Measurements of Absorption Spectra of Viral Lipids and Proteins in Vacuum-UV Regions with Synchrotron Radiation

Bull. Univ. Osaka Pref., Ser. B, Vol. 43,
大阪府立大学紀要，農学・生物学，1991

Measurements of Absorption Spectra of Viral Lipids and Proteins in Vacuum-UV Regions with Synchrotron Radiation

Tsuneo MEGUMI[1], Shinichi FUJITA[1], Mikio SAITO[2] and Takashi ITO[3]

[1]Research Institute for Advanced Science and Technology,
University of Osaka Prefecture, Sakai, Osaka 593
[2]Biophysical Laboratory, Rikyo University, Toshima-Ku, Tokyo 171
[3]Institute of Physics, College of Arts and Sciences, University of
Tokyo, Meguro-Ku, Tokyo 153,
(Received, 1990)

Summary

Using synchrotron radiation as a light source, absorption spectra of membrane lipids and spike proteins, viral components of Sendai virus, were obtained in the wavelength regions from 130 nm to 320 nm. The absorption spectrum of lipids have a broad peak at 190 nm and a steep rise below 160 nm, while purification of lipids caused a decrease in absorption at the wavelength longer than 170 nm and an increase below 160 nm. From this result, the rather flat absorption of crude lipids in the longer wavelength region is concluded to originate from diffused reflection. Spike proteins show a small peak at 280 nm and a shoulder at 230 nm in addition to a more pronounced peak at 190 nm and a slight increase below 160 nm. They give more diffused reflection of light than lipids. This is ascribed to the fact that they have no better solvent to transform themselves into thin films.

Introduction

Since synchrotron radiation was developed as a source of vacuum UV, action spectra of microorganisms such as phages[1)], viruses[2)], bacteria[3)] and yeasts[4)], have been elucidated. Comparison between action spectra of these living substances and absorption spectra of their components, like membrane lipids and proteins, has been a long standing problem. Few data of absorption spectra, however, were available excluding DNA[5)].

We have been trying to measure the absorption spectra of membrane lipids and spike proteins from Sendai virus using synchrotron radiation (SR) in the wavelength from 130 nm to 320 nm[6)]. This technique is accompanied by some technical difficulties of the measurement especially in the vacuum-UV region. The samples have to be made into thin films, and be transparent without giving diffused reflection as possible. Their optical properties have also to be unchanged in a high vacuum.

The viral lipids used here are not vaporized in a vacuum, and could be made into a thin layer. On the other hand spike proteins have no better solvent to form transparent films. In the present report, we discuss technical points for measuring absorption spectra of both types of substances with SR.

Materials and Methods

Virus The Z strain of Sendai virus grown in the allantoic sac of 10-day-old embryonated eggs was used throughout this study as described elsewhere[2]. The viruses were pelleted from the allantoic fluid harvested at 72 hr after incubation by centrifugation at 43, 000 g for 30 min with a Hitachi RP50−2 rotor. By repeating the same procedures viruses were purified for the use of experiments.

Preparation of viral lipid and spike proteins Spike proteins were prepared with a dialysis of a supernatant containing spike proteins and lipid bilayers according to the procedures described previously[7,8,9]. Viral lipids were extracted with chloroform-methanol (2:1) from virions. Some optical experiments were made for samples extracted with pure methanol. In this case, some dark precipitate remained a little in the solution.

