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Interaction of HIV-1 with susceptible lymphoblastoid cells

^{1}H NMR studies

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Different strains of HIV susceptible lymphoblastoid cells have been infected by HIV-1 and examined by means of ^{1}H NMR spectroscopy at different times after infection, taking advantage of the presence of high resolution lipid signals from the plasma membrane of tumor cells. A transient decrease in intensity of fatty acid signals, originated by changes in membrane structure, has been observed early after viral infection. Marked alterations in membrane-dependent steps of phospholipid synthesis can also be inferred by the observed transient depression in peaks from choline-based metabolites. Spectral modifications deriving from changes in lipid metabolism are also produced both in infected cells a few days after infection and in permanently infected cells. ^{1}H NMR can, therefore, monitor structural and metabolic effects induced by HIV infection.

HIV-1; Lymphoblastoid cell; Membrane organization; Lipid metabolism; ^{1}H NMR spectroscopy

1. INTRODUCTION

HIV has been recognised as the etiological agent causing the acquired immunodeficiency syndrome (AIDS) in humans, being trophic and cytopathic for the subpopulation helper/inducer of T lymphocytes characterized by the CD4 protein structure acting as virus receptor on plasma membrane [1,2]. Several established CD4+ cell lines of tumoral origin have also been demonstrated to be susceptible to HIV infection, developing rapid cytopathic effects [3–5].

Recently, ^{1}H NMR has been used to elucidate structural and metabolic events following HIV-1 infection in human peripheral blood lymphocytes (PBL) [6,7], taking advantage of the presence of mobile lipid signals of triglycerides in the plasma membrane of actively proliferating cells [8,9].

^{1}H NMR spectral patterns similar to those of stimulated lymphocytes are displayed by lymphoblastoid [10] and promonocytic cells. We have therefore taken advantage of the presence of these signals to study HIV-1 cell interaction to verify that the effects observed on PBL are a general pattern of HIV-1 interaction with susceptible cells. Structural changes, evidenced in the NMR signals of triglyceride pools of the plasma membrane, have actually been observed also for CD4+ lymphoblastoid cells in the early stages and within few days after infection, though with different time kinetics. The ^{1}H NMR spectra produced by HIV-1 carrying lymphoblastoid and promonocytic cells display a low intensity of lipid signals which can be related to the presence of the virus in the membrane. Finally, spectral modifications originated by changes in lipid metabolism due to virus–cell interaction appear to mimic those found in PBL [6,7].

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2. MATERIALS AND METHODS

The examined cell lines are: CEM-ss and HUT-78, human T-lymphoid cell lines; the producer (D10) and non-producer (F12) HIV-1 infected HUT-78 cell clones [11]; U937, human monoblastoid cell line; U937-III, a producer HIV-1-infected U937 clone. Both uninfected and HIV-1 infected cells were grown in RPMI 1640 (Flow Laboratories, Scotland) supplemented with 10% foetal calf serum (FCS) and split biweekly. Exponentially growing uninfected cells were infected with $10^{4.5}$ $TCID_{50}/10^6$ cells of HIV-1 (HTLV-III$_B$ strain), by incubating ultracentrifuged virus preparation on packed cells for 1 h at 37°C. Considering that 0.1 $TCID_{50}$/cell is sufficient to infect more than 90% of cells [13], it may be calculated that in these experiments one out of five cells was infected with one infectious particle. This infection yielded approximately 8 defective or infectious virus particles per cell [12]. Infection was monitored by detecting reverse transcriptase (RT) activity in the supernatant of infected cultures [11]. Perchloric acid (PCA) extracts were prepared as previously described [14].

^{1}H and ^{31}P NMR spectra were run at the probe temperature (27°C) on a Bruker AM 400 spectrometer at 400.13 and 161.98 MHz, respectively. For the ^{1}H NMR measurements, 5×10^6 washed cells, resuspended in 200 μl of PBS, were introduced in the internal compartment of a coaxial 5/2 mm tube system. 1 mM TMSP in ^2H_2O in the external compartment was used as chemical shift and area reference.

