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FOURIER TRANSFORM INFRARED SPECTRA OF CELLS TREATED WITH THE DRUG ADRIAMYCIN

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SUMMARY : Fourier Transform Infrared Spectroscopy (FT-IR) is used to study the interaction of adriamycin molecule with DNA and/or cells. For the drug-DNA complexes, the data show that adriamycin interacts not only with the bases pair but also with the sugar-phosphate of DNA within intercalating process. In the case of treated tumor cells, spectra suggest that adriamycin could be interacting also with the proteins of the membrane. The obtained results show that FT-IR is a powerful technique in the study of biological system, say cells.

Adriamycin, an antitumor drug has a quinonoid structure coupled to a saturated ring and to an aminosugar. Recently (1,2) we have studied the Raman and resonance Raman spectra of adriamycin and its interaction with a pharmacological target DNA and K562 tumor cells (3). We have observed spectral modifications which were related to structural and conformational changes of both the target and the drug. In that study (2) only the chromophore vibrations could be observed and analysed : the sugar vibrations were not shown in the resonance spectra. It was therefore interesting to study also the interactions of the unsaturated moiety and the sugar with the target molecule.

In this work, Fourier Transform Infrared Spectroscopy (FT-IR) has been employed to study the adriamycin molecule and its interactions with the pharmacological target DNA and cells. The FT-IR spectrum of adriamycin was recorded and its bands have been assigned to the molecular vibrations of the

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molecule. It is suggested (2,4) that nuclear DNA is the main target of the drug. First, spectra of DNA-adriamycin water solutions were recorded and then spectra of cells treated with adriamycin.

EXPERIMENTAL

The spectra have been recorded with a DIGILAB FTS-15/C Fourier Transform Michelson infrared interferometer equipped with a high sensitivity HgCdTe detector (Infrared Associates, New Brunswick, NJ) and a KBr beam splitter. Normally, 125 to 1000 interferograms were recorded with an optical velocity of $1.2 cm.s^{-1}$, co-added and Fourier transformed with a spectral resolution of 2 to 4 cm^{-1}. The frequencies are accurate and reproducible to better than $\pm$ 1 cm^{-1}.

Adriamycin was obtained from Roger Bellon Laboratories in Paris, France and was used as received. Fresh solutions were used for the experiments. Calf thymus DNA (type I) was purchased from Sigma Chemical Company and was purified on a CsCl gradient. Stock aqueous solutions were prepared containing 10^{-2}M $NaClO_4$ and DNA (5 mg/ml). The adriamycin-DNA solutions were prepared by mixing DNA and adriamycin aqueous solutions containg 10^{-2}M $NaClO_4$ at room temperature ($\sim$20°C). The concentration of adriamycin was 10^{-5}M and the ratio r = nucleotide / adriamycin was between 5 and 200. The samples were recorded as films between KRS-5, CaF_2 and BaF_2 windows. A human erythroleukemia K562 cell line was used. Cells were grown in Dulbecco medium with 10% heat-inactivated fetal calf serum (FCS) supplemented with 2 mML-glutamine in 5% CO_2 in air at 37°C. For the preparation of the cell samples see reference 3.

RESULTS AND DISCUSSION

The spectrum of free DNA (concentration 4 mg/ml) in 10^{-2}M $NaClO_4$ solution was obtained and is shown in Fig. 1A. Fig.1B illustrates the FT-IR