Measurements of absorption spectra Synchrotron radiation ring of Solid State Institute of University of Tokyo was used for the light source of vacuum-UV. The method for absorption measurements of thin films followed the previous one, which was used for ATP and other nucleic acid-related compounds[5], with some improvement for the beam to be defined precisely. Figure 1 shows the scheme of the sample arrangement. A sample film was prepared by vaporizing a solution (usually 4 μl) of the corresponding substance with a known concentration placed on a 3×3 mm area defined by silicone oil on a CaF_2 plate (thickness, 0.5 mm) in a vacuum system. Immediately behind the CaF_2 plate a diaphragm of 2×2 mm made of aluminum was placed in order to center light path to the sample area. The beam size at this position was 7×9 mm. Monochromatic light,

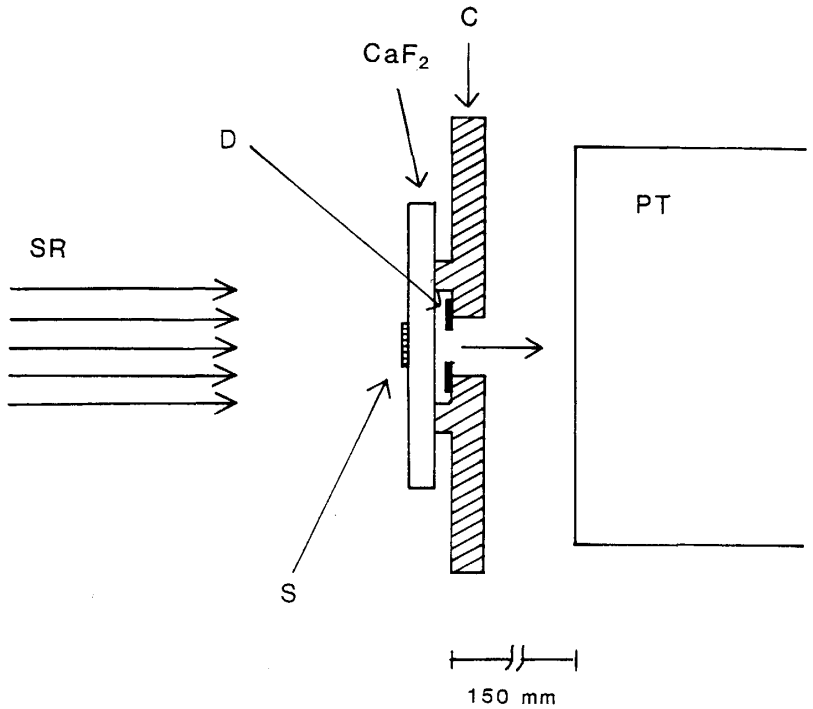

Fig. 1. Arrangement for absorption measurements of thin films. Symbols: SR, synchrotron radiation; s, sample film (3×3 mm); CaF_2 plate (thickness 0.5 mm); D, diaphragm (2×2 mm); C, cassette; PT, photomultiplier.

thus, goes through the sample film, the CaF_2 plate, and the diaphragm, and reaches a photomultiplier with a salicylate coat placed at the distance of 150 mm from the diaphragm.

The absorbance $[\log (I_0/I)]$ of the material was calculated using photomultiplier readings with and without sample films at a given wavelength. Scanning wavelength over the region from 130 to 320 nm required about 20 minutes. The readings at 5 nm intervals were fed to a desk top computer for the necessary calculations. The bank width of monochromatic light was estimated to be about 3 nm under the present conditions[5]. The molar extinction coefficient could not be calculated with the present materials because of the undefined molecular weight. The variation of incident photon flux due to the slow decay of ring current was automatically corrected[5].

Results and Discussion

Figure 2 shows absorption spectra of lipids depending on various extraction procedures. Crude lipids (film of type 1), prepared from a solution after the first extraction with chloroform-methanol, gives a spectrum with a peak at 190 nm, which is increasing with decreasing wavelength below 170 nm (squares). With purer lipids (film of type 2), prepared from a solution after three times of extraction, a little lower absorption is found

