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Interaction of ferulic acid derivatives with human erythrocytes monitored by pulse field gradient NMR diffusion and NMR relaxation studies

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Abstract

Ferulic acid (Fer), a natural anti-oxidant and chemo-protector, is able to suppress experimental carcinogenesis in the forestomach, lungs, skin, tongue and colon. Several Fer derivatives have been suggested as promising candidates for cancer prevention, being the biological activity related also to the capacity of partitioning between aqueous and lipid phases. In the present work, pulsed field gradient (PFG) NMR diffusion measurement and NMR relaxation rates have been adopted for investigating the interaction of three Fer derivatives (Fer-C11, Fer-C12 and Fer-C13) with human erythrocytes. Binding to the erythrocyte membrane has been shown for all derivatives, which displayed a similar interaction mode such that the aromatic moiety and the terminal part of the alkyl chain were the most affected. Quantitative analysis of the diffusion coefficients was used to show that Fer-C12 and Fer-C13 display higher affinity for the cell membrane when compared with Fer-C11. These findings agree with the higher anti-oxidant activity of the two derivatives.

Keywords: Ferulic acid derivatives; PFG NMR; Relaxation; Erythrocytes

1. Introduction

Ferulic acid (3-hydroxy-4-methoxycinnamic acid) is a ubiquitous compound in nature, found especially in wheat, corn, rice, tomatoes, spinach, cabbage and asparagus. Together with its alkyl esters it (Fig. 1) elicits

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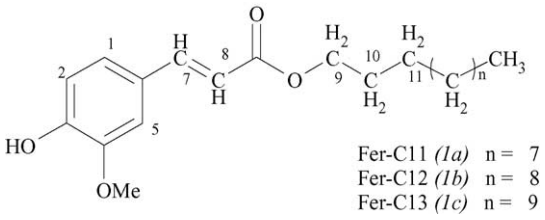

Fig. 1.

interesting anti-oxidant properties (Graf, 1992; Rice-Evans et al., 1996; Niki, 1997; Kikuzaki et al., 2002; Trombino et al., 2004), that are potentially useful not only to prevent lipid oxidation in food but, also, to prevent free-radical-induced diseases such as cancer and atherosclerosis or aging caused by oxidative tissue degeneration (Niki, 1997; Kikuzaki et al., 2002; Anselmi et al., 2001; Balasubashini et al., 2004). The anti-oxidant properties rely on the radical scavenging activity (Niki, 1997) and, as a consequence, ferulic acid is by far the most effective among hydroxycinnamic acids, due to the resonance stabilization of the phenoxy radical by an extended side-chain coniugation. Different group at the end of the carbon side chain are also able to affect anti-oxidant behavior (Nenadis et al., 2003). In particular esterification of ferulic acids with medium chain alcohols (Niki, 1997; Kikuzaki et al., 2002) determines a further increase in activity and suggests that also preferential localization and relative partitioning ability between aqueous and lipophilic interfaces may be related to the anti-oxidant potential.

The anti-oxidant properties of a series of *n*-alkyl ferulates of natural source or originating from chemical modification of naturally occurring compounds were recently investigated (Anselmi et al., 2004a) in order to define (i) the optimum chain length of the molecule for anchoring to the membrane binding sites; (ii) the orientation of the phenoxy group with respect to the membrane bilayer; and (iii) the effect of side-chain folding upon the orientation or positioning at the membrane. It came out that the dodecyl and the tridecyl ferulates were the most active among the even and odd members derivatives, respectively. It was, in fact, stressed that the three dimensional arrangement relative to the membrane bilayer plays a key role in dictating the anti-oxidant ability of *n*-alkyl ferulates and furnishes a reasonable explanation of the mechanism of action. Moreover it was found out that, besides the conformational parameters, also the alkyl chain length plays a critical role; in fact the anti-oxidant activity decreases from tridecyl ferulates onward.

Moreover, the evaluation of the protective effect of ferulic acid and some *n*-alkyl derivatives against hemolysis of an erythrocyte suspension induced by free radical promoter CuOOH showed that the *n*-dodecyl ferulate is more active than the *n*-octyl and *n*-hexadecyl compounds (Anselmi et al., 2004b).

