle due tirosine dell'enzima esposte al solvente.

INTRODUCTION

As is well known, in the hydration of globular proteins it is possible to distinguish two separate steps [1], [2]. In the first step, below 80 % relative humidity, the water is adsorbed on some hydrophilic groups as a single molecule. In a second step, above 80 % of relative humidity, the water condensates on the water first bound to specific groups [1], [2].

The amount of water bound to each hydrophilic group as a function of relative humidity has been calculated [3], [4] and measured [2] for model substances and the values so obtained have been extrapolated to globular proteins.

In this work we show that using a suitable technique [5] we can follow spectrophotometrically in the U. V. region the hydration of the tyrosines in the lysozyme. This enzyme has been object of a systematic study through the differential spectrophotometry in the near U. V. under different conditions [7], [10]. So that it is possible to correlate the effects due to a new perturbant, such as hydration, with the physical and chemical structure.

EXPERIMENTAL

Materials.

The enzyme used is a commercial purified preparation purchased from Boehing Mannheim G.m.b.H. preparation number 15351 E.L.G.B., three times recrystallized and salt free. For the preparation of solutions we used Millipore filtered bidistilled water. The stock solution was 3×10^{-5} M lysozyme.

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Methods.

For the U.V. absorption studies we prepared some films obtained by depositing 12 drops of the solution on a 22 mm quartz plate and evaporating the solvent in a dry box with a continuous flux of dry nitrogen. Using these techniques we obtained homogenous and well transparent films.

The quartz plate was suspended on the arm of a microbalance model Chan. R 100, using a rigid quartz wire so that the quartz plate could not rotate.

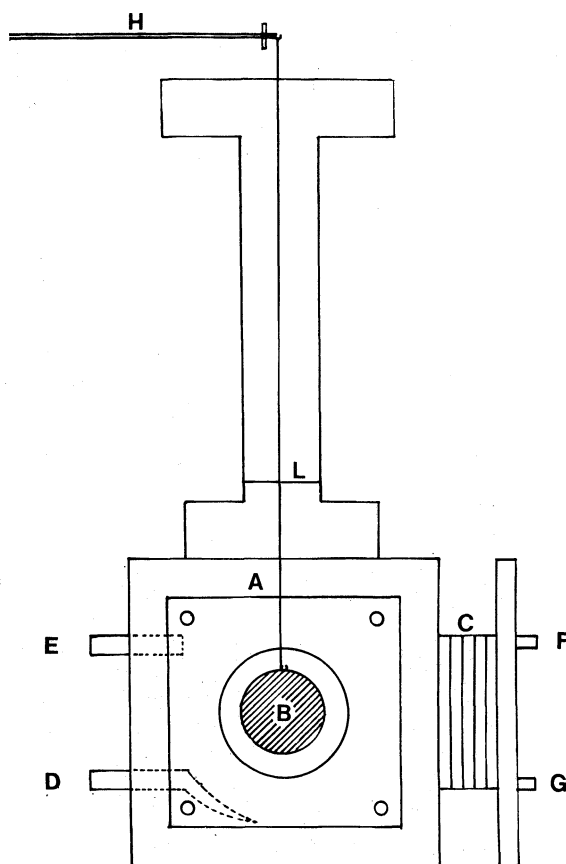


Fig. 1. - Experimental setup: A: measuring cell; B: sample; C: thermostat; D: input gas; E: output gas; F: input thermostatic fluid; G: output thermostatic fluid; H: balance arm; L: diaphragm.

The quartz plate, so suspended, was contained in a cell, shown in fig. 1, equipped with two quartz windows and with a thermostat. The cell was also furnished with an input and an output for flushing gases.

The amount of hydration is immediately seen from the actual weight of the sample, provided we know the weight of the dry film (2 mgr.). Different values of humidity in the measuring cell described above, are obtained by mixing a flux of dry nitrogen and a flux of water vapor saturated

nitrogen. The dry nitrogen is obtained by allowing ultrapure nitrogen to pass through a trap filled with liquid nitrogen.

We monitored the humidity of the entering gas with an electrical hygrometer sensible to 1 pmm of water (Show Igrometer model S).

The measuring cell is easily introduced into the sample compartment of a Cary 15 spectrophotometer. The absorption spectra are digitized and recorded. Due to difficulties in obtaining two identical films we could not use a direct differential technique.

RESULTS AND DISCUSSION

Fig. 2 shows the hydration differential spectra of lysozyme in the region 320–240 nm and the parental spectra. Many features are evident from the differential spectrum. As is known [8], the contribution at 293–94 nm is due

