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The interaction of the polyphenylacetylene surface with biological environments studied by XPS, RAIRS and biological tests

G. Iucci · L. Rossi · N. Rosato · I. Savini · G. Duranti · G. Polzonetti

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Abstract A π-conjugated polymer, polyphenylacetylene or PPA, has been tested for its possible applications as biosensor or biomaterial. Protein adsorption was investigated by incubating PPA films in solutions of bovine serum albumin (BSA) dissolved in phosphate buffer (PBS) having increasing protein concentration. Investigations on the PPA films were carried out by means of two surface analysis techniques, X-ray photoelectron spectroscopy (XPS) and reflection-absorption infrared spectroscopy (RAIRS). Desorption of BSA from the PPA surface was also investigated.

Finally, the cytototoxicity of the PPA surface was checked by measuring viability and proliferation of lymphoma macrophages and SAOS osteoblasts grown in the presence of the polymer.

Introduction

The use of biosensors for monitoring chemicals concentration in medical or environmental applications has recently raised considerable interest owing to their selective and fast response. Many biosensors use a polymer membrane [1], capable of immobilizing (covalently or not) a biological system (protein, enzyme or cell), interfaced with a proper transducer of the biological signal. Π-conjugated polymers (mostly polypyrrole, polyaniline and their derivatives) have been used in biosensors preparation, owing to their peculiar property of electrical conductivity. Biosensors containing various types of enzymes immobilized by different techniques on the surface of conjugated polymers have been recently reported [2–5].

The immobilization of a biological component on a specific support is a complex process, and needs a preliminary study aimed at understanding the required characteristics of the support in connection with the immobilized biological component and with the immobilization mechanism.

Polyphenylacetylene (PPA), whose structure is shown in Fig. 1 (top right corner), is a conjugated polymer that has been used in the preparation of humidity sensors [6–7]. XPS investigations [8] have shown that the surface of PPA films is covered by a layer of –OH groups that have a part in water adsorption and might play a role in the immobilization of biological components.

The present study is a preliminary analysis of the possibility to use PPA films as polymeric support for protein immobilization; for this purpose, protein adsorption on the PPA surface was investigated. The selected protein, bovine serum albumin (BSA), that has been chosen as a model system, is the main component of blood serum and the first plasma protein to be adsorbed on surfaces [9]; BSA is therefore a suitable system for the simulation of the biological environment typical of biosensors used in the medical field.

BSA adsorption on the PPA surface was monitored by means of two surface analysis techniques, XPS (X-ray photoelectron spectroscopy) and RAIRS (reflection-absorption infrared spectroscopy), having a sampling depth of 5–10 nanometers and of few micrometers, respectively.

G. Iucci (✉) · L. Rossi · G. Polzonetti
Dipartimento di Fisica E. Amaldi, Universit à "Roma Tre", Unità INFM, INSTM, and CISDiC, Via della Vasca Navale 84, 00146 Roma
e-mail: iucci@uniroma3.it

N. Rosato · I. Savini · G. Duranti
Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università di Roma "Tor Vergata", Via Montpellier 1, 00133 Roma

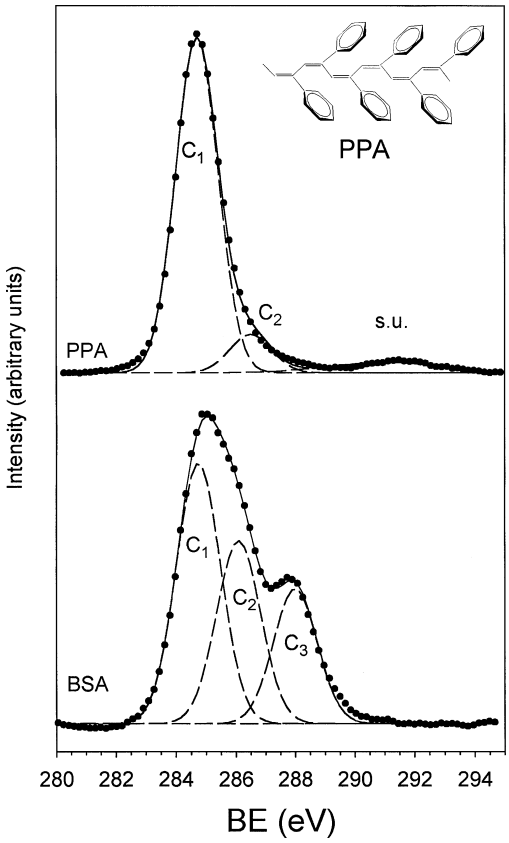

Fig. 1 Curve-fitting analysis of the C1s experimental spectra of pristine BSA and PPA film; markers represent the experimental points, continuous lines the calculated spectra, dashed lines the fitting components. Chemical structure of *cis*-PPA is also shown.

In a previous paper [10] we reported investigations on BSA adsorption on the PPA surface as a function of incubation time; we now present a study of protein adsorption as a function of concentration. BSA desorption from the PPA surface was also investigated by incubation of the PPA/BSA samples with sodium dodecyl sulphate (SDS), a methodology aimed at investigating the strength of protein-surface interactions [11–12].