The interaction of undecyl, dodecyl and tridecyl ferulates (Fig. 1) with human erythrocytes are here presented with the aim of ascertaining whether the anti-oxidant activity correlates with the mode of such interaction. An improvement in delineating the anti-oxidant potential is also expected since the last is often tested by measuring the protection offered by drugs against hemolysis induced by hydroxyl or peroxyl radicals. Among the several methods developed to investigate drug-membrane interactions, the rapid and precise evaluation of self-diffusion coefficients, allowed by pulse field gradient (PFG) NMR (Canet, 1997), was believed to provide quite suitable approach. In fact, during the so-called diffusion delay time (vide infra), the drug rapidly exchanges between the membrane bound state(s) and the bulk solution such that the measured diffusion coefficient maintains memory of both environments in a weighted average way. As a matter of fact, the PFG NMR technique has successfully been applied to drug-macromolecule associations (Canet, 1997; Atkinson et al., 2003; Lin et al., 1997; Fielding, 2000; Waldeck et al., 1997; Price et al., 2002; Bleicher et al., 1998) and to molecular aggregation in solution (Mansfield et al., 1998, 1999; Viel et al., 2002; Yao et al., 2000; Price et al., 1999). A further advantage arises from measuring NMR relaxation rates in the same sample, thus yielding independent and complementary information on the system under study.

2. Materials and methods

The ferulic derivatives were synthesized according to standard procedure by reaction of ferulic acid (of natural origin) (Taniguchi et al., 1999) with the corresponding alkanol in the presence of an acid catalyst (Taniguchi et al., 1998). In a three-necked flask, 0.1 mol of ferulic acid, and 0.1 mol of alkanol were dissolved in 200 ml of toluene and 1.0 g of *p*-toluensulphonic acid

was added. The reaction mixture was stirred at 80 °C for 16 h.

After cooling, the mixture was washed with a saturated aqueous solution of sodium hydrogen carbonate. The organic layer was dried over anhydrous sodium sulfate. The solvent was fully evaporated off from the organic layer under reduced pressure. The crude products were purified on silica gel column and identified by TLC, UV and NMR analysis.

Ferulic acid was supplied by Tsuno Rice Fine Chemicals (Wakayama, Japan); toluene (analytical grade), *p*-toluensulfonic acid, sodium hydrogen carbonate, anhydrous sodium sulfate, 1-undecanol, 1-dodecanol and 1-tridecanol were purchased from Aldrich (Milan, Italy).

Human erythrocytes of group A were collected from healthy volunteers, suspended in anti-coagulant solution (heparin and EDTA), and centrifuged at $1000 \times g$ for 5 min. The cells were washed five times with Tris–HCl buffer (172 mM, pH 7.6) and centrifuged at $1600 \times g$ for 10 min. The buffy coat of white cells was removed carefully after each wash. The erythrocytes were resuspended at 10% hematocrit in 10 mM phosphate buffered saline (PBS, pH 7.4 containing 137 mM NaCl and 2.7 mM KCl) and stored at 4 °C for 3 days maximum.

^{1}H NMR spectra were obtained at 14.1 T with a Bruker Avance 600 Spectrometer operating at controlled temperature (±0.2 K) and using a 5 mm triple resonance probe with gradients along *x*, *y*, *z* directions. Chemical shifts were referenced to external tetramethylsilane (TMS). COSY, TOCSY, NOESY and ROESY spectra were obtained by using standard pulse sequences. TOCSY experiments were acquired with a total spin-locking time of 75 ms using a MLEV-17 mixing sequence. NOESY and ROESY spectra were acquired with a mixing time ranging between 100 and 350 ms. Spin lattice relaxation rates were measured with inversion recovery pulse sequences. The same sequence was also used to measure the single-selective relaxation rates by means of suitably shaped π-pulses instead of the usual non-selective π-pulse. All rates were calculated by regression analysis of the initial recovery curves of longitudinal magnetization components leading to errors not larger than ±3%. The diffusion coefficients were measured at 298 K by a PFG longitudinal eddy-current delay (LED), pulse sequence with bipolar gradients incorporating spoil gradients during both longitudinal storage periods (Wu et al., 1995; Gibbs and Johnson, 1991; Dingley et al., 1995; Johnson, 1999).

The gradient strength was incremented (with an initial value of 0.86 G cm^{-1} and a step size of 2.65 G cm^{-1} for 2 ms), while the separations of the field gradients and the total echo time were kept constant. A series of 16 spectra, with a number of scans ranging from 16 to 32, was recorded in 2D mode for each measurement, with a recycle time of 10 s between scans. The diffusion values were calculated by regression analysis of the signal decay leading to errors not larger than ±2–5%. The strength of the B_0 field gradient was calibrated by measuring the self-diffusion coefficient of the residual HDO signal in a 100% D_2O sample at 298 K (Callaghan et al., 1983; Price, 1998). The measurement of the DMSO diffusion coefficient was used to determine the hydrodynamic radius of DMSO at 0.148 nm using a viscosity for DMSO at 298 K of 2.00×10^{-3} kg m^{-1} s^{-1}. A diffusion experiment was performed on a sample of TMS in DMSO at 298 K in order to evaluate its hydrodynamic radius *r* by the ratio (Longsworth, 1960):

$$r_{\mathrm{TMS}} = \frac{r_{\mathrm{DMSO}} D_{\mathrm{DMSO}}}{D_{\mathrm{TMS}}} = 0.150\,\mathrm{nm} \quad (1)$$

Such value was then used to measure the viscosity of the solution containing the ferulic derivatives by measuring the diffusion of TMS (vide infra).