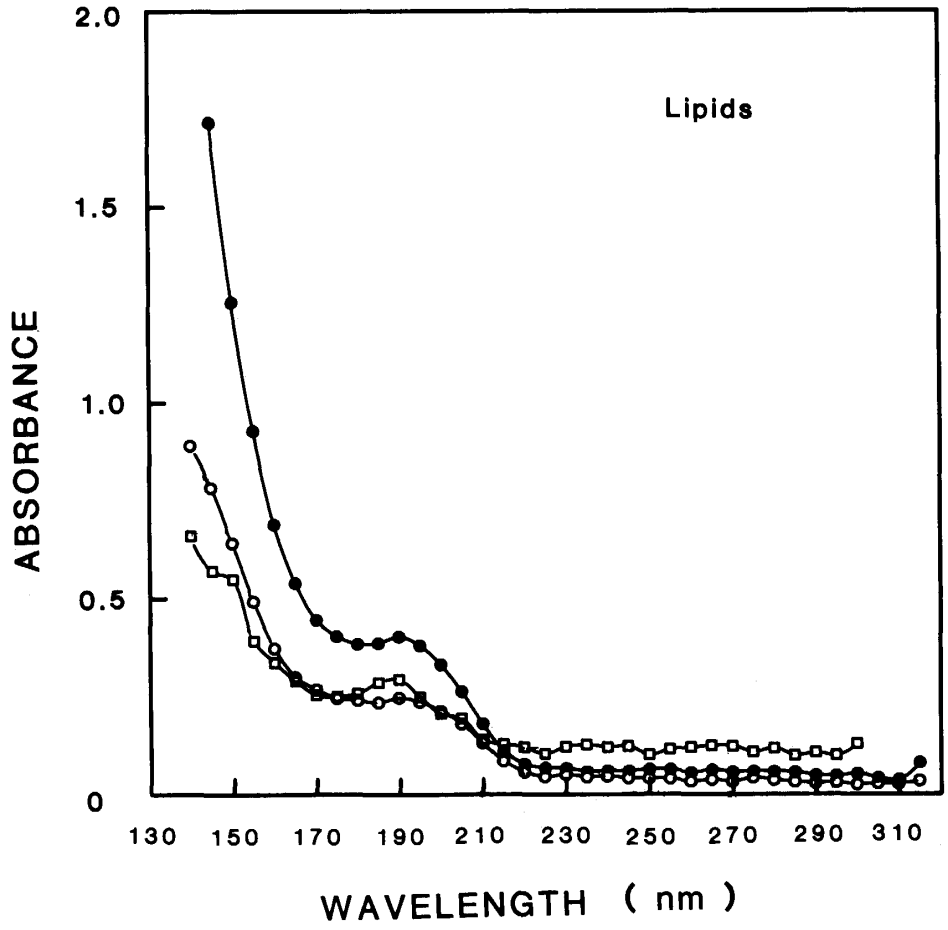

Fig. 2. Absorption spectra of crude lipids (film of type 1, □), purified lipids (film of type 2, ○), and methanol-extracted lipids (film of type 3, ●).

in the wavelength region of higher than 180 nm excluding 195−210 nm. On the other hand, absorption increases at wavelength shorter than 160 nm (open circles). The decrease in absorbance at 190 nm may come from reduction of proteins contaminated.

In the case of film of type 2, further extraction of these lipids with pure methanol[10] results in somewhat strong enhancing of absorption at the vacuum-UV region (filled circles). It is noted that the extraction with pure methanol gives a little dark precipitate in solution. Viral lipids are known to be composed of various kinds of lipids. Some of them must be removed from the solution with this extraction process. Hence, our experimental result may reveal that the optical absorption of structurally mixed substances is strengthen by decrease of the number of components. This argument is not opposed to the results that the absorption of the film of type 2 at shorter wavelength is stronger than that of the film of type 1. In this case, some proteins may be reduced by the process of repeated extraction.