The possibility to use PPA as a biomaterial (i.e. a non living material used in medicine and capable of interacting with biological systems) was also investigated by analyzing lymphoma macrophages (U937) and SAOS osteoblasts viability and proliferation after incubation with PPA.

Materials and methods

Chemicals

BSA was purchased from Sigma. Phosphate buffered saline (PBS) solution was prepared, according to literature methodology, by dissolving 8.00 mg/ml NaCl, 0.200 mg/ml KCl, 1.150 mg/ml Na_2HPO_4 and 0.200 mg/ml KH_2PO_4, in distilled water [13]. Solutions with increasing BSA concentration (BSA/PBS) were prepared by dissolving 0.00 (solution A), 1.00 (B), 2.00 (C), 4.00 (D) or 8.00 (E) mg/ml BSA in PBS.

The culture medium (RPMI 1640), bovine fetal serum, glutamine and penicillin-streptomycin were obtained from GIBCO INVITROGEN.

Sample preparation

PPA was prepared by catalytic polymerization (catalyst $[Rh(cod)Cl]_2$ cod = cyclooctadiene) from freshly distilled phenylacetylene precursor (Fluka), as previously described [14]; in the reported experimental conditions, *cis-transoid* PPA (i.e. a polymer having the backbone structure shown in Fig. 1) is obtained.

Thin films of PPA for XPS and RAIRS analysis were prepared by casting from freshly prepared chloroform solutions (2.5 mg/ml) onto gold plated Si(111) substrates; the sample surface prepared is quite hydrophilic [8], due to the presence of chemisorbed –OH groups (see Results section). PPA films were then treated in one of the following ways:

- incubation with one of the BSA/PBS solutions A-E at 25° C for 24 hours (adsorption experiment);
- incubation with solution C for 24 h, followed by washing with PBS and subsequent incubation with 3% or 6% w/w aqueous solution of SDS for 24 or 48 hours (desorption experiment); in a control experiment PPA films were incubated directly with SDS solutions;
- incubation with the culture medium at 25 °C for 4 hours and 40 minutes or for 24 hours.

After incubation, the samples were washed thrice with PBS and once with distilled water and dried in vacuum for 24 hours in a $CaCl_2$ dryer.

PPA films prepared by spin-coating (1000 rounds per second) on the Au substrate from $CHCl_3$ solutions (2.5 mg/ml) were investigated for comparison, in order to obtain information about BSA adsorption on a more hydrophobic PPA surface, containing less chemisorbed –OH groups [15]. Incubation with PBS (solution A) and BSA/PBS (C) was performed; no significant difference was evidenced with the results obtained for the hydrophilic films prepared by casting, probably because incubation in aqueous solution produces in any case water chemisorption on the PPA surface. Therefore, the data presented in the "Results" section refer to PPA films prepared by casting.

Thin films of BSA were prepared by casting from aqueous solutions (2.5 mg/ml) onto gold-coated silicon substrates, and dried in vacuum.

XPS measurements

XPS analyses were performed in an instrument of our own design consisting of a preparation and an analysis chamber separated by a gate valve. The analysis chamber is equipped with a manipulator having 6 degrees of freedom and with a 150 mm mean radius hemispherical electron analyzer with a five lens output system combined with a 16-channel detector.

The PPA samples were prepared and treated as described in the preceding section; the spectra of pristine BSA were also recorded for comparison. Samples were introduced in the preparation chamber and left outgassing overnight at a base pressure of about 10^{-8} torr, before introduction in the analysis chamber. Typical vacuum in the analysis chamber during the measurements was in the 10^{-9}–10^{-10} torr range.

Non-monochromatised Al Kα X-ray radiation ($h\nu$ = 1486.6 eV) was used; the instrumental resolution is 1.0 eV, as measured for the $Ag3d_{5/2}$ line. Core level spectra of PPA (C1s, O1s), of BSA (C1s, O1s, N1s, S2p), of PBS (O1s, P2p, C12p, Na1s) and of the culture medium (C1s, O1s, N1s, P2p, C12p, Na1s) were recorded on the respective samples.

The spectra of PPA were energy referenced to the C1s signal located at a BE (binding energy) of 284.7 eV, as expected for aromatic carbons [8, 16]; the spectra recorded for BSA were energy referenced to the N1s signal of the peptide nitrogen located at 400.0 eV BE [16–18]. Atomic concentration ratios were calculated from peak intensities by using Scofield's cross section values [19]. The standard deviation of the measured XPS atomic ratios is ± 10%. A curve-fitting analysis of the C1s spectra was performed by using gaussians as fitting functions, after subtraction of a straight line as background.

RAIRS measurements

Reflectance-absorbance spectra were recorded by means of a VECTOR 22 (Bruker) FT-IR interferometer operating in the wavenumber range 400–4000 cm^{-1}, equipped with a Specac P/N 19650 series monolayer/grazing angle accessory, operating in the "grazing incidence" at 70° reflection angle and with a DTGS detector. The use of the gold-coated silicon substrates is necessary in order to increase sample reflection.