3. Results and discussion

With the exception of a different intensity of the merged methylene resonances, the NMR spectra of the three derivatives were identical (Table 1). Also NOESY and ROESY spectra (not shown) were identical, showing only trivial dipolar connectivities consistent with the absence of stabilized conformations.

The extremely low water solubility of ferulic acid esters led to the choice of DMSO as a solvent in view of an experimental protocol requiring addition of red blood cells to the NMR sample. It is, in fact, known that DMSO is used for cell cryopreservation (Rowley, 1992). The integrity of cells was anyway continuously checked by microscopy inspection (data not shown).

Several NMR methods have been suggested for investigating ligands interacting at macromolecular sites. All methods rely on changes induced by slowing down

Table 1
^{1}H NMR chemical shifts (600 MHz) (δ, ppm), diffusion coefficients ($D \times 10^{10}$, $m^2\,s^{-1}$) for the three ferulic acid esters 21 mM free and in presence of erythrocytes 10% in volume, in DMSO-d_6 at $T = 298$ K

	δ (ppm)			D ($m^2\,s^{-1}$)					
	1a	1b	1c	Free			+RBCs		
				1a	1b	1c	1a	1b	1c
H_1	7.10	7.10	7.10	2.73	2.72	2.78	1.88	1.73	1.67
H_2	6.78	6.78	6.78	2.79	2.72	2.77	1.86	1.73	1.69
OH	9.62	9.62	9.62	nd	nd	nd	nd	nd	nd
H_5	7.31	7.31	7.31	2.72	2.74	2.79	1.86	1.67	1.66
OMe	3.81	3.81	3.81	2.75	2.74	2.79	nd	nd	nd
H_7	6.46	6.46	6.46	2.79	2.71	2.76	1.86	1.71	1.65
H_8	7.52	7.52	7.52	2.77	2.72	2.77	1.88	1.69	1.65
H_9	4.11	4.11	4.11	2.77	2.74	2.79	nd	nd	nd
H_{10}	1.62	1.62	1.62	2.66	2.67	2.77	1.82	1.70	1.67
H_{11}	1.34	1.34	1.34	2.62	2.69	2.79	1.85	1.70	1.70
$(CH_2)_n$	1.24	1.24	1.24	nd	nd	nd	nd	nd	nd
CH_3	0.85	0.85	0.85	2.76	2.73	2.81	1.78	1.68	1.64

nd: not determined.

of the reorientational dynamics of the ligand when going from the free to the bound environment. From this point of view, the PFG NMR method of measuring changes in the diffusion rates of the ligand has been shown to provide a very good piece of information on small molecules interacting with biological or model membranes (Waldeck et al., 1997, 1995; Price et al., 2002; Guy et al., 1986; Price and Kuchel, 1990; Lennon et al., 1994).

The following experimental protocol was therefore used:

a. The diffusion coefficients of the ferulic acid derivatives were measured in DMSO solution at different concentration in order to ascertain eventual aggregation phenomena and provide the basis of subsequent comparison.
b. The same diffusion coefficients were then measured upon the addition of variable volumes of the aqueous erythrocyte suspension to the NMR samples.
c. Since addition of water to DMSO yields unpredictable effects on the solution viscosity, that, in turn, strongly affects the NMR features, the diffusion coefficients of the free ligand had to be somehow normalized before attempting any comparison with the effects induced by the presence of cells. As a consequence, diffusion coefficients were also measured after addition of variable volumes of water alone to the three derivatives NMR samples.

As shown in Table 1, the diffusion coefficients measured by PFG NMR on different resonances of all the ferulic acid esters 21 mM in DMSO-d_6 are, within the experimental error limit, consistent with a unique diffusion behavior of the three molecules. As also shown in Table 1, the addition of aliquots of the erythrocytes aqueous suspension yields slower diffusion coefficients. Such change is directly related to the volume of added RBC and reaches a ca. 40% decrease upon addition of 60 μl (Table 1). Quite interestingly the three derivatives are diversely influenced by the presence of erythrocytes, being 1b and 1c much more affected than 1a.