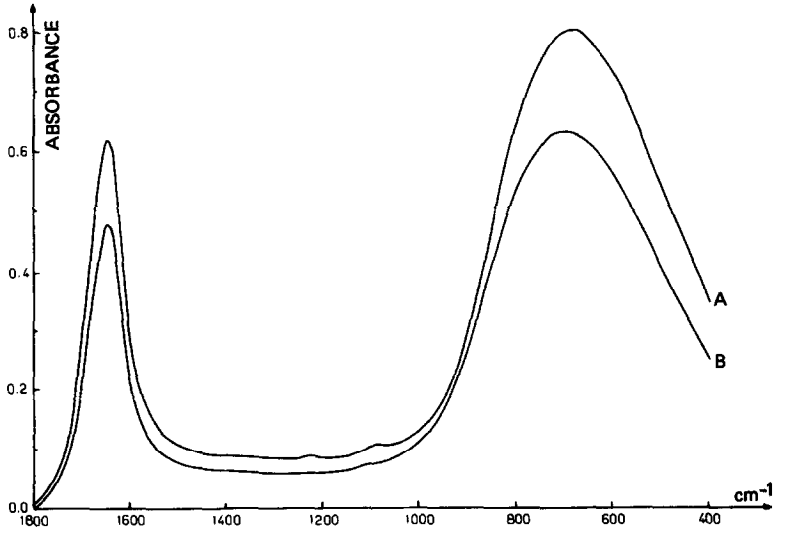


Fig.1 FT-IR spectra of (A) calf thymus DNA 5 mg/ml in H_2O, 10^{-2}M $NaClO_4$ and (B) solvent H_2O, 10^{-2}M $NaClO_4$.

spectrum of the solvent, 10^{-2}M $NaClO_4$ in H_2O. Only two strong vibrations of water are clearly seen in these spectra. The power of the present FT-IR spectrometer equipped with a computer is shown when the spectrum of the solvent is subtracted and the complete spectrum of the DNA and DNA-adriamycin (r = 200) in the region 1800-900 cm^{-1} is revealed in Figs. 2 & 3. The well known C = 0 stretch, the bending NH_2 and other skeletal vibrations are observed in the spectra. Considerable changes are shown in the region 1500-1800 cm^{-1}, which are due to the intercalation of the drug into the G-C planes of the double helix (2). Furthermore, the band at 1715 cm^{-1} in the spectrum of the complex DNA-adriamycin, assigned to the carbonyl vibration, which is a function of base stacking or magnesium depletion (5) indicates that DNA is still in the B form. In the region 1400-900 cm^{-1} the intense bands of the sugar and phosphate vibrations are observed. The FT-IR spectra of DNA-adriamycin complexes with nucleotide/adriamycin ratio r = 200 which corresponds to a concentration of 10^{-5}M adriamycin do not show the presence of adriamycin; the concentration is too low. However, comparison of the spectra of DNA and DNA-adriamycin, after computer subtraction reveals some

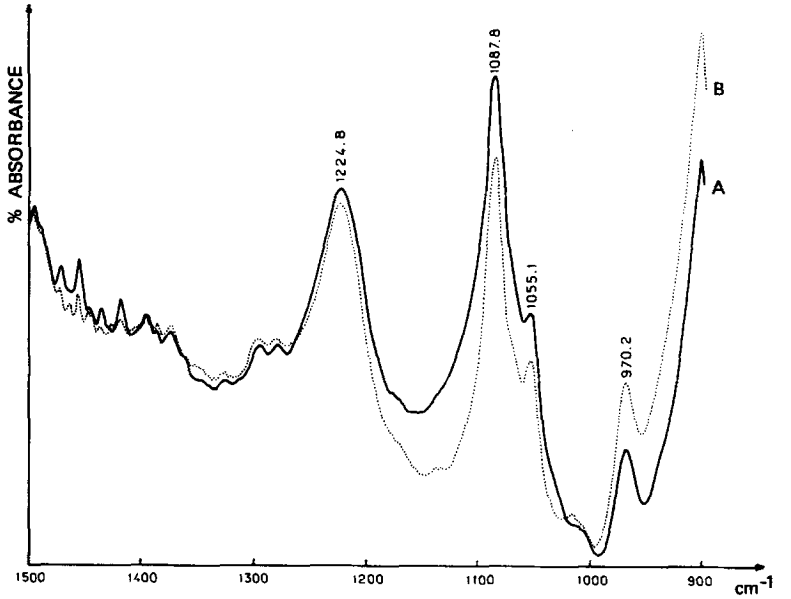


Fig.2 FT-IR difference spectra of (A) DNA 4 mg/ml obtained by subtraction of the solvent and (B) (DNA + adriamycin)-(solvent) (r = 200) in the region 1500-900 cm^{-1}.