Cell cultures

U937 lymphoma macrophages (purchased from ATCC) were grown in RPMI 1640 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2.5 mM glutamine and 0.1 mg/ml penicillin-streptomycin in a humidified atmosphere with 5% (v/v) CO_2 at 37°C. SAOS osteoblasts (purchased from ATCC) were grown in Dulbecco's MEM mixed F12 supplemented with 10% (v/v) heat-inactivated FBS, 2.5 mM glutamine and 0.1 mg/ml kanamycin in a humidified atmosphere with 5% (v/v) CO_2 at 37°C.

Biological assays

PPA films were prepared by depositing 2.0 ml of a freshly prepared chloroform solution (2.5 mg/ml) onto Petri glass dishes (inner diameter 48 mm) previously dried in oven at 250°C for 12 hours and left cooling to room temperature (RT) in the oven. The freshly prepared PPA films were aged at RT for 12 hours, then dried in oven for 72 hours at 50°C. This procedure is required in order to improve polymer film adhesion during the tests.

Every dish was seeded with a suspension of $5.0 \cdot 10^5$ cells/ml lymphoma macrophages in culture medium for a total amount of $2.5 \cdot 10^6$ cells/dish and incubated for 270 minutes. A suspension of $2.5 \cdot 10^5$ cells/ml was used for 24 hours incubation experiments.

After incubation at 37°C, cells were washed twice in PBS and resuspended in 1 ml PBS. Cell viability was measured by the trypan blue exclusion assay in a Neubauer hematocytometer chamber. Each experiments was performed in triplicate.

SAOS cells were seeded (10^5/ml) on PPA surface or in Petri dishes (control experiment), and maintained in culture medium. SAOS cells growing on the PPA surface were monitored by optical microscopy. After 48 hours incubation at 37°C images of the SAOS cells were taken using a Nikon Optronic CCD camera and optical Nikon microscopy (100×). Images were elaborated using a Arkon-Fish Nikon software.

Results

XPS results

Modifications of the XPS spectra of PPA as a function of incubation with BSA have been studied. Before discussing the spectra of the PPA/BSA systems, we will quickly examine the C1s spectra of the pristine materials BSA and PPA shown in Figure 1; the chemical structure of PPA is also displayed in the top right corner.

The overall C1s spectrum of BSA (bottom figure) results from three major contributions, labeled C_1, C_2 and C_3: the main peak occurring at 285.0 eV (C_1) corresponds to aliphatic carbons of the amino acid pending groups, peak C_2 (286.5 eV) is assigned to the OC-CH-N carbons of the peptide chain and to C-N and C-O carbons of the pending groups, peak C_3 at 288.2 eV to –CO-NH- peptidic carbons and to –COOH carbons of the pending groups [20].

In the spectrum of PPA, the main signal labeled C_1 (BE = 284.7 eV) is due to the sp^2 carbons of the polymer backbone and of the phenyl pending groups. The high BE shoulder

Table 1 Atomic ratios measured from XPS data of PPA films incubated with BSA/PBS solutions A–E; data concerning pristine PPA and pure BSA are also presented for comparison. The ratios C_3/C_1, N/C_1, N/C_3 were determined form curve-fitting deconvolutions of the experimental C1s spectra.

Sample		Atomic ratios					
		N/C	O/C	S/C	C_3/C_1	N/C_1	N/C_3
BSA:	measured	0.250	0.294	0.013	0.5	0.48	0.95
	theoretical	0.27	0.313	0.013			
PPA			0.088				
PPA+ PBS (A)			0.111				
PPA+ BSA/PBS 1.00 mg/ml (B)		0.0781	0.217	0.002	0.10	0.11	1.1
PPA+ BSA/PBS 2.00 mg/ml (C)		0.11	0.24	0.0025	0.16	0.15	0.94
PPA+ BSA/PBS 4.00 mg/ml (D)		0.149	0.32	0.0029	0.22	0.23	1.03
PPA+ BSA/PBS 8.00 mg/ml (E)		0.149	0.32	0.0031	0.21	0.23	1.07

C_2 (286.4 eV) is associated to the C-OH groups present on the polymer film surface as a result of water chemisorption. The shake-up satellites (s.u.), at about 7 eV towards high BE from the main photoemission peak, are generated by $\pi \rightarrow \pi^*$ transitions, mainly from the phenyls.