The relatively slow diffusion features of the free ferulic compounds are apparently due to the viscosity of the solvent ($\eta = 2.00 \times 10^{-3}\,kg\,m^{-1}\,s^{-1}$ as compared to $\eta = 8.94 \times 10^{-4}\,kg\,m^{-1}\,s^{-1}$ of water at the same temperature) rather than to aggregation. In fact, diluting the sample (Table 2) was not accompanied by faster diffusion coefficients or changes in chemical shifts.

As already stated, the effect of the viscosity change due to water was independently checked by measuring the diffusion coefficient of the 1b derivative (averaged over all protons), DMSO, H_2O and TMS upon addition of 60 μl of water to three DMSO solutions having different amounts of dissolved 1b (Table 2).

It is apparent that addition of water leads to substantial slowing of diffusion features of all molecules in solution consistently with a sizeable increase in solvent

Table 2
Diffusion coefficients ($D \times 10^{10}$, $m^2\ s^{-1}$) and the $\eta \times D$ product for the Fer-C12 (1b) derivative at different concentrations in DMSO-d^6 at $T = 298$ K

	D ($m^2\ s^{-1}$) $\times 10^{10}$			$\eta \times D$ ($kg\ s^{-2}$) $\times 10^{12}$		
	21 mM	9 mM	4.5 mM	21 mM	9 mM	4.5 mM
No water						
1b	2.72	2.88	2.86	0.55	0.56	0.56
DMSO	7.30	7.54	7.54	1.47	1.48	1.47
H_2O	9.70	9.79	9.92	1.96	1.92	1.94
TMS	7.20	7.42	7.42	1.45	1.45	1.45
+60 μl H_2O						
1b	2.13	2.17	2.05	0.56	0.57	0.55
DMSO	5.58	5.66	5.51	1.47	1.48	1.48
H_2O	7.38	7.40	7.34	1.95	1.94	1.97
TMS	5.51	5.55	5.44	1.45	1.45	1.46

The viscosity η ($kg\ m^{-1}\ s^{-1}$) was calculated in absence of water: 2.02×10^{-3} (21 mM), 1.96×10^{-3} (9 mM), 1.96×10^{-3} (4.5 mM), and in presence of 60 μl of water: 2.64×10^{-3} (21 mM), 2.62×10^{-3} (9 mM), 2.68×10^{-3} (4.5 mM).

viscosity. Such change is well documented in the literature, where it is reported that the presence of H_2O as a cosolvent yields a further increase in the viscosity that reaches a maximum at χ_{DMSO} ca. 0.36 as a consequence of well-defined intermolecular interactions (Mizuno et al., 2000; Kirchner and Reiher, 2002; Catalán et al., 2001). Since H-bonding of 1b with one or the other solvent may contribute to the viscosity, while TMS is not expected to be involved in H-bonding, the diffusion coefficient of TMS was used to calculate the medium viscosity, as reported in the caption of Table 2, by using the equation (Rice-Evans et al., 1996):

$$D = \frac{k_B T}{6\pi\eta r_H} \quad (2)$$

where $k_B = 1.380658 \times 10^{-23}\ J\ K^{-1}$ is the Boltzmann's constant, η is the viscosity of the medium and r_H is the hydrodynamic radius of the molecule, here assumed of spherical shape (Stilbs and Lindman, 1981).

The use of TMS as internal diffusion reference allows to take into account the variation in solvent properties while comparing solutions differing for solute concentration or solvent composition, making it possible to correct the measured diffusion values for any change in the viscosity (Cabrita and Berger, 2001). The analysis of the $\eta \times D$ product (Table 2) is an easy way to understand if a change in diffusion values is due to viscosity or not: if that product, measured in different conditions, has the same value, then the decrease of diffusion coefficient depends only on viscosity.

It follows that the effects of adding water to DMSO solutions of the three ferulic acid derivatives are completely accounted for by the change in viscosity and

Table 3
Diffusion coefficients ($D \times 10^{10}$, $m^2\ s^{-1}$) and the $\eta \times D$ product for the three derivatives (1a, 1b, 1c) 21 mM in DMSO-d^6 at $T = 298$ K

	D ($m^2\ s^{-1}$) $\times 10^{10}$			$\eta \times D$ ($kg\ m\ s^{-2}$) $\times 10^{12}$		
	1a	1b	1c	1a	1b	1c
Free						
TMS	7.24	7.20	7.46	1.45	1.45	1.45
Fer	2.74	2.72	2.78	0.55	0.56	0.54
+60 μl RBCs						
TMS	5.11	5.11	5.00	1.46	1.46	1.45
Fer	1.85	1.70	1.67	0.53	0.48	0.49

Fer: ferulic derivatives. The viscosity η ($kg\ m^{-1}\ s^{-1}$) of the solution was calculated in the free samples: 2.01×10^{-3} (1a), 2.02×10^{-3} (1b), 1.95×10^{-3} (1c), and in presence of 60 μl of RBCs: 2.85×10^{-3} (1a), 2.85×10^{-3} (1b), 2.91×10^{-3} (1c).

preferential solvation can be ruled out, at least at the measured solvent composition.