NMR signals from cells were obtained with a flip angle of 90°, an acquisition time of 1.1 s, and an interpulse delay of 2.5 s. Typically, 600 scans for a total time of approximately 20 min were used to run a single ^{1}H NMR cell spectrum. The H_2O and H^2HO signal was gated irradiated.

Variations in fatty acid signals were determined on the methylene peak at 1.26 ppm (FA) because the signal from methyl groups at 0.9 ppm has a relevant contribution also from cholesterol and proteins. The small variability in area measurements due to cell sedimentation during the time course of NMR experiments was included in the experimental error. The contribution of dead cells to fatty acid signals at 1.26 ppm has been estimated from many samples with different percentages of non viable cells, resulting in 30% signal intensity with respect to that from viable cells.

Signal intensities were therefore corrected taking into account this contribution. No contribution from dead cells was observed for the signals from choline based metabolites.

3. RESULTS AND DISCUSSION

^{1}H NMR spectra have been run for two different leukemic T-cell lines, namely HUT-78 and CEM-ss. Figs. 1aa′ and 2aa′ show the ^{1}H NMR spectra of HUT-78 and CEM-ss cells, respectively, and Figs. 1bb′ and 2bb′ those from the corresponding PCA extracts. Cell spectra are characterized by the presence of the intense methylene (1.26 ppm - hereafter indicated as FA) and methyl (0.9 ppm) signals from fatty acid chains, mainly from triglycerides. A doublet from lactate (LA) is also present (1.33 ppm) in spectra from both cells and PCA extracts (see upper inserts of Figs. 1 and 2). An intense peak at 3.2 ppm in the $-N(CH_3)_3$ choline (Cho) region is observable. This signal is split into two components in the PCA extracts (see lower inserts of Figs. 1 and 2). The central peak at 3.22 ppm is attributed to glycerophosphorylcholine and/or phosphorylcholine and the signal at 3.19 ppm to choline. The presence of glycerophosphorylcholine and phosphorylcholine is confirmed by ^{31}P NMR (not shown). It is worth noting that HUT-78 cells are characterized by a very low glycerophosphorylcholine signal in comparison to that of CEM-ss. As a consequence, the contribution to the ^{1}H NMR signal at 3.22 ppm in HUT-78 cell spectra is mainly from phosphorylcholine. The region around 1.9–2.5 ppm from acetyl groups has been attributed to surface carbohydrates [10]. On the other hand, the correspondence between the patterns of this region in cells and PCA extracts is indicative of the presence of acetate, glutamate and glutamine from cytoplasmic metabolites, in agreement with other authors [15,16]. The detailed analysis of the entire ^{1}H NMR spectrum of PCA extracts from these cells is beyond the purpose of the present work and will be reported elsewhere.

Fig. 3a shows the ^{1}H NMR spectra of samples from CEM-ss cells at different times after HIV-1 infection (b,c,d), as compared to an uninfected sample (a). An impressive decrease of the FA and Cho signals is visible in the time range 30–60 min (Fig. 3b,c). The signals have completely recovered 2 h post-infection (Fig. 3d). Data from HUT-78 cells infected with HIV-1 (not shown) strictly resemble those of CEM-ss.