Table 1 shows the atomic ratios determined from XPS results obtained on PPA films incubated with BSA solutions (PPA/BSA) having increasing protein concentration; data concerning the pristine materials (BSA and PPA) are also shown for comparison. The measured O/C ratio in the pristine PPA film reveals the presence, on average, of one –OH group per 1.5 monomer units. When PPA films were incubated with only PBS (sample A) adsorption of PBS salts (containing chlorine, sodium and phosphates) took place only to a very limited extent. Addition of BSA to the PBS solutions (samples B-E) produces protein adsorption and co-adsorption of the salts contained in PBS as already discussed in [10]. The N/C, O/C and S/C atomic ratios, indicative of protein adsorption, increase with BSA concentration up to 4.00 mg/ml, after which saturation is obtained. The plot of the measured atomic ratios as a function of BSA concentration in solutions A–E is shown in Fig. 2a. Moreover, no change is detected in the N1s and S2p BEs (not shown in the table) with respect to pristine BSA, suggesting that protein adsorption takes place without significant modifications in the chemical structure. When BSA adsorption was studied as a function of incubation time a similar effect (increasing protein adsorption followed by saturation) was evidenced [10].

Curve-fitting deconvolution of the C1s spectra of PPA/BSA films as a function of increasing BSA concentration in the range 1–4 mg/ml (samples B-D) is shown in Fig. 3; the spectrum of sample E (8.0 mg/ml BSA), not significantly different from the spectrum of sample D (4.0 mg/ml BSA), is not shown. BSA adsorption is evidenced by the appearance in the spectra of peak C_3 (related to peptide carbons) and by the increase in intensity of peak C_2 (C-O, C-N carbons), both signals being present in the BSA spectrum. Increasing protein adsorption as a function of BSA concentration is evidenced by the growing intensity of signals C_2 and C_3 with respect to signal C_1, which is the main component of the PPA spectrum.

The curve-fitting analysis allowed calculations of the areas of the component peaks; Table 1 and Fig. 2b show the

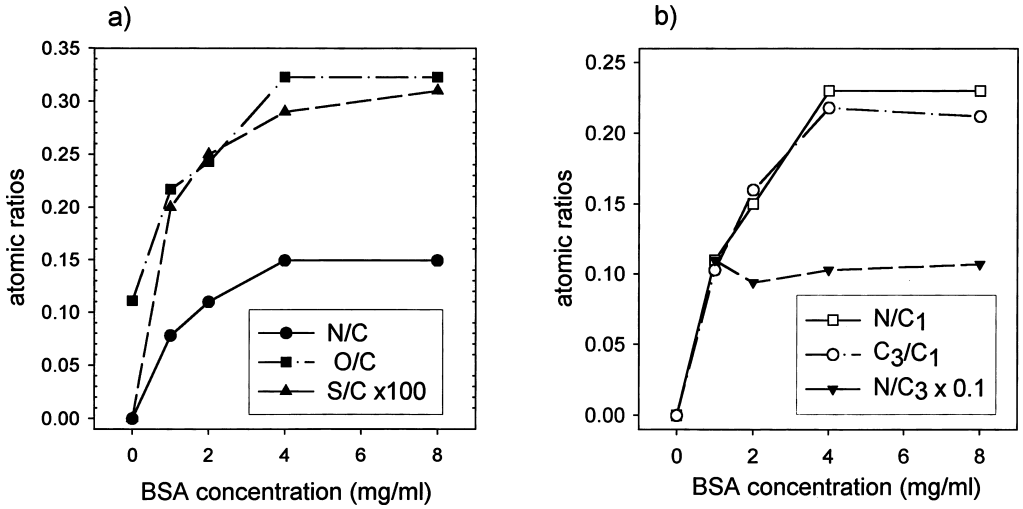

Fig. 2 BSA adsorption on the PPA surface; plot of the measured atomic ratios as a function of BSA concentration: N/C, O/C, S/C (2a), C_3/C_1, N/C_1, N/C_3 (2b).

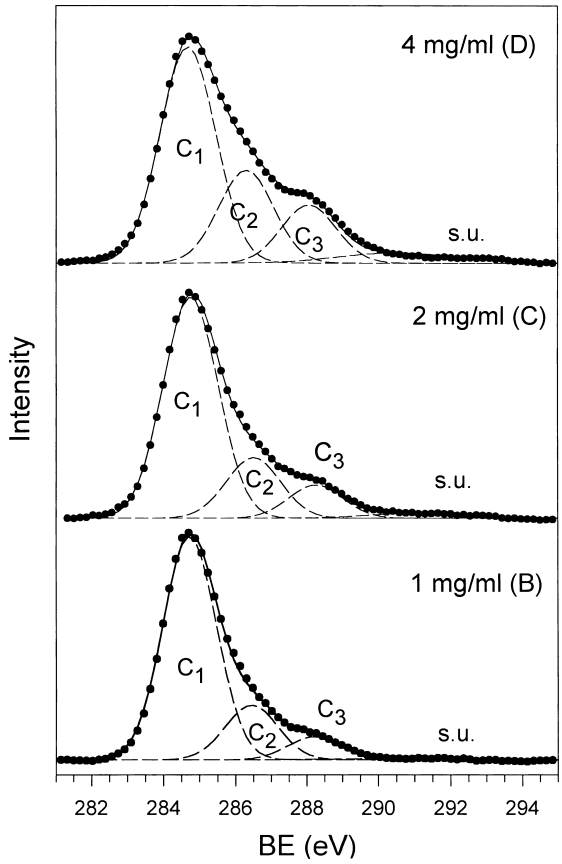

Fig. 3 Curve-fitting analysis of the C1s experimental spectra of PPA film after incubation with BSA/PBS solutions having 1.0, 2.0 or 4.00 mg/ml concentration (samples B-D); markers represent the experimental points, continuous lines the calculated spectra, dashed lines the fitting components.