If, with this in mind, we reconsider the effect of erythrocytes upon the observed diffusion coefficients of the three ferulic acid derivatives it is now evident that, while the $\eta \times D$ values of TMS are not changed, those of 1a, 1b, and 1c (again averaged over all proton resonances) are somehow and differentially slowed down, being, again, 1b and 1c more affected than 1a (Table 3).

When a molecule self-aggregates or binds a macromolecule site, and binding involves rapid exchange, on the time scale of NMR diffusion experiment ($\Delta \gg k_{off}^{-1}$, where Δ is the separation between the leading edges of the gradient pulses and k_{off} is the kinetic constant for the dissociation process), then the estimated diffusion coefficient is a weighted average of diffusion coefficients of free and bound states, being the weighting factors the corresponding occupational probabilities (Stilbs and Lindman, 1981, 1982; Stokkeland and Stilbs, 1985).

Ligand binding studies are commonly analyzed using the simplest two-site model in which the ligand can bind to any of the n identical independent binding sites on a macromolecule (Luo et al., 1999). In this case the observed ligand diffusion coefficient, D_{obs}, is the population-weighted average of the free, D_f, and the bound, D_b, values (Price et al., 2002):

$$D_{obs} = p_f D_f + p_b D_b \quad (3)$$

Such approach suitably probes relatively weak interaction only; the exchange rate between the two environments must, in fact, be fast enough to provide significant modulation. Eq. (3) can be exploited by considering that (i) D_f is likely much larger than D_b and (ii) D_b can be approximated by the diffusion coefficient of the macromolecule which is not likely to be affected by ligand binding (Fielding, 2000). In the case of erythrocytes, the D_b value is expectedly very small (the lateral diffusion is very slow with a limit value of $10^{-12}\,m^2\,s^{-1}$) (Eisinger and Scarlata, 1987; Kuba et al., 2002); in addition, also p_b is small, being the ligand concentration exceedingly larger than the likely concentration of cellular binding sites. In such conditions the term $p_b D_b$ can be neglected and Eq. (3) becomes:

$$D_{obs} = p_f D_f \quad (4)$$

Table 4
Molar fraction of the three ferulic derivatives

	p_f	p_b
1a	0.96	0.04
1b	0.88	0.12
1c	0.90	0.10

Errors in calculated fractions are in the range 4–10%.

As already stated, we considered the product $\eta \times D$ instead of the diffusion value in order to take the viscosity changes into account. Equation (Trombino et al., 2004) has been therefore considered:

$$(\eta \times D)_{obs} = p_f(\eta \times D)_f \quad (5)$$

From such equation we could calculate the values of p_f and p_b of all three derivatives (Table 4), thus estimating the strength of the interaction. It is now evident that 1b and 1c have almost the same affinity for the erythrocyte membrane, whereas the association of 1a is weaker by a factor of ca. 3.

Such trend correlates with the different anti-oxidant activity of the three ferulates being the 1b and 1c compounds more active than 1a*:* the concentration able to inhibit the lipid peroxidation process in rat liver microsomal membranes (IC_{50} (μM)) was for undecyl derivatives two or three times larger than that for the other two compounds (Anselmi et al., 2004a).

The independent measurements of spin-lattice relaxation rates provided further support to the observed binding interaction and also allowed some inferences on the interaction mode. Such rates were measured after selective excitation of proton resonances, since it is well ascertained that the R_i^{sel} parameter is a very sensitive tool for detecting binding of relatively small ligands to macromolecular binding sites (Fielding, 2000; Luo et al., 1999; Valensin et al., 1982; Delfini et al., 2000; Rossi et al., 1999; Berti et al., 2000). The methoxy group could be of primary importance to map the interaction between the cells and ferulic acid derivatives but unfortunately this signal is almost masked by the broad water resonance in the presence of erythrocytes. Nevertheless significant information comes from the analysis of relaxation effects of the other resonances belonging to the aromatic ring. When plotting the normalized proton relaxation enhancement $\Delta R_i^{sel}/R_{if}^{sel}$, where ΔR_i^{sel} is the measured change in selective relaxation rate ($= R_{iobs}^{sel} - R_{if}^{sel}$) and R_{if}^{sel} is the corresponding rate

