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Research Article

Influence of Fluorescent Labelling of Polystyrene Particles on Phagocytic Uptake, Surface Hydrophobicity, and Plasma Protein Adsorption

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Purpose. To investigate the influence of fluorescent labelling of polystyrene particles on phagocytic uptake, surface hydrophobicity and protein adsorption.

Methods. Phagocytic uptake was analysed using chemiluminescence. Hydrophobicity was quantified by adsorption measurements of a hydrophobic dye. Protein adsorption was evaluated by two-dimensional electrophoresis.

Results. Commercially available fluorescently labelled particles showed marked differences when compared to unlabelled particles: phagocytic uptake and surface hydrophobicity of labelled particles were diminished. Also the plasma protein adsorption pattern was found to be different from the unlabelled particles: for example, the amount of fibrinogen adsorbed was strongly reduced on the labelled particles. On the other hand, some unknown proteins could be detected on the fluorescently marked particles. In contrast, plain polystyrene particles and labelled ones could be successfully synthesised by Paulke which did not show any considerable differences in phagocytic uptake, surface hydrophobicity and protein adsorption. Polysorbate 20 added as stabilizer to particle suspensions led to completely different behaviour of the particles: the particles showed altered protein adsorption patterns, dominated by immunoglobulins and especially by apolipoproteins. Furthermore, these particles were not phagocytized at all.

Conclusions. Surface hydrophobicity and phagocytic uptake in vitro as well as the interactions with plasma proteins of commercially available polystyrene particles were strongly affected by fluorescent labelling. Particles synthesised by Paulke remained unchanged after labelling. The results show the importance of thorough surface characterization for using particles in test systems *in vitro* and *in vivo*.

KEY WORDS: polystyrene particles; fluorescent labelling; phagocytic uptake; hydrophobicity; protein adsorption; 2-D PAGE.

INTRODUCTION

Colloidal carriers such as liposomes, emulsions, microspheres and nanoparticles have been studied as a means of delivering drugs to selected sites in the body (1–5). By far the greatest attention has been focused on the intravenous route. To target drugs intravenously to various sites of the body, it is necessary to avoid the recognition by the macrophages of the mononuclear phagocytic system (MPS). Cultures of phagocytic cells are employed to screen for intravenously injectable colloidal carriers potentially avoiding the MPS uptake *in vivo* (6–8). These *in vitro* test systems are used to study the effect of physicochemical properties, e.g. particle size, charge, surface hydrophobicity and the presence or absence of complement activating groups, on the phagocytic uptake (6). A prerequisite for such studies are particles well defined in their properties. Very often polystyrene "standard" particles are employed, for the ease of detection fluorescently labelled particles are broadly used. It is assumed that the fluorescence marker has none or little effect on the physicochemical surface properties and consequently on the phagocytosis. To study size effects on the uptake *in vitro* and *in vivo*, the differently sized particles—both non-labelled or labelled—need to possess identical properties such as surface hydrophobicity and charge.

It was reported that attachment of a fluorescent label on the particle surface or variation of particle size can alter the surface hydrophobicity (6). This can lead to altered particle-cell contact and thus to a different phagocytic uptake *in vitro*, depending on the extent of change in surface hydrophobicity. Simultaneously, in *in vivo* studies the interactions of i.v. injected particulate carriers with blood-components are likely to be different. Plasma protein adsorption strongly depends on the surface characteristics of the particles (9,10) and is regarded as the determining factor for their *in vivo* behaviour after i.v. administration ("differential adsorption"(11)).

The aims of this study were firstly to assess the extent to which the *in vitro* phagocytic uptake of non-labelled and labelled polystyrene particles differs and is affected by surface

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hydrophobicity. Different particle sizes were used. Secondly, the effect on adsorption of plasma proteins due to the presence or absence of a label was investigated. This is of particular interest for the performance of reliable *in vivo* studies with model particles. Thirdly, it was intended to minimize possible effects of labelling on the surface properties by modifying the production method of the particles, e.g. incorporation of a fluorescent marker in the particle volume.