evolution of the C_3/C_1, N/C_1 and N/C_3 ratios as a function of BSA concentration. The corresponding measured ratios for pure BSA (in good agreement with the calculated values) are also shown in Table 1. Nitrogens and C_3 carbons are found only in the BSA molecule, while C_1 carbons are present in higher concentration in PPA than in BSA. Both the C_3/C_1 and N/C_1 ratios confirm increasing protein adsorption up to a concentration of 4.00 mg/ml, followed by saturation at higher concentration, while the N/C_3 ratio remains constant, within the experimental error (approximately $\pm$ 10%), as expected.

The thickness of an overlayer adsorbed on a substrate can be calculated using the equation:

$$I = I_\infty[1 - \exp(-d/\lambda)] \quad \text{(a)}$$

Where I is the intensity of a photoemission signal from an overlayer, I_∞ the intensity of the same signal in a layer of infinite thickness, d the overlayer thickness and λ the inelastic mean free path (IMPF). λ can be calculated for a photoelectron having a kinetic energy KE by equation [21]:

$$\lambda(nm) = B \cdot KE^{1/2} \quad \text{(b)}$$

where $B = 0.096$ nm $\cdot$ $eV^{-1/2}$ for an organic overlayer.

For the N1s photoelectrons of BSA, using the MgKα source we obtain a IMPF $\lambda \approx 30$ Å. The thickness of the BSA overlayer can be roughly estimated from the N1s peak average intensities measured for PPA/BSA samples D, E (I) and for the pristine BSA film (I_∞), considered in a first approximation to be "infinitively thick" (few μm) compared to the BSA overlayer in the PPA/BSA systems. On the basis of equation (a), the overlayer at saturation (samples D, E) was found to be approximately 40 Å thick. The BSA molecule was postulated to be an oblate ellipsoid with measured dimensions of 140 $\times$ 40 Å [22, 23]; X-ray crystallography measurements revealed a heart-shaped molecule, with similar dimensions [24]. On the basis of the experimental data we can assume that saturation of BSA adsorption corresponds to a complete coverage of the PPA surface by a BSA overlayer, probably without protein denaturation.

RAIRS results

Fig. 4 shows the RAIRS spectra of BSA, PPA and PPA/BSA (sample D). The main peaks in the BSA spectrum are the amide-I band (C = O stretching vibration) localized at 1650 cm^{-1}, as expected for a protein containing a high percentage of α-helical conformation [25], and the amide-II band (N-H bending vibration) at 1540 cm^{-1}; N-H and O-H stretching vibrations are found in the range 3000–3500 cm^{-1}. The peaks present in the PPA spectrum are typical of aromatic systems: C-H stretching vibrations (3100–3000 cm^{-1}), C=C stretching (1600 cm^{-1} and 1500–1450 cm^{-1}, C-H bending vibrations (760, 740, 700 cm^{-1}), overtones and combination bands in the range 1700–2000 cm^{-1}. Concerning the C-H bending, the presence of a band located at 740 cm^{-1} is typical of *cis-transoid* PPA, [14], as expected on the base of the catalyst used for the polymer synthesis.

In the PPA/BSA spectrum, BSA adsorption on the PPA surface is evidenced by the appearance of the amide (I) and amide (II) bands and of the O-H stretching vibration; the new peaks corresponding to vibrational modes of BSA, labeled with an asterisk in the picture, can be clearly detected only at high protein concentration (samples D and E). The peaks typical of pristine PPA are not affected by protein adsorption, indicating that the polymer structure is not modified, as already evidenced in [10].

Desorption experiments

In order to evaluate the strength of the BSA/PPA interaction, a desorption experiment was carried out by incubating sample C (PPA/BSA 2 mg/ml) with sodium dodecyl sulphate