Absorption of film of type 1 at longer wavelength is rather flat, and largely reduced by further extraction with chloroform-methanol. Since this process removes proteins from the samples, as noted above, the transparency must be improved. The absorption of film of type 1 at longer wavelength region, thus, may originate from diffused reflection of the light. In fact lipids are known to have no absorption band at longer than 250 nm. As a matter of fact, it was proved with a conventional spectrophotometer that the sample

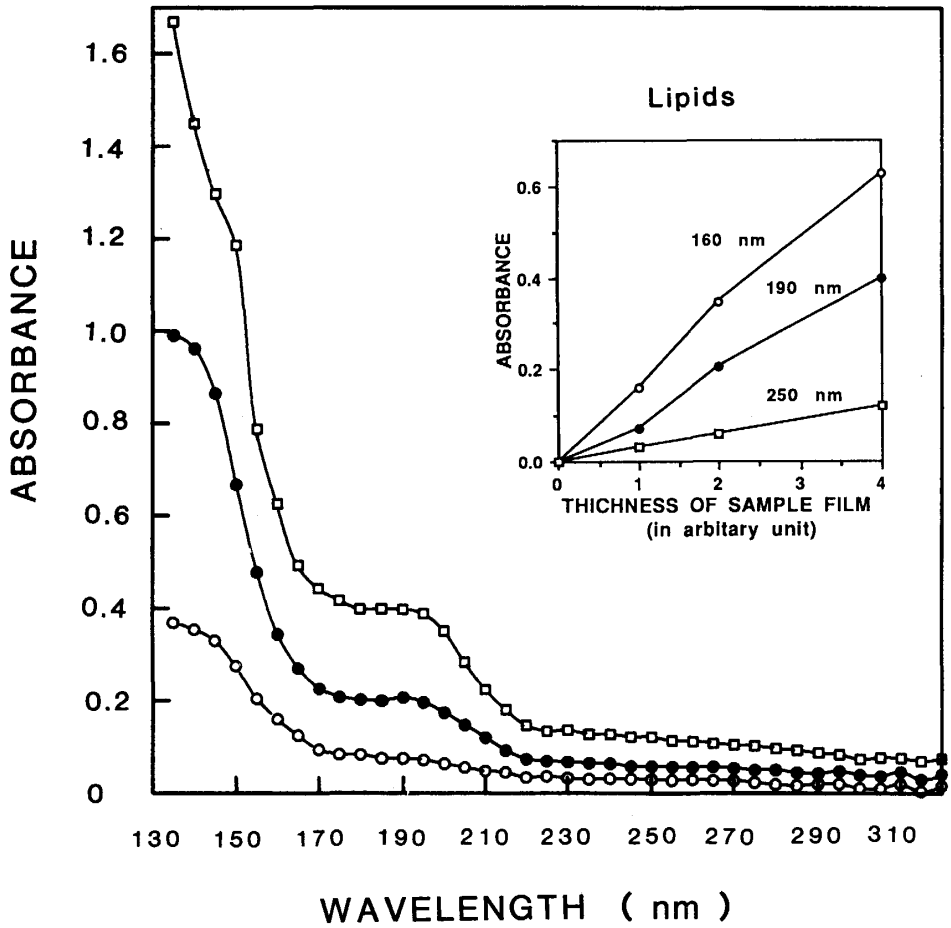

Fig. 3. Absorption spectra of thin films of lipids, thickness, 2 μl (○), 4 μl (●) and 8 μl (□). The inset shows the relationship between thickness and absorbance at 160, 190 and 220 nm.

solution for the film of type 2 have no such absorbance. On the basis of arguments above, film of type 2 is taken to be the best sample of the three, though the diffused reflection could not be completely excluded. The peak of absorption at 190 nm is pointed out to be from unsaturated double bonds[11]. The sharp increase below 160 nm may arise from saturated bonds[11].

Figure 3 represents three absorption spectra of lipid films of type 2 with different thicknesses which was controlled by changing the amount of sample solution. In the inset is shown relationship between the thickness of the film and the absorbance for 160, 190 and 250 nm. Plots for every wavelength were on a good linear line. This result means that the absorption spectrum obtained within the maximum thickness is not deformed in the whole wavelength range employed.

