# NOTIZEN

## Electron-Beam Induced Conformational Changes in Polypeptide Layers: an Infrared Study

W. Baumeister, M. Hahn

Institut für Biophysik und Elektronenmikroskopie der Universität Düsseldorf

and

U. P. Fringeli

Laboratorium für Physikalische Chemie der ETH Zürich

(Z. Naturforsch. **31 c**, 746–747 [1976]; received August 17, 1976)

Electron Microscopy, Radiation Damage, Polypeptide Monolayers, Infrared Dichroism, Conformational Changes

Langmuir-Blodgett type polypeptide monolayers have been irradiated under conditions simulating the hazards of electron microscopic imaging. The damaging effect, *i. e.* the randomization of the α-helical layers, which occurs in the dose range between 50 and 200 $e^-/nm^2$, has been monitored by infrared dichroism.

The beam of an electron microscope is besides being a flux of short wavelength radiation suitable for high resolution structure determination, also an intense flux of ionizing radiation which inevitably deteriorates the structure under investigation. For organic and biomolecules radiation damage is the most serious obstacle in approaching resolution levels which allow to deduce their molecular structure. Meaningful high resolution information is only to be expected if the damaging effects can be minimized. This in turn requires a thorough knowledge of the damaging effects involved, which at present does not exist.

In an attempt to elucidate the especially intriguing response of proteins to irradiation [1–3] we have concentrated our efforts onto relatively simple polypeptide systems. It has been shown in the pioneering work of Malcolm [4, 5] and Loeb [6] that some high molecular weight synthetic homopolypeptides can be spread as monolayers at water-air interfaces and deposited onto solid supports by conventional Langmuir-Blodgett-techniques. The possibility to manipulate the conformation assumed at the interface by changing the spreading solvent and the liquid subphase [7] as well as the high degree of longe range order in these monolayers make them attractive for studying the effects of radiation damage on the three basic secondary structures of proteins, *i. e.* to measure the relative sensitivities of α-helices, β-structures and random coil structures. Infrared spectroscopy is diagnostic for transitions between these conformations and has the advantage that it can be performed under solid state conditions, i. e. under conditions relevant to electron microscopy. With the ATR-IR-technique [8] radiation

Requests for reprints should be sent to Dr. W. Baumeister, Institut für Biophysik und Elektronenmikroskopie der Universität Düsseldorf, Moorenstraße 5, *D-4000 Düsseldorf*.

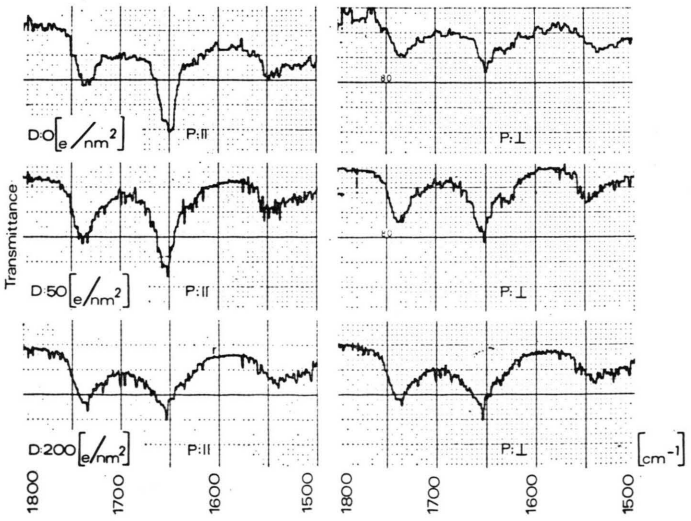


Fig. 1. Polarized ATR-IR spectra of poly-α-methyl-L-glutamate bilayers (P: ∥ parallel polarized light; P: ⊥ perpendicular polarized light). The strong polarization of the amide I band (~1650 $cm^{-1}$) in the non-irradiated control reflects the long range order in the α-helical layers. An electron dose of 50 $e^-/nm^2$ significantly diminishes the dichroic ratio and with 200 $e^-/nm^2$ absorption is almost isotropic, *i. e.* the layers are completely randomized.

damage effects can be measured in extremely thin ($\sim 2$ nm) layers [9].

The polypeptide choosen for this study was poly-α-methyl-L-glutamate which was spread from chloroform/dichloracetic acid (10:1) on water and transferred to germanium internal reflection plates at film pressures closely below the collapse pressure (18 dyn·cm$^{-1}$). The pronounced infrared dichroism reflects the high degree of long-range order of the α-helices. These layers were irradiated with electron doses between $0-400\,e^-/nm^2$ simulating the imaging conditions of electron microscopy. The degradation of order was followed by measuring the decrease of the dichroic ratio of the initially sharp amide I ($\sim 1650\,cm^{-1}$) band. Though we have not yet attempted a quantitative band-shape analysis it becomes immediately apparent from the polarized spectra (Fig. 1) that the polypeptide layers are randomized by doses as low as $50\,e^-/nm^2$, which is comparable to the critical dose for lipid layers [9]. It should be kept in mind that this dose is approximately 2 orders of magnitude lower than the minimum dose needed for single heavy atom detection in monolayers [10] and at least 3 orders of magnitude lower than the doses conventionally applied to obtain high resolution pictures of isolated protein molecules [11]. This stresses the importance of matching the e.m. resolution to a level governed by the radiation sensitivity of the specimen [11].

It should be mentioned in this context that the investigation of the structure of polypeptide layers has still another impact upon electron microscopy: The high degree of long-range order in polypeptide films built up at interfaces lets seem it feasible to design spatially defined charge patterns at interfaces, by spreading polypeptides with charged side chains. These charge patterns could serve as templates for adsorbing proteins in a regular fashion which would allow to perform their structural analysis at significantly lower doses as if they were in a random orientation.

The authors wish to thank Miss M. Fringeli, Miss S. Wiedemann and Miss M. Kikhoefer for technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (RU 5/17), the Schweizer Nationalfonds (Proj. No. 3.521-0.75) and the Fritz Hoffmann-La Roche Stiftung (Proj. No. 127). An EMBO Short Term fellowship (W. Baumeister) is also gratefully acknowledged.

[1] M. Hahn, J. Seredynski, and W. Baumeister, Proc. Nat. Acad. Sci. U.S. **73**, 823 [1976].
[2] W. Baumeister, M. Hahn, J. Seredynski, and L. M. Herbertz, Ultramiscroscopy, in press.
[3] W. Baumeister, U. P. Fringeli, M. Hahn, and J. Seredynski, Biochim. Biophys. Acta, in press.
[4] B. R. Malcolm, Biochem. J. **110**, 733 [1968].
[5] B. R. Malcolm, Nature **219**, 929 [1968].
[6] G. I. Loeb, J. Colloid Interface Sci. **26**, 236 [1968].
[7] G. I. Loeb and R. E. Baier, J. Colloid Interface Sci. **27**, 38 [1968].
[8] N. J. Harrick, Internal Reflection Spectroscopy, Interscience Publ. 1967.
[9] W. Baumeister, U. P. Fringeli, M. Hahn, F. Kopp, and J. Seredynski, Biophys. J. **16**, 791 [1976].
[10] W. Baumeister and M. Hahn, in: Progress in Surface and Membrane Science (J. F. Danielli and D. Cadenhead, eds.), Academic Press, New York 1976, in press.
[11] W. Baumeister and M. Hahn, Hoppe-Seyler's Z. Physiol. Chem. **356**, 1313 [1975].