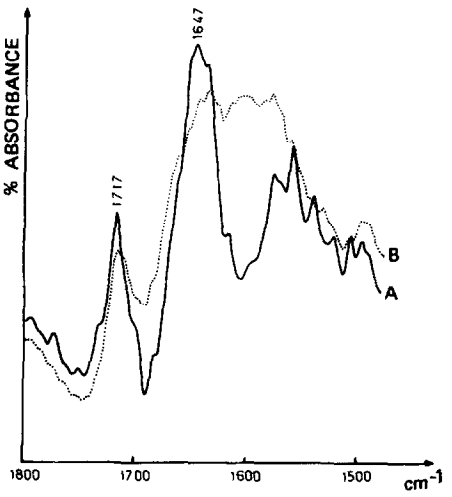
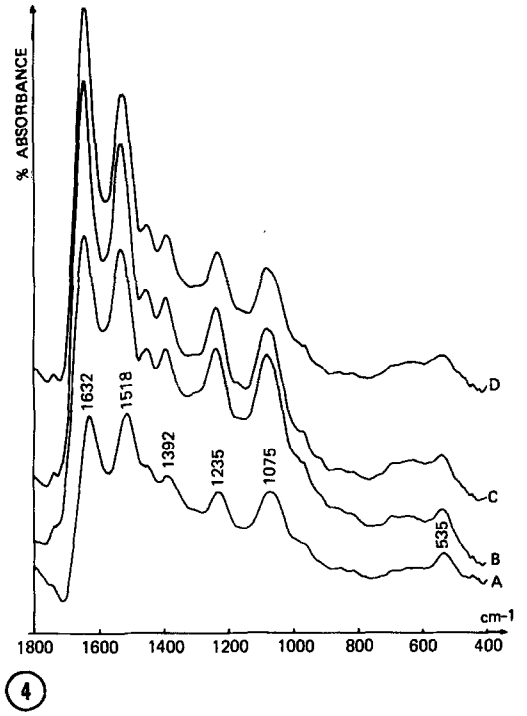


Fig.3 FT-IR difference spectra of (A) DNA 4 mg/ml and (B) (DNA + adriamycin)-(solvent) (r = 300) in the region 1800-1500 cm^{-1}.

changes mainly for the sugar-phosphate bands. Particularly, the band at 1087 cm^{-1} assigned to ν_s PO_2^- decreases in intensity indicating a drug-phosphate interaction with the backbone in the DNA double helix. A similar interaction has been seen previously in the FT-IR spectra of *cis*-platinum (an antitumor drug) interacting with DNA (4). On increasing the amount of adriamycin to give r = 5 we see more important changes in the phosphate-sugar bands. These *in vitro* studies indicate that it is the amino-sugar moiety of the drug adriamycin which interacts with the backbone of DNA.

These model studies of the mechanism of action of a drug are interesting. They were followed by *in vivo* experiments in which cancer cells from a human erythroleukemia K562 cell line were treated with adriamycin. The results for different times of incubation and drug concentration are shown in Figs. 4 & 5. Considerable changes are observed in the intensities of the bands which are mainly the Amide I and II bands of the cellular proteins, particularly the Amide I band at 1632 cm^{-1}. It should be pointed out that the intensity increase of the bands is a function of incubation time and drug concentration. The intensity increase is most probably due to an adriamycin-protein interaction. The 1632 and 1518 cm^{-1} bands are also slightly shifted to higher frequencies. This could be due to a different intracellular concentration of