Fig. 3B shows the spectra of an infected sample of CEM-ss, with respect to the control one, at day 4 after infection, when high levels of RT activity in culture medium indicated active viral production. While the

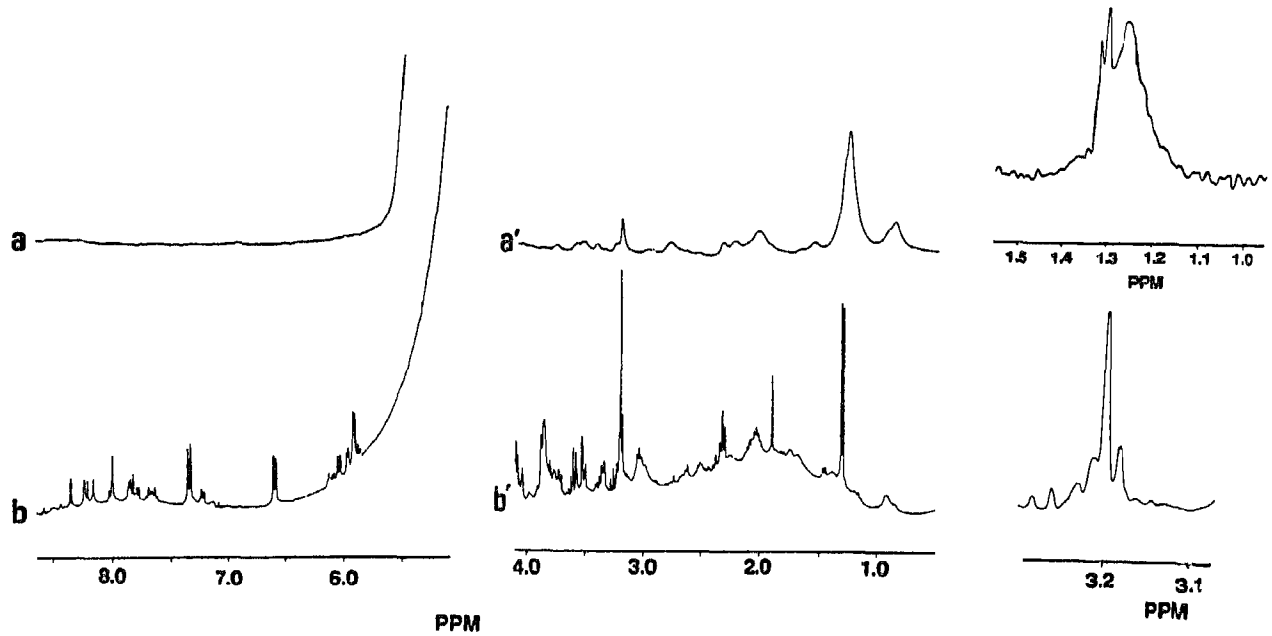

Fig. 1. ^{1}H NMR spectra 7 × 10^6 (a,a′) and PCA extracts from 5 × 10^8 (b,b′) HUT-78 cells (water region around 4.8 ppm was omitted). The insert of the region around 3 ppm is relative to the expanded Cho region of the PCA extracts (lower trace); the insert of the region around 1.3 ppm is relative to the expanded FA and LA region of cells (upper trace).