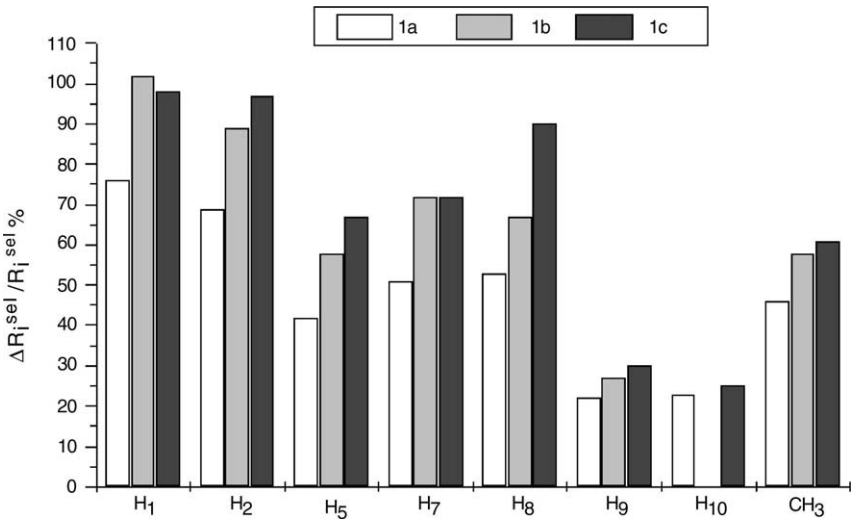

Fig. 2. Normalized selective relaxation enhancements of protons of the three ferulic acid derivatives 21 mM in DMSO at $T = 298$ K in the presence of 60 μl of RBCs.

in the free state, the diverse affinity of the three derivatives can be immediately viewed (Fig. 2). Moreover some inferences on the binding mode can be laid based on the following. An increase in R_i^{sel} may arise from either a slowing down of molecular motions or an increase in the number of ^1H–^1H dipole–dipole interactions contributing to relaxation or both. From this point of view, the data in Fig. 2 indicates that:

(1) 1a, 1b and 1c bind in the same way, though with different affinities;
(2) the strongest effect experienced by aromatic or olefinic protons suggests either location close to a relatively more extended proton-rich environment or a more pronounced slowing down of internal motions. By the same way, the alkyl side chain, which most likely is embedded in the membrane double layer (thus determining the relative binding affinity), can be hypothesized to retain higher degrees of internal flexibility.

The observed effects on the ethylenic protons H_7, H_8 and on aromatic proton H_1 and H_2 support the previous evidence that the most effective anti-oxidants are characterized by main mean folded conformations, where the *n*-alkyl chain bends towards the ethylenic protons which are in turn coplanar to the aromatic moieties (Anselmi et al., 2004a).

The observed findings suggest that PFG NMR diffusion measurements provide a suitable tool for delineating the interaction between whole cellular double bi-layer and such that (i) some quantitative aspects of binding can be inferred; (ii) structure-activity correlations can be attempted; and (iii) inferences on the interaction modes can be made.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemphyslip.2004.12.005.

References

Anselmi, C., Centini, M., Andreassi, M., Carini, M., Maffei Facino, R., 2001. New future promising cosmetic ingredient: ferulic acid. Yushi 54, 53–59.

Anselmi, C., Centini, M., Granata, P., Sega, A., Buonocore, A., Bernini, A., Maffei Facino, R., 2004a. Antioxidant activity of ferulic acid alkyl esters in a heterophasic system: a mechanistic insight. J. Agric. Food Chem. 52, 6425–6432.

Anselmi, C., Centini, M., Andreassi, M., Buonocore, A., La Rosa, C., Maffei Facino, R., Sega, A., Tsuno, F., 2004b. Conformational analysis: a tool for the elucidation of the antioxidant properties of ferulic acid derivatives in membrane models. J. Pharm. Biomed. 35, 1241–1249.

Atkinson, C.E., Aliev, A.E., Motherwell, W.B., 2003. Binding site optimisation for artificial enzymes by diffusion NMR of small molecules. Chem. Eur. J. 9, 1714–1723.

Balasubashini, M.S., Rukkumani, R., Viswanatha, P., Menon, V.P., 2004. Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytother. Res. 18, 310–314.

Berti, F., Gaggelli, E., Valensin, G., 2000. ^{1}H NMR relaxation investigation of herbicides interacting with photosystem II preparations from *Spinacia Oleracea* L. Appl. Magn. Reson. 18, 289–301.