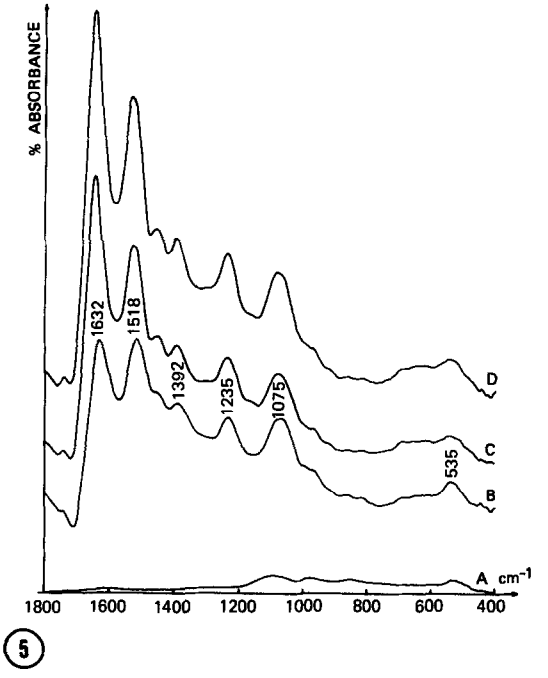


Fig.4 FT-IR spectra of (A) untreated K562 cells, (B) treated cells with adriamycin 50 μg/ml, incubation time 2 mn, (C) 5 mn, (D) 3h.

Fig.5 FT-IR spectra of (A) PBS buffer, (B) untreated K562 cells, (C) treated with adriamycin 50 μg/ml, 3 hours and (D) 10 μg/ml, 10 hours.

adriamycin entering the cell and interacting at the cell surface (6) with the proteins. It is difficult to interpret at present the FT-IR spectra. However, they are promising and it is shown here that FT-IR can be useful in studying these complex biological systems. The spectra show that the drug adriamycin when entering cells interacts with the proteins at the cell surface and when entering the cell it interacts with the nucleic acids inside the cell. We have also applied the powerful FT-IR technique to another system, Candida albicans with a polysaccharide structure treated with various concentrations of cytohelicase, an enzyme which interacts with the protein at the surface of the membrane and allows adriamycin to enter in the cell and become cytotoxix (7). The spectra are of excellent quality and could be useful in studies of protein-enzyme interactions. The FT-IR spectra of the above system as a function of cytohelicase concentration are shown in Fig. 6. Work is being continued on these systems in order to decide if adriamycin which was thought

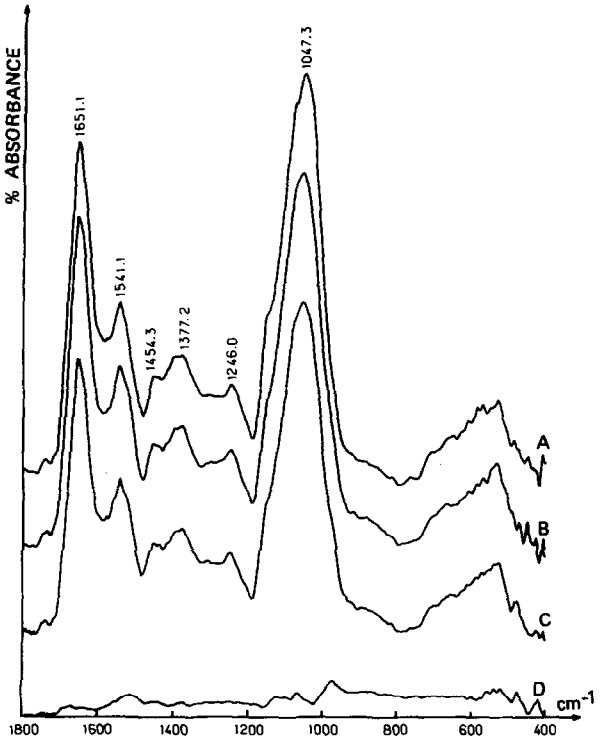


Fig.6 FT-IR spectra of (A) Candida albicans, (B) Candida albicans + cytohelicas enzyme (7 I.U/ml), (C) (14 I.U/ml), (D) difference spectrum (C-A).

to interact only with DNA, can also cause damage at the cell surface by interacting either with the proteins and/or with the phospholipids of the membrane.

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