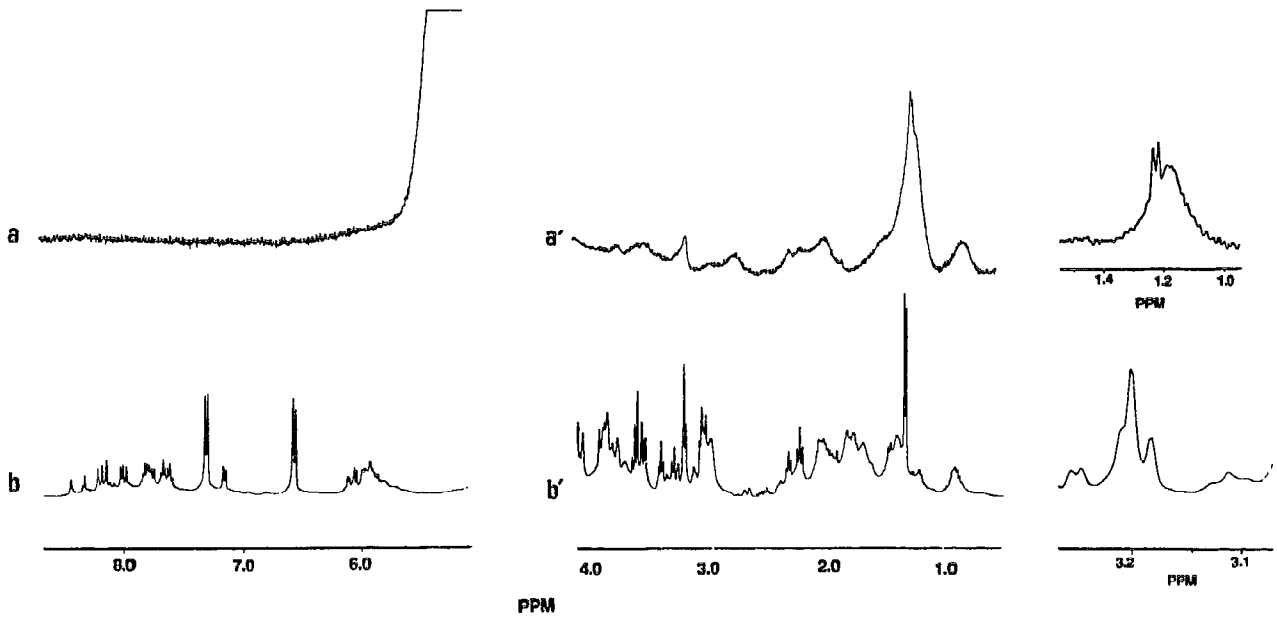

Fig. 2. ^{1}H NMR spectra of 7×10^6 (a,a') and PCA extracts from 5×10^8 (b,b') CEM-ss cells (water region around 4.8 ppm was omitted). The insert of the region around 3 ppm is relative to the expanded Cho region of the PCA extracts (lower trace); the insert of the region around 1.3 ppm is relative to the expanded FA and LA region of cells (upper trace).

lipid chain signal is strongly decreased, Cho signal is enhanced by a factor of 3.

Plots for FA peak intensities vs time are shown in Fig. 4A for CEM-ss cells and in Fig. 4B for HUT-78 cells. Corresponding values of RT activity in the supernatants are shown in Table I.

Peak intensity decreases and subsequent recovery shortly after HIV infection can be related to viral parti-