Bleicher, K., Lin, M., Shapiro, M.J., Wareing, J.R., 1998. Diffusion editing NMR: screning compound mixture by affinity NMR to detect binding ligands to vancomycin. J. Org. Chem. 63, 8486–8490.

Cabrita, E.J., Berger, S., 2001. DOSY studies of hydrogen bond association: tetramethylsilane as a reference compound for diffusion studies. Magn. Reson. Chem. 39, S142–S148.

Callaghan, P.T., Gros, M.A.L., Pinder, D.N., 1983. The measurement of diffusion using deuterium pulsed field gradient nuclear magnetic resonance. J. Chem. Phys. 79, 6372–6381.

Canet, D., 1997. Radiofrequency field gradient experiments. Prog. Nuclear Magn. Reson. Spectrosc. 30, 101–135.

Catalán, J., Díaz, C., García-Blanco, F., 2001. Characterization of binary solvent mixtures of DMSO with water and other cosolvents. J. Org. Chem. 66, 5846–5852.

Delfini, M., Gianferri, R., Dubbini, V., Manetti, C., Gaggelli, E., Valensin, G., 2000. ^{1}H NMR relaxation investigation of inhibitors interacting with Torpedo californica acetylcholinesterase. J. Magn. Reson. 144, 129–133.

Dingley, A.J., Mackay, J.P., Chapman, B.E., Morris, M.B., Kuchel, P.W., Hambly, B.D., King, G.F., 1995. Measuring protein self-association using pulsed-field-gradient NMR spectroscopy: application to myosin light chain 2. J. Biomol. NMR 6, 321–328.

Eisinger, J., Scarlata, S.F., 1987. The lateral fluidity of erythrocyte membranes temperature and pressure dependence. Biophys. J. 28, 273–281.

Fielding, L., 2000. Determination of association constant (K_a) from solution NMR data. Tetrahedron 56, 6151–6170.

Gibbs, S.J., Johnson Jr., C.S., 1991. A PFG–NMR experiment for accurate diffusion and flow studies in the presence of eddy currents. J. Magn. Reson. 93, 395–402.

Graf, E., 1992. Antioxidant potential of ferulic acid. Free Radic. Biol. Med. 13, 435–448.

Guy, R.D., Razi, M.T., Rabenstein, D.L., 1986. Measurements of rates of transport across erythrocytes membranes by ^{1}H nuclear magnetic resonance spectroscopy. J. Magn. Reson. 66, 434–444.

Johnson Jr., C.S., 1999. Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. Prog. Nuclear Magn. Reson. 34, 203–256.

Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K., Taniguchi, H., 2002. Antioxidant properties of ferulic acid and its related compounds. J. Agric. Food Chem. 50, 2161–2168.

Kirchner, B., Reiher, M., 2002. The secret of dimethyl suldoxide–water mixture. A quantum chemical study of 1DMSO-*n* water clusters. J. Am. Chem. Soc. 124, 6206–6215.

Kuba, J., Li, L., Gryczynski, I., Piszczek, G., Johnson, M., Lakowicz, J.R., 2002. Lateral diffusion coefficients in membranes measured by resonance energy transfer and a new algorithm for diffusion in two dimensions. Biophys. J. 82, 1358–1372.

Lennon, A.J., Scott, N.R., Chapman, B.E., Kuchel, P.W., 1994. Hemoglobin affinity for 2,3-bisphosphoglycerate in solutions and intact erythrocytes: studies using pulsed-field gradient nuclear magnetic resonance and Monte Carlo simulations. Biophys. J. 67, 2096–2109.

Lin, M., Shapiro, M.J., Wareing, J.R., 1997. Diffusion-edited NMR-affinity for direct observation of molecular interaction. J. Am. Chem. Soc. 119, 5249–5250.

Longsworth, L.G., 1960. The mutual diffusion of light and heavy water. J. Phys. Chem. 64, 1914–1917.

Luo, R.S., Liu, M.L., Mao, X.A., 1999. NMR diffusion and relaxation study of drug–protein interaction. Spectrochim. Acta A 55, 1887–1901.

Mansfield, S.H., Jayawickrama, D.A., Timmons, J.S., Larive, C.K., 1998. Measurement of peptide aggregation with pulsed-field gradient nuclear magnetic resonance spectroscopy. Biochim. Biophis. Acta 1382, 257–265.

Mansfield, S.L., Gotch, A.J., Harms, G.S., Johonson, C.K., Larive, C.K., 1999. Complementary analysis of peptide aggregation by NMR and time-resolved spectroscopy. J. Phys. Chem. B 103, 2262–2269.