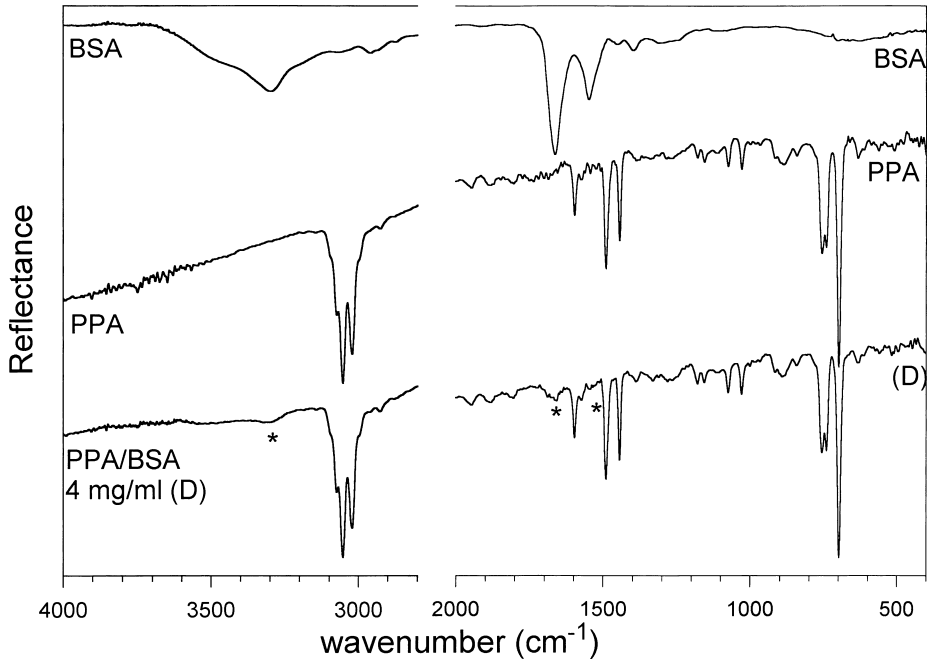

Fig. 4 RAIRS spectra in the range 4000-2800 and 2000-400 cm^{-1} of BSA, pristine PPA film and PPA film after incubation with BSA/PBS solution having 4.00 mg/ml concentration (PPA/BSA D).

(SDS) solutions having 3% or 6% w/w concentration for 24 or 48 hours. Under the reported experimental conditions partial desorption of BSA from the PPA surface took place. Table 2 shows the measured atomic ratios indicating the amount of BSA adsorbed on the PPA surface (C/N, C_3/C_1, N/C_1) for different incubation times and SDS concentrations. A plot of the experimental results is also shown in Fig. 5; Fig. 5a shows the evolution of the atomic ratios as a function of time upon incubation with 3% SDS, Fig. 5b for 6% SDS. It is worth noticing that about half of the amount of protein initially adsorbed is removed after 24 hours incubation with 3% SDS (5a); further exposure for 24 more hours yields a further decrease of the adsorbed BSA. However, even after 48 hours incubation the XPS data yield evidence of BSA molecules still bound to the PPA surface. It was not possible to expose the PPA surface for longer times, due to possible degradation of the PPA film for long incubations. Therefore, we tried to improve BSA desorption by increasing to 6% the SDS percentage in the solution used for incubation (5b). However, no strong difference is evidenced between the samples treated with 3% or 6% SDS; the higher SDS concentration improves only slightly the removal of BSA. The experimental data indicate that most of the BSA molecules are simply physisorbed on the PPA surface, and therefore removed in the desorption experiment. However, part of the adsorbed BSA seems more strongly bound to PPA, since complete desorption cannot be obtained even after 48 hours incubation with SDS.

Table 2 Atomic ratios measured from XPS data for PPA/BSA (sample C) after increasing incubation times with aqueous SDS 3% w/w or 6% w/w. The ratios C_3/C_1 and N/C_1 were determined form curve-fitting deconvolutions of the experimental C1s spectra.

Incubation time (h)	SDS (%)	Atomic ratios		
		N/C	C_3/C_1	N/C_1
0		0.11	0.16	0.15
24	3	0.044	0.093	0.06
48	3	0.031	0.063	0.04
24	6	0.043	0.075	0.054
48	6	0.024	0.053	0.03

Biological assays

Cytotoxicity tests were performed on PPA films prepared by casting from $CHCl_3$ solutions onto Petri dishes and aged for three days at 50°C in order to increase its stability. The viability of the lymphoma macrophages incubated for 4 hours 30 minutes or for 24 hours with PPA films was investigated (for experimental details see the "Materials and methods" section). Lymphoma macrophages were chosen for their limited adhesion to the PPA surface.

The experiments evidenced survival of 93% (± 2%) of the cells after 4 hours 30 minutes incubation; up to 24 hours the percentage of lively cells was not significantly modified (91% ± 3%). Cell growth rate, measured after 24 hours incubation with PPA, was found to be about 90% (± 5%) with respect to control cells.

Furthermore, we evaluated PPA cytotoxicity by using adherent SAOS osteoblasts. Cells growing on the PPA surface were monitored, and pictures were taken 48 hours after seeding. SAOS cells showed strong adhesion to the PPA surface