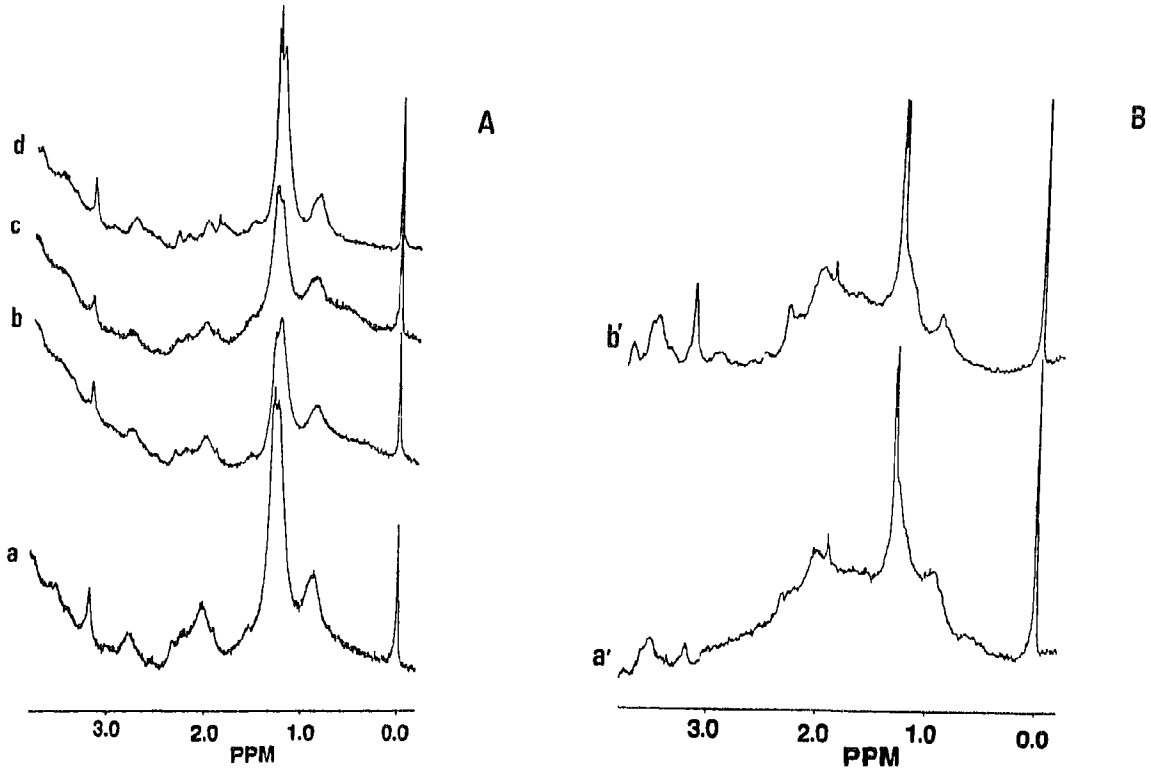

Fig. 3. (A) ^{1}H NMR spectra of CEM-ss cells for different times after HIV-1 infection: (a) uninfected, (b) 30 min, (c) 60 min, (d) 120 min. (B) ^{1}H NMR spectra of CEM-ss cells at day 4 after HIV-1 infection: (a') uninfected; (b') infected.

Table I

Reverse transcriptase (RT) activity (cpm/ml) in the supernatants of lymphoblastoid cell lines HUT-78 and CEM-ss after HIV-1 infection

Days post-infection	HUT-78	CEM-ss
1	35 568	nd
2	<1 000	<1 000
3	21 545	nd
4	21 224	21 560
5	267 760	nd
6	nd	190 820

nd = not determined

cle internalization by cells: this hypothesis is supported by previous evidence indicating that HIV internalization and uncoating occur within 30 min after infection of CEM-ss cells [12], in agreement with the time kinetics here reported for this cell line (Fig. 4A). This behavior is different from that observed by Nicolau [17] on the interaction of other retroviruses which produce permanent modifications of FA signals at very high particle/cell ratios (about 1000:1). The strong effect on cell membrane structure observed in our experiments at much lower viral particles/cell ratio (8:1) suggests that other phenomena, besides a localized virus-receptor interaction, may be involved.

The FA signal from infected cells increases its intensity already a few hours after infection in both cell lines, remaining high with respect to controls in the following days. This would indicate higher triglyceride concentration, in agreement with what has been observed by biochemical techniques in HIV-infected ERIC cells [18]. This signal is again decreased in intensity after a few days from infection (Figs. 3B and 4A,B), when cells start to bud and release the progeny viral particles, as demonstrated by the elevated RT activity values detectable in the medium (Table I). The effects on NMR signals do not occur in concomitance with syncytia formation. In fact, FA signal decreases also in PBL where the number of syncytia induced by HIV is considerably lower.

Two different phenomena take therefore place at the level of the triglyceride pool, affecting the FA signal in opposite directions. The presence of viral particles inside the cells induces higher production of triglycerides, thus giving a positive contribution to the corresponding peak. On the contrary, when the virus crosses the cell membrane, causing changes in its structure, the FA signal is depressed.