Mizuno, K., Imafuji, S., Ochi, T., Ohta, T., Maeda, S., 2000. Hydration of the CH groups in dimethyl sulfoxide probed by NMR and IR. J. Phys. Chem. B 104, 11001–11005.

Nenadis, N., Zhang, H.-Y., Tsimidou, M.Z., 2003. Structure-antioxidant activity relationship of ferulic acid derivatives: effect of carbon side chain characteristic groups. J. Agric. Food Chem. 51, 1874–1879.

Niki, E., 1997. Free radicals, antioxidants, and cancer. In: Ohigashi, H., Osawa, T., Terao, J., Watanabe, S., Yoshikawa, T. (Eds.), Food Factors for Cancer Prevention. Springer, Tokyo, Japan, pp. 55–57.

Price, W.S., Kuchel, P.W., 1990. Restricted diffussion of bicarbonate and hypophosphite ions modulated by transport in suspension of red blood cells. J. Magn. Reson. 90, 100–110.

Price, W.S., Tsuchiya, F., Arata, Y., 1999. Lysozyme aggregation and solution propetries studied using PSGE NMR diffusion measurements. J. Am. Chem. Soc. 121, 11503–11512.

Price, W.S., Elwinger, F., Vigouroux, C., Stilbs, P., 2002. PGSE-WATERGATE, a new tool for NMR diffusion-based studies of ligand macromolecule binding. Magn. Reson. Chem. 40, 391–395.

Price, W.S., 1998. Pulsed-field gradient nuclear magnetic resonance as a tool for studying translational diffusion. Part II. Experimental aspects. Concepts Magn. Reson. 10, 197–237.

Rice-Evans, A.C., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.

Rossi, C., Bastianoni, S., Bonechi, C., Corbini, G., Corti, P., Donati, A., 1999. Ligand–protein recognition studies as determined by nuclear relaxation analysis. Chem. Phys. Lett. 310, 495–500.

Rowley, S.D., 1992. Hematopoietic stem cell cryopreservation: a review of current techniques. J. Hematother. 1, 233–250.

Stilbs, P., Lindman, B., 1981. Determination of organic counterion binding to micelles through Fourier transform NMR self-diffusion measurements. J. Phys. Chem. 85, 2587–2589.

Stilbs, P., Lindman, B., 1982. FT NMR self-diffusion for the study of counterion binding in polyelectrolyte solutions. J. Magn. Reson. 48, 132–137.

Stokkeland, I., Stilbs, P.A., 1985. Multicomponent self-diffusion NMR study of aggregation of nucleotides, nucleosides, nucleic acid bases and some derivatives in aqueous solution with divalent metal ions added. Biophys. Chem. 22, 65–75.

Taniguchi, H., Nomura, E., Tsuno, T., Minami, S., 1998. Ferulic acid ester antioxidant/UV absorbent. European Patent App., 0681 825 A2.

Taniguchi, H., Hosoda, A., Tsuno, T., Maruta, Y., Nomura, E., 1999. Preparation of ferulic acid and its application for the synthesis of cancer chemopreventive agents. Anticancer Res. 19, 3757–3762.

Trombino, S., Serini, S., Di Nicuolo, F., Celleno, L., Andò, S., Picci, N., Calviello, G., Palozza, P., 2004. Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta-carotene, and ascorbic acid. J. Agric. Food Chem. 52, 2411–2420.

Valensin, G., Kushnir, T., Navon, G., 1982. Selective and nonselective proton spin-lattice relaxation studies of enzyme–substrate interactions. J. Magn. Reson. 46, 23.

Viel, S., Mannina, L., Segre, A., 2002. Detection of π–π complex by diffusion-ordered spectroscopy (DOSY). Tetrahedron Lett. 43, 2515–2519.

Waldeck, A.R., Nouri-Sorkhabi, M.H., Sullivan, D.R., Kuchel, P.W., 1995. Effects of cholesterol on transmembrane water diffusion in human erythrocytes measured using pulsed field gradient NMR. Biophys. Chem. 55, 197–208.

Waldeck, A.R., Kuchel, P.W., Lennon, A.J., Chapman, B.E., 1997. NMR diffusion measurements to characterise membrane transport and solute binding. Prog. Nuclear Magn. Reson. Spectrosc. 30, 39–68.

Wu, D., Chen, A., Johnson Jr., C.S., 1995. An improved diffusion ordered spectroscopy experiment incorporating bipolar gradient pulses. J. Magn. Reson. A 115, 260–264.

Yao, S., Howlett, G.J., Norton, R.S., 2000. Peptide self-association in aqueous trifluoroethanol monitored by pulsed field gradient NMR diffusion measurements. J. Biomol. NMR 16, 109–119.