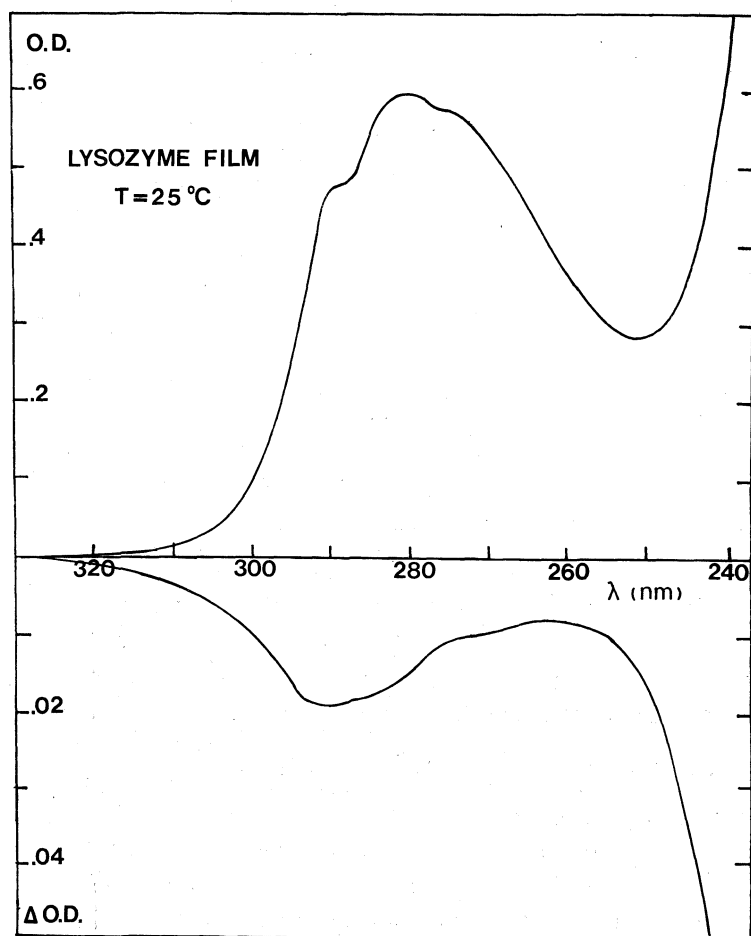


Fig. 2. — Upper curve: dry lysozyme film U. V. spectrum; lower curve: U. V. differential spectrum obtained by subtracting the spectrum of the lysozyme film at maximum hydration from the spectrum of the dry sample.

to tryptophan residues. The tryptophan groups of the enzyme are not the major sites of hydration. However, it is possible to observe in the spectrum of this chromophore a modification generated by the hydration of some charged groups [5] which are in the proximity of the tryptophans. Since the behaviour of this band (293–294 nm) can reflect only indirectly the hydration of the charged groups, we concentrate our studies on the band at 288 nm in the differential spectra [7]. Such a band comes from a perturbation in the $\pi - \pi^*$ transition of the phenolic chromophore because of formation of a hydrogen bond between tyrosine hydroxyl and the water molecule. The beginning of a strong negative differential band at 250–240 nm comes from the hydration of the amide groups of the backbone [11].

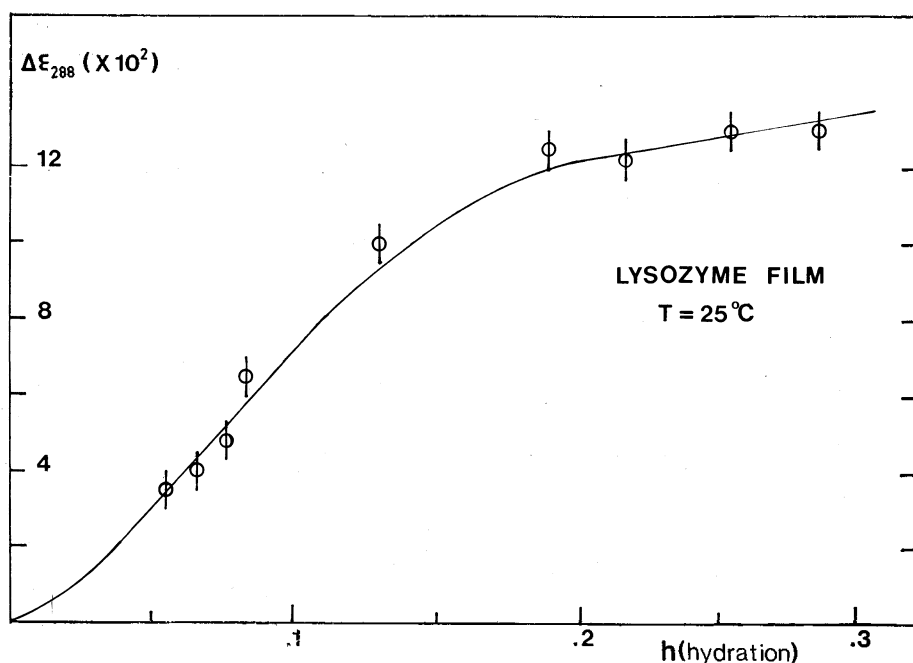


Fig. 3. - Variation of the extinction coefficient at 288 nm as a function of hydration.

In fig. 3 the variation of the absorption coefficient at 288 nm as a function of hydration is shown and two different regions are evident: the first one below $h = .25$, and the second one above $h = .25$, $\left(h = \frac{\text{mg of water}}{\text{mg of protein}}\right)$. The first region is due, as we said before, to the direct interaction of water molecules with the phenolic groups. In the lysozyme molecules there are three tyrosines and only one (Tyr 33) is inaccessible to the solvent [12], while the tyrosines in the positions 20 and 23 can be reached by the solvent [12].

For hydration below $h = .25$, the amount of water associated with the tyrosine for each degree of hydration has been estimated to be about 2 molecules of water per tyrosine residue [3], [4]. If such a value is true we

obtain that the water causes a change in absorbance $\epsilon_{288} = 600/\text{molecule}$ of bound water. For hydration above $h = .25$ we observe the condensation of a water multilayer, and we do not have any further change in the differential spectrum.

CONCLUSION

Our technique allows the measurement of hydration and U. V. absorption on the same sample and at the same time. We applied this technique to lysozyme and we were able to measure the spectral changes due to hydration of the two accessible tyrosines.

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