This interpretation is confirmed by the results obtained on chronically HIV-infected T-lymphoblastoid cells. Fig. 5A shows the ^{1}H NMR spectra of HUT-78, control cells (a), of D10 permanently HIV-infected virus-producing cells (b) and of F12 permanently infected non-producer cells (c). From the comparison of the three spectra, it can be seen that the FA signal is strongly decreased in its intensity in D10 cells which are actively producing viral particles, with respect to both HUT-78 and F12 samples. The spectra have strong similarities with the only exception of the peak from choline based metabolites which undergoes changes probably related to the viral presence in the cells. However, this peak is modulated by a number of different factors including proliferation rates (our unpublished observations) that affect the study of the correlation of its intensity with the viral presence in different cell lines. Fig. 5B reports the values of FA intensities for samples of HUT-78, D10 and F12 cells as a function of days after first seeding. Signal intensities from D10 samples are always lower than those from both HUT-78 and F12 cells. On the other hand, spectra from F12 cells which contain and express a full-length HIV-1 genome but do not assemble HIV virions, show some variability with respect to the parental cell line (HUT-78). Effects observed on these chronically infected cells were reproducible in independent experiments.

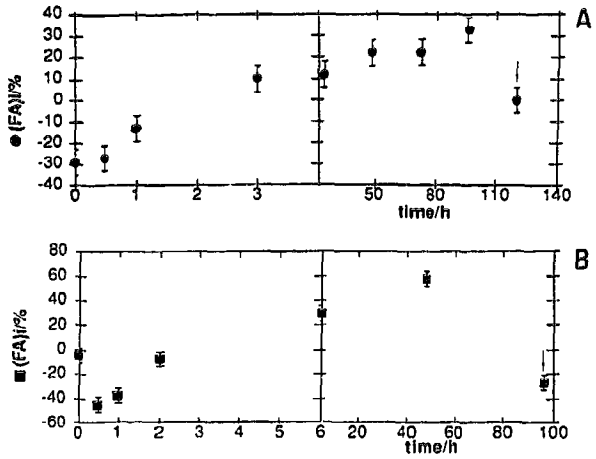

Fig. 4. (A) CEM-ss cells and (B) HUT-78 cells: percent intensity variations of FA signals as a function of time after infection. Arrows indicate the time of the maximum RT activity.

Finally, some spectra have been run with the promonocytic cells U937. Fig. 6b shows that permanently infected HIV-producing cells have a very small FA signal underimposed to the intense lactic acid doublet, while Cho signal, similarly to what was observed for D10 cells (Fig. 5Ab), is remarkably more intense with respect to its parental uninfected cell line.

The selected effects in peaks originated by the choline based metabolites can also be related to modulation of enzymatic reactions involved in lipid metabolism induced by HIV.

In both CEM-ss and HUT-78 samples, this peak is deeply depressed shortly after HIV-1 internalization, recovering its original value within 2 h (Fig. 3A). The same signal is enhanced in infected cells at the time corresponding to maximum viral replication and HIV release (Fig. 3B). This seems to be a general feature of

