Mikrochim. Acta [Wien] 1988, I, 255—258

Mikrochimica Acta

Investigation of Intramolecular Processes in Proteins by Time Resolved FTIR-Difference Spectroscopy

Klaus Gerwert* and Benno Hess

Max-Planck-Institut für Ernährungsphysiologie, Rheinlanddamm 201, D-4600 Dortmund 1, Federal Republic of Germany

Abstract. A new technique is developed by which intramolecular processes of proteins can be investigated time-resolved in the infrared spectral region. By this technique the transduction mechanism of light energy into chemical energy in chromoproteins is explored on an atomic resolution.

Key words: time-resolved FTIR, intramolecular, proteins, light energy.

The elucidation of protein functions on an atomic level challenges life science today. Great progress in this direction was achieved by X-ray diffraction analysis which shows the structure of proteins at nearly atomic resolution [1]. In order to elucidate the mechanism of such complex structures a non-invasive technique is needed which shows the reactions of the individual chemical groups on a molecular level time resolved. In principle infrared spectroscopy fulfills this condition. In order to select the absorbance bands of functionally relevant groups from the background absorbance of the whole protein, difference spectra between different states of the protein have to be taken. An active state of the protein can be stabilized e.g. at a deep temperature. Since the observed absorbance changes are in the order of 10^{-2} to 10^{-3}, conventional infrared spectroscopy cannot be used because the baseline distortions are too large. The multiplex advantage of the FTIR method enables much shorter measuring times, and thereby highly reproducible difference spectra can be taken. By this static method difference spectra are achieved between protein states which are stabilized (if possible at all) mostly under unphysiological conditions. In order to measure the absorbance changes at room temperature time resolved, one can apply the flash photolysis method in the infrared range [2]. In this case the absorbance change of one selected wavelength element is measured only and therefore a large measuring time is needed for the whole spectrum.

* To whom correspondence should be addressed

In order to combine the advantages of static FTIR and time-resolved IR spectroscopy a time resolved FTIR apparatus has been developed (Fig. 1). The FTIR apparatus is a Bruker IFS88 and the NYD/YAG pulsed dye laser system is a YG481 of Quantel. The time course is shown in Fig. 2. As