Incidentally, the absorbance of the film of type 2 above 250 nm is ascribed to diffused reflection as pointed above. Our result reveals that this effect also depends linearly on the thickness of the film within the experimental condition. It means that when we subtract the absorption at 250 nm from the whole spectrum as a base line, the resultant absorption at every wavelength depends linearly on the thickness. This discussion may be useful to determine the absorption coefficient of those samples in the vacuum-UV region.

The absorption spectrum of spike proteins is shown in Figure 4. Since the proteins are not soluble, but suspended in water, the resultant film appears opaque. In spite of

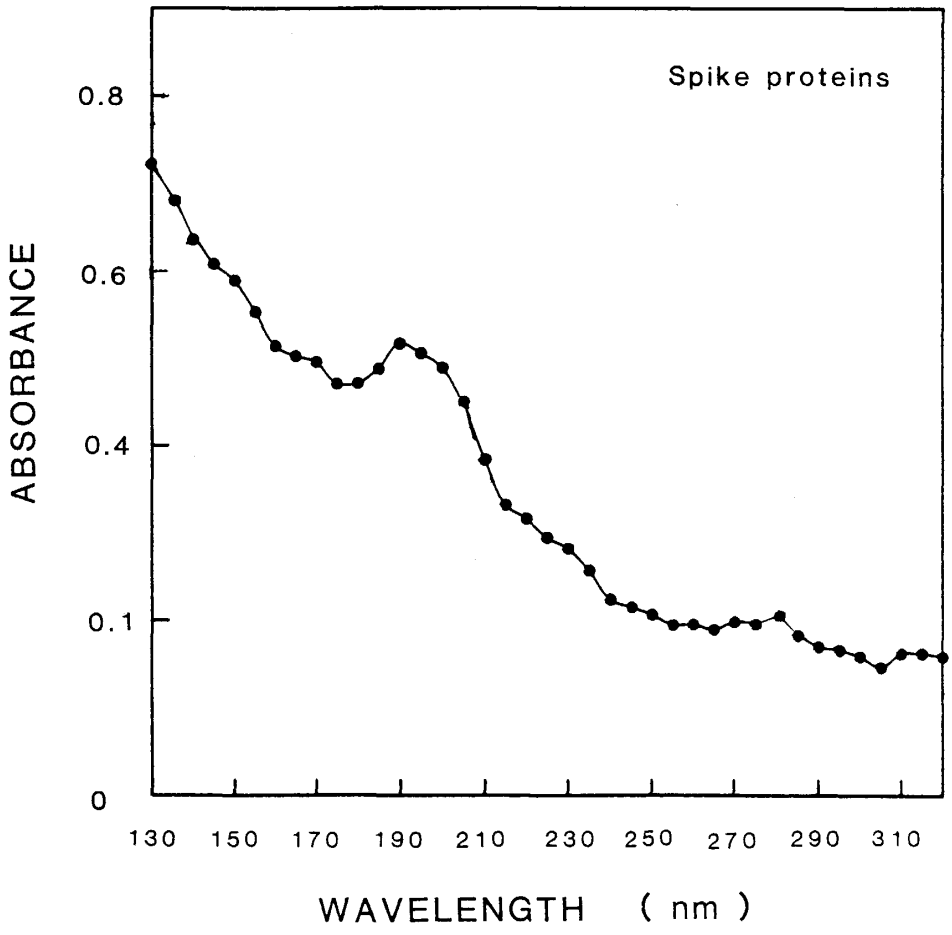

Fig. 4. Absorption spectra of spike proteins (●) The films was made from suspension.

that, the spectrum obtained have some features characteristic to the aromatic amino-acid residues of protein (280 nm), double bonds or conjugated double bonds (230 nm) and unsaturated carbon (190 nm)[11,12],.