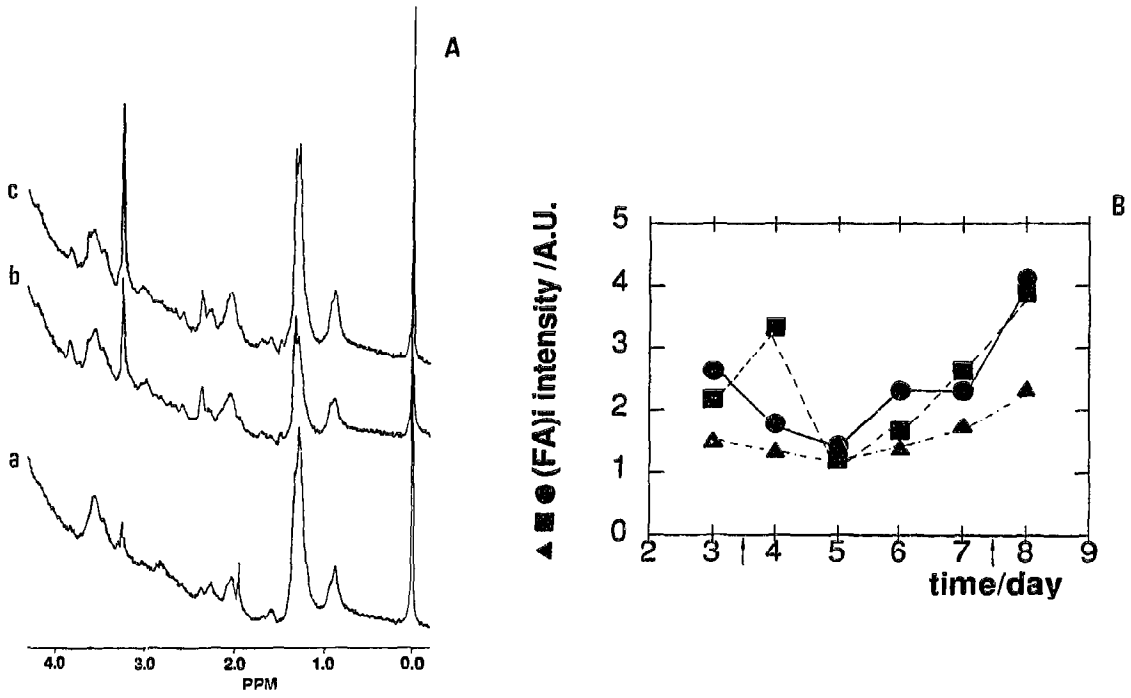

Fig. 5. (A) ^{1}H NMR spectra of different cell lines: (a) HUT-78 cells, uninfected; (b) D10, chronically infected and virus-producing cells; (c) F12, chronically infected, not virus producing cells. (B) Percent intensity variations of FA signals as a function of time after initial seeding: (•) HUT-78, (▲) D10, (■) F12. Arrows indicate successive seedings.

cells carrying HIV-1 because both producer and non-producer chronically infected cells present spectra characterized by a very intense Cho peak (Figs. 5 and 6). This latter alteration is attributed to the slowing down of the cytidyltransferase driven reaction [6–7,18], although an increased phosphatidylcholine breakdown cannot be completely ruled out.

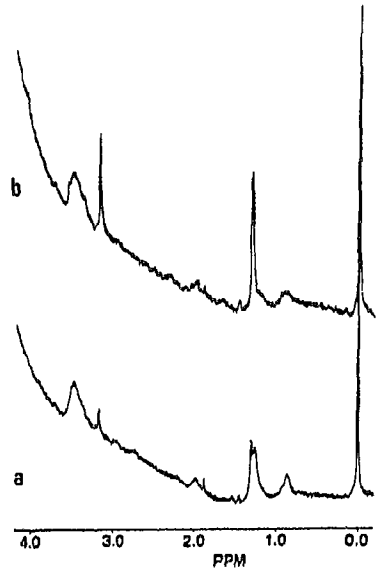

Fig. 6. ^{1}H NMR spectra of promonocytic cells: (a) U937 cells, uninfected; (b) U937-III cells, chronically infected and virus-producing cells.

4. CONCLUSIONS

The morphological characterization of the early stages of HIV infection of susceptible cells is usually carried out by means of electron microscopy [19–21]. We have shown that ^{1}H NMR spectroscopy can monitor HIV-cell interaction providing information, at molecular level, on deep perturbations occurring in cell membrane structure.

Important events leading to lipid synthesis and breakdown occur in cell membrane [22]: ^{31}P NMR has been proposed as a method more suitable for studying these processes with respect to traditional biochemical techniques involving membrane disruption [23]. In the present paper, we have used ^{1}H NMR spectroscopy to provide information on changes in lipid metabolism accompanying HIV infection.

The observed effects are present, though with different kinetics, in all examined cell lines as well as in stimulated peripheral blood lymphocytes [6,7], thus demonstrating that they are a general feature of the interaction of HIV with susceptible cells.

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