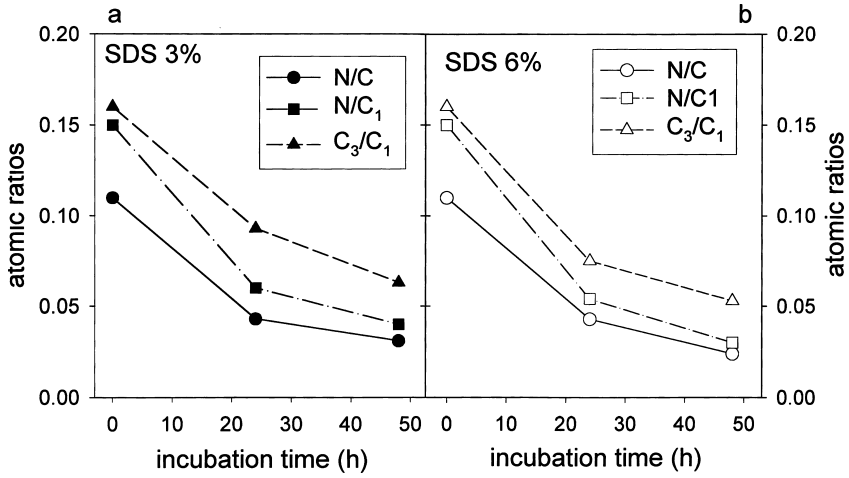

Fig. 5 BSA desorption with SDS: plot of the measured atomic ratios (N/C, C_3/C_1, N/C_1) as a function of incubation time with 3% SDS (5a) or 6% SDS (5b).

without any morphological change with respect to control cells (Fig. 6). Cell viability and growth rate were unaffected by the presence of the polymer; indeed we measured only a small percentage of floating cells (about 3%). An exact quantification of cell survival and growth rate was not possible, as trypsin, required to detach cells from PPA, damaged the polymer film, thus interfering with the measurements.

This results yield good perspectives for possible use of PPA in the preparation of biosensors for chemical applications. The positive outcome of the tests performed allows to consider PPA as a biomaterial, i.e. a "non living material used in medicine capable of interacting with biological systems".

In order to understand the modifications induced on the PPA surface by incubation with the culture medium (CM) used in the biological tests XPS and FTIR investigations have been carried out. The culture medium contains a mixture of different salts, mainly sodium chloride and phosphates; the main organic components are the twenty amino acids and glucose.

Table 3 shows the XPS results obtained in the analysis of PPA samples incubated with the culture medium (PPA/CM) for 4 hours 30 minutes or 24 hours. XPS analysis reveals a strong adsorption of the amino acids and glucose (see O/C, N/C and S/C atomic ratios); coadsorption of the salts present in the CM also takes place, as evidenced by the P/C, Na/C, Cl/C atomic ratios.

The C1s spectra of PPA/CM after 4 h 30' or 24 hours incubation (Fig. 7) are very similar to the spectra of the PPA/BSA systems and result from the superimposition of three main signals, labeled C_1, C_2 and C_3. The interpretation of signal C_1 (284.7 eV) and of the shake-up satellite (s.u., 291 eV) has been already discussed; peak C_2 results from the C-O carbons of glucose and the C-N carbons of the amino acids; C_3 is due to the carboxyls (CO_2^-) of the adsorbed amino acids, in their zwitterionic form.

The RAIRS spectrum (not shown) of PPA/CM after 4 hours 30 minutes incubation does not show significant

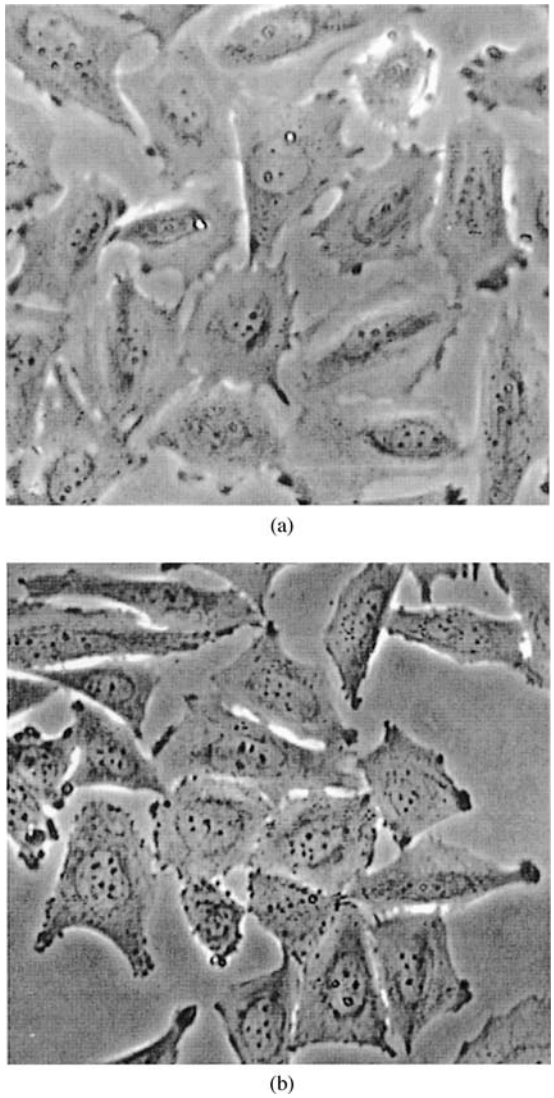

Fig. 6 Images of the SAOS cells maintained in normal culture conditions, seeded on the PPA surface (6a) or in normal Petri dishes (6b) 48 hours after seeding.