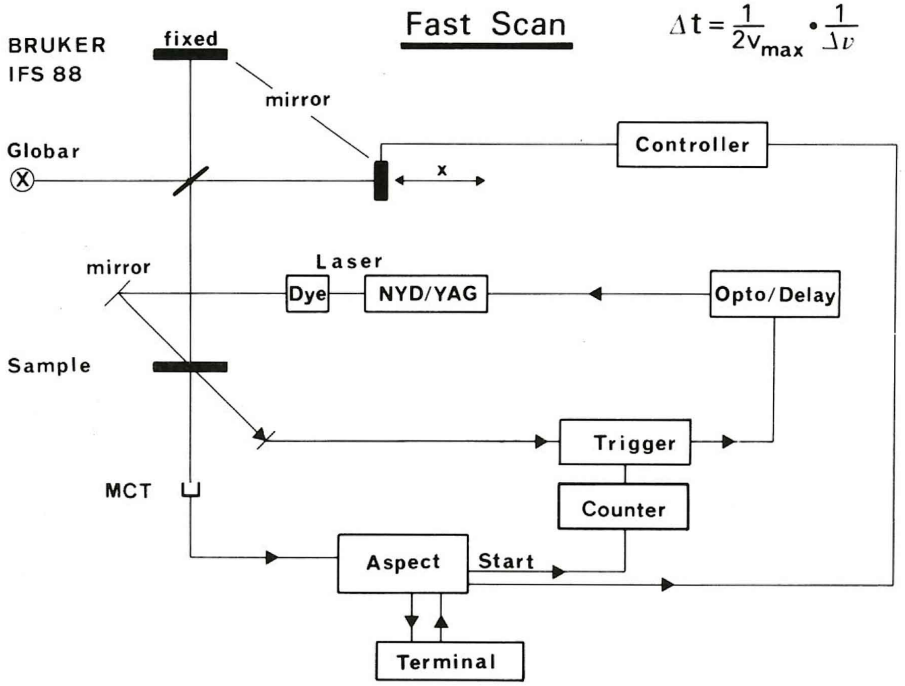

Fig. 1. Apparatus for time resolved measurements

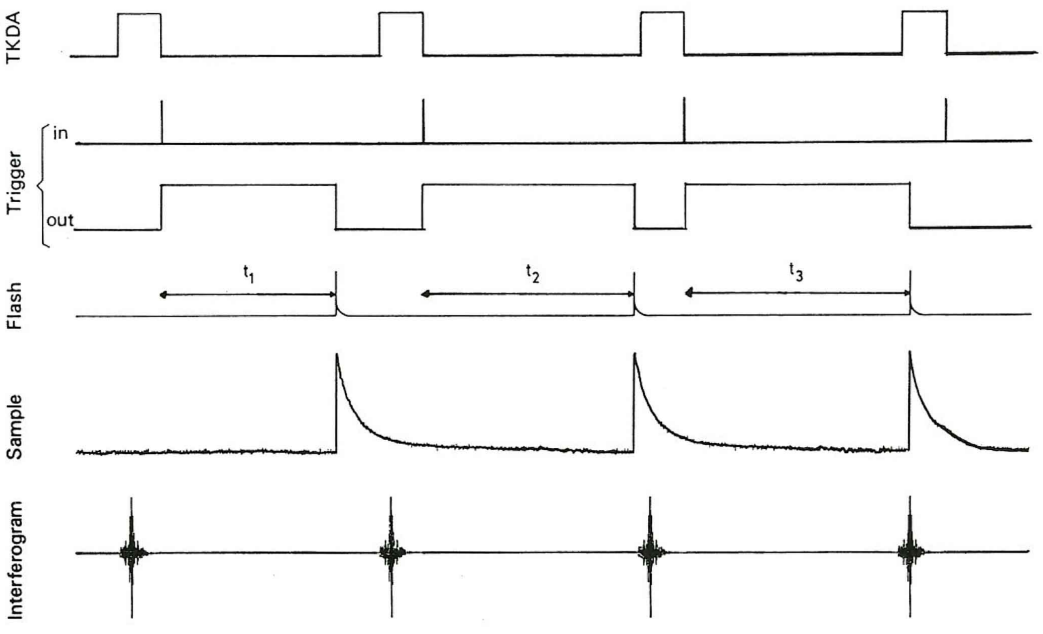

Fig. 2. Time course of the apparatus

reference the "Take Data" signal of the FTIR apparatus is used. When data of the FTIR apparatus are taken (collect sample) a pulse of the aspect computer triggers the laser flash after a variable delay time t_i. The LED signal triggers the pulse of the laser. By this method interferograms of different states of chromoproteins can be measured. The time resolution ΔT is determined by the velocity of the scanner v_{max}, the scan length Δx and the spectral resolution Δv,

$$\Delta T = \frac{1}{v_{max}} \Delta x = \frac{1}{2v_{max}} \frac{1}{\Delta v}.$$

At the maximum velocity of 6.3 cm/s a time resolution of 10 ms at 8 cm^{-1} spectral resolution is achieved.

With this apparatus intramolecular processes of the lightdriven protonpump bacteriorhodopsin are investigated. The retinal protein bacteriorhodopsin has a molecular weight of 26 700 Dalton. After light illumination a photocycle of about 10 ms half-time is initiated which is accompanied by a vectorial proton transport. In Fig. 3 a deep temperature difference spectrum (200 K) between the ground state (BR) and a photointermediate (M) is shown. The difference spectrum indicates e.g. protonation of internal aspartic acids [3] (1762 cm^{-1}) and alltrans configuration of the chromophore retinal (1200 cm^{-1}, 1169 cm^{-1}) in BR [4]. By the new time resolved technique a similar difference spectrum is obtained at room temperature (Fig. 4). For the difference spectrum 500 scans with and