The relatively high absorbance at longer wavelengths represents the influence of the low transparency of the film. Most of them may be brought from diffused reflection. It is a future problem to know how the scattering influences absorption spectrum in the vacuum-UV region.

Conclusion: Some viral components could be made into films. Since they are usually not entirely transparent, diffused reflection intervenes, more or less, in the absorption spectra. In spite of that, we obtained absorption spectra characteristic to the molecular structures of the film substances. This technique will help to elucidate more absorption data useful for the comparison of them with action spectra of many living substances, though it remains still a problem how to control the effect of diffused reflection.

Acknowledgment

We acknowledge the use of synchrotron radiation facilities at the Synchrotron Radiation Institute, University of Tokyo. We thank the Radiation Biology Group for the cooperation in performing the experiments.

References

1) MAEZAWA, H., ITO, T., HIEDA, K., KOBAYASHI, K., ITO, A., MORI, T., and SUZUKI, K. (1984). Action spectra for inactivation of dry phage T1 after monochromatic (150−254 nm) sychrotron irradiation in the presence and absence of photoreactivation and dark repair, *Radiat. Res.* **198**, 227−233.

2) MEGUMI, T., ITP, T. and ITO, A. (1982). Some characters of Vacuum-UV Effects on Sendai Virus, *J. Radiat. Res.*, **23** (1982) 272−282.

3) MUNAKATA, K., HIEDA, K., KOBAYASHI, K., ITO, A. and ITO, T. (1986). Action spectra in ultraviolet wavelength (150−250 nm) for inactivation and mutagenesis of *Bacillus subtilis* spores obtained with synchrotron radiation, *Photochem. Photobiol.*, **44**, 385−390.

4) ITO, T., ITO, A., HIEDA, K. and KOBAYASHI, K. (1983). Wavelegngth dependence of inactivation and membrane damage to Saccharomyecs cerevisiae cells by monochromatic synchrotron vacuum-UV radiation, *Radiat. Res.***96**, 532−548.

5) ITO, A. and ITO, T. (1986). Absorption spectra of deoxyribose, ribosephosphate, ATP and DNA by direct transmission measurements in the vacuum-UV (150−190 nm) and Far-UV (190−260 nm) regions using synchrotron radiation as a light source, *Photochem. Photobiol.*, **44**, 355−358.

6) ITO, T., KADA, T., OKADA, S., HIEDA, K., KOBAYASHI, K., MAEZAWA, H. and I TO, A. (1984). Synchrotron system for monochromatic uv irradiation (>140 nm) of biological material, *Radiat. Res.*, **89**, 65−73.

7) HOSAKA, Y. and SHIMIZU, Y.K. (1972). Artifical assembly of envelope particles of HVJ (Sendai Virus) I. Assembly of hemolytic and fusion factors from envelopes, *Virology*, **49**, 627−639.

8) HOSAKA, Y. and SHIMIZU, Y.K. (1972). Artifical assembly of envelope particles of HVJ (Sendai Virus) II. Lipid components for formation of the active hemolysin, *Virology*, **49**, 640–646.
9) HOSOKAWA, Y. (1978). Reassembly of Sendai virus (HVJ) envelope by stirring, *Ann. Rep. Radiat. Ctr. Osaka Pref.*, **19**, 105–108.
10) ROSOFF, I.I., PLATT, I.R., KLEVEVENS, H.B. and BURR, G.O. (1945). Extreme ultraviolet absorption spectra of the fatty acids, *J. Am. Chem. Soc.*, **67**, 673–678.
11) PAINTER, L.R., ATTEREY, J.S., HUBEBELL, H.H., Jr., and BIRKHOFF, R.P. (1984). Vacuum ultraviolet optical properties of squalane and squalene, *J. Appl. Phys.*, **55**, 756–759.
12) PREISS, J.W. and SETLOW, R. (1956). Spectra of some amino acids, peptides, nucleic acids and protein in the vacuum ultraviolet, *J. Chem. Phys.*, **25**, 138–141.