Table 3 Atomic ratios measured from XPS data for PPA films incubated with culture medium (CM).

Sample	Atomic ratios				
	N/C	O/C	P/C	Na/C	C1/C
PPA + CM 4 hours 30 min	0.107	0.286	0.01	0.05	0.02
PPA film + CM 24 hours	0.112	0.27	0.02	0.06	0.05

modifications, revealing that the structure of the polymer film is not modified upon incubation with CM and that the adsorption of chemicals is limited to the topmost surface layer (the RAIRS spectroscopy has a sampling depth of few micrometers, compared to few nm of the XPS technique). After 24 hours incubation, low intensity OH stretching (3300 cm^{-1}) and C = O stretching (1670 cm^{-1}) bands are detected, clearly resulting from adsorption of the organic components in the medium.

Discussion

The adsorption of a well-characterized protein (BSA) on the surface of a π-conjugated polymer (PPA) was investigated with the aim of ascertaining the possibility to use PPA as a material compatible with proteins. For this purpose, surface investigation techniques such as XPS (sampling depth up to 10 nm) or RAIRS (few μm) spectroscopies were used.

Hypothesizing a physical interaction (Van der Waals forces, hydrogen bonds) between the PPA surface and the adsorbed BSA molecules, we have studied protein adsorption as a function of concentration. The chemical structure of the polymer film and of the protein is not modified upon adsorption. Increasing protein adsorption as a function of concentration followed by saturation for concentrations higher than 4.0 mg/ml was evidenced; saturation probably corresponds to complete coverage of the PPA surface by a BSA layer.

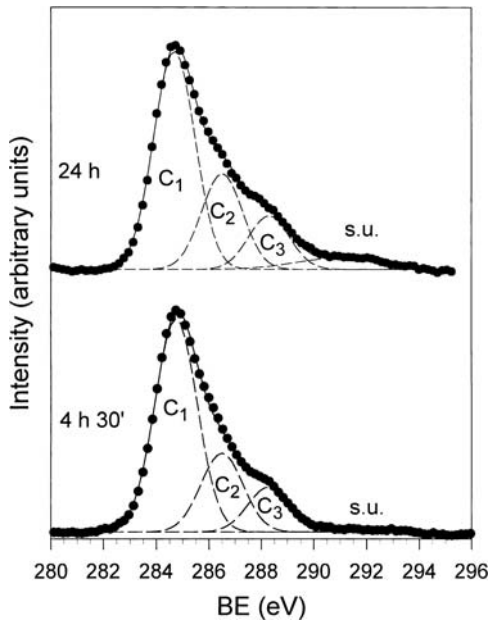

Fig. 7 Curve-fitting analysis of the C1s experimental spectra of PPA film after incubation with CM for 4 hours 30' or 24 hours; markers represent the experimental points, continuous lines the calculated spectra, dashed lines the fitting components.

BSA desorption from the PPA surface was also investigated by incubation with SDS. A partial protein desorption was evidenced, the amount of desorbed protein depending on incubation time. The experimental results suggest that the interaction of the BSA with the PPA surface takes place mainly by physisorption. However, complete BSA desorption could not be obtained even for high incubation times with concentrated SDS, suggesting that a strong interaction takes place between BSA and the PPA surface.

The surface of PPA films, when prepared by casting, is covered by chemisorbed –OH groups (approximately one per 1.5 monomer units) which are actually confined to the topmost surface layer, as evidenced by comparison between XPS and RAIRS results. The PPA surface is therefore more hydrophilic compared to other polymers used in biosensors or as biomaterials, that can be made hydrophilic by surface treatments [19, 26–29]; the acquired hydrophilicity of the PPA surface can play a role in protein adsorption and desorption. It is worth noticing, however, that an increase in the hydrophilicity of the polymer surface should deplete albumin adsorption, as recently shown in [30]. Nevertheless, since albumin has amphiphilic properties, the presence of chemisorbed –OH groups on the surface of a hydrophobic polymer could actually favor protein adsorption.

Finally, biological tests, carried out in order to check the interaction of the polymer with cultured cells, evidenced survival and normal cell proliferation of lymphoma macrophages grown in the presence of PPA.

Conclusions

The modifications induced on the surface of thin films of polyphenylacetylene (PPA), a π-conjugated polymer, upon incubation with solutions containing bovine serum albumin (BSA) have been investigated by XPS and RAIRS spectroscopy. Experimental results yield evidence of increasing BSA adsorption upon increasing protein concentration in the solution, followed by saturation when the PPA surface is completely covered by a BSA overlayer.

BSA desorption from the PPA surface took place upon incubation with SDS, but complete desorption could not be achieved even after 48 hours incubation, suggesting that a

strong interaction takes place between BSA and the PPA surface.

Moreover, biological assays preformed by measuring the viability and proliferation of lymphoma macrophages and SAOS osteoblasts grown in the presence of PPA yielded evidence of cell survival.

PPA is therefore a non cytotoxic polymer capable of protein adsorption and hence a possible candidate for biosensors preparation.

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