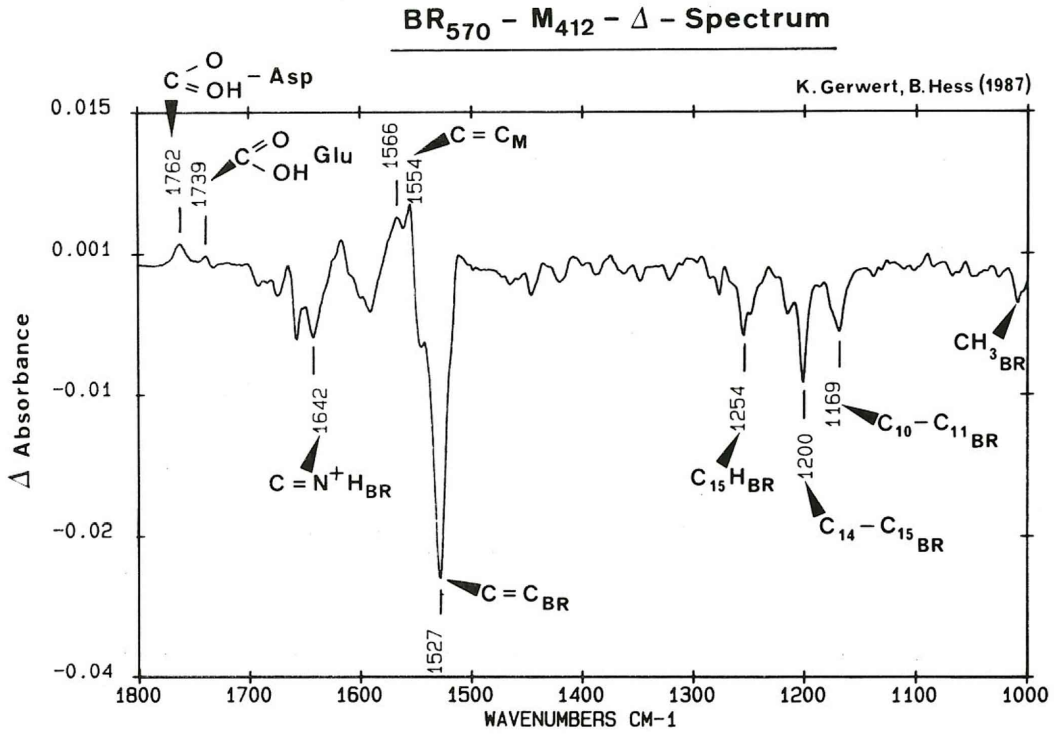

Fig. 3. Deep temperature static BR-M difference spectrum

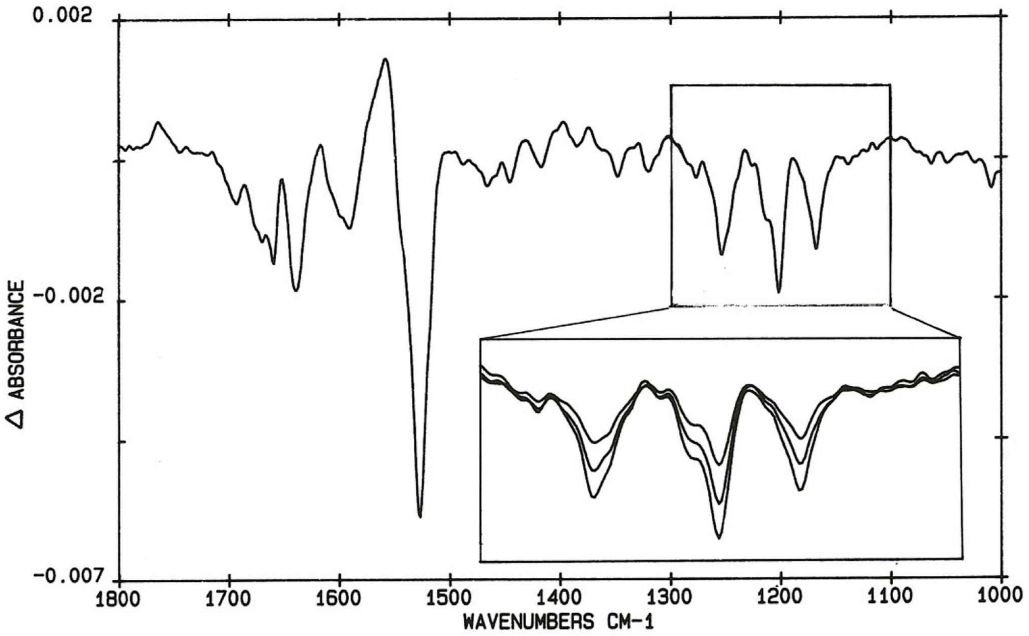

Fig. 4. Room temperature time resolved BR-M difference spectrum (4 cm^{-1}, 20 ms). Insert shows difference spectra for three different delay times t_1, t_2, t_3

without laser flashes are averaged. A spectral resolution of 4 cm^{-1} and a time resolution of 20 ms are used. The time resolved spectrum shows clear differences above 1640 cm^{-1}. These differences indicate that the protein shows a distinct behaviour at room temperature compared to the frozen state. With this new technique the proton transport mechanism will be investigated. In order to assign the absorbance bands in the difference spectra isotopically labelled proteins are used.

Acknowledgement. We would like to thank Bruker, especially Dr. Gronholz, Mr. Weil and Dr. Simon, for kind support. Further useful help by R. Eichas-Nell, M. Schubert and H. Schlüter is gratefully acknowledged.

References

[1] B. Alberts, D. Brag, J. Lewis, M. Raff, K. Roberts, J. D. Watson, *Molecular Biology of the Cell,* Garland, New York, 1983.

[2] K. Gerwert, R. Rodriquez-Gonzalez, F. Siebert, *Time-Resolved Vibrational Spectroscopy* (M. Stockburger, A. Laubereau, eds.), Springer, Berlin - Heidelberg - New York - Tokyo, 1985, pp. 263—268.

[3] M. Engelhard, K. Gerwert, B. Hess, W. Kreutz, F. Siebert, *Biochemistry* **1985,** *24,* 100.

[4] K. Gerwert, F. Siebert, *EMBO Journal* **1986,** *5,* 805.

Received August 28, 1987.

Printed in Austria