MATERIALS AND METHODS

Polystyrene Particles

Non-biodegradable polystyrene particles were used as in vitro test system in order to avoid any influence of biodegradation (overview in Table I). Non-labelled and fluorescently labelled polystyrene latex particles with different diameters were purchased from Polysciences (Eppelheim, Germany). Furthermore, a range of polystyrene latex particles of different sizes was synthesised by Paulke at the Max-Planck-Institute (MPI): The non-labelled particles synthesised by Paulke with nominal diameters of 100 nm and 200 nm were prepared by classical emulsion polymerization, particles of 500 nm by emulsifier-free aqueous styrene polymerization (12,13). The variation of particle dimensions made it necessary to modify the method of synthesis. Rhodamine B was used as fluorescent label. The labelled polystyrene latex particles were produced with sizes similar to the non-labelled particles by emulsion polymerization in a water/ethanol mixture (3:1). The solvent was used to drag the label into the volume phase of the colloids. (1 mg label/g polymer). Purification procedures (dialysis and ultrafiltration) were employed to remove as many molecules as possible which were localized at the surface of the particles. During purification the latex particles lost surface charges by desorption of charged molecules leading to instability. Therefore, two of the first latexes had to be re-stabilised by the addition of polysorbate 20. In the further series the charge density at the surface was increased by increasing the polymerization temperature. Particle size was analysed using photon correlation spectroscopy (PCS) (Zetasizer 4, Malvern Instruments, Malvern, UK). Particle charge was quantified as zeta potential. The measurements were performed at a field strength of 20 V/cm in cell culture medium (Zetasizer 4, Malvern Instruments).

Cell Line and Culture Conditions

The human promyelocytic cell line HL60 was employed to study the phagocytic uptake of particles. These cells can differentiate into mature granulocytes by treatment with a variety of chemical agents (14–18). Here, retinoic acid (RA) was used (10^{-6} M, 5 day incubation). The cells were cultured in RPMI-1640 (Biochrom, Berlin, Germany) with addition of fetal calf serum (10%) (Biochrom) and 0.2% penicillin/streptomycin (Boehringer Mannheim, Mannheim, Germany). All other reagents according to (7) were purchased from Sigma-Aldrich GmbH (Deisenhofen, Germany).

Experimental Details

The differentiated HL60 cells were centrifuged (400 g, 7 min, 22°C), washed, counted and then adjusted with PBS to 2 $\times$ 10^6 cells/ml. Chemiluminescence (CL) was used to quantify the phagocytic uptake of particles as described previously (19). For the CL measurement 100 µl of the cell suspension were given into wells of microtitre plates and incubated with 100 µl luminol solution per well for 30 min (1×10^{-4} M in PBS). The background intensity at the end of this incubation period was typically below 0.1 arbitrary units of the luminometer. Then 100 µl suspension of polystyrene particles were added to each well. The CL intensity was measured within 240 minutes with an CL Amerlite Analyser (Amersham, UK). Plotting the CL intensity versus the time yielded intensity/time profiles. It could be shown that the AUC of the intensity/time profiles correlates with the total mass of polystyrene particles internalized (20). The shape of the profile provides information about the uptake kinetics, the area under the curve (AUC) about the extent of uptake (20,21). The AUC was calculated in arbitrary AUC units (arbitrary intensity unit of the luminometer $\times$ time in min).

Surface hydrophobicity was quantified by measuring the adsorption of the hydrophobic dye Rose Bengal on the particles at increasing surface area in the suspension as described previously (6). Suspensions with constant Rose Bengal concentration (20 µg/ml) but increasing latex particle concentrations were prepared. The aqueous phase and the surface of the particles are considered as two phases. Rose Bengal undergoes partitioning between these phases. At each latex concentration the partitioning quotient (PQ) was calculated as

Table I. Non-Labelled and Rhodamine B Labelled Polystyrene Particles Used in the Study

	Non-labelled particles		Fluorescently (F) labelled particles		
Size [nm]	Purchased from Polysciences (PS)	Synthesis by Paulke (P)	Purchased from Polysciences (PS)	Synthesis by Paulke (P)	Synthesis by Paulke, + polysorbate (P)
100	PS100 [101nm, PI: 0.09]	P100 [108nm, PI: 0.02]	PSF100 [109nm, PI: 0.09]	PF100 [120nm, PI: 0.02]	PFP100 [108nm, PI: 0.09]
200	PS200 [202nm, PI: 0.08]	P200 [228nm, PI: 0.09]	PSF200 [210nm, PI: 0.11]	PF200 [220nm, PI: 0.04]	PFP200 [214nm, PI: 0.10]
500	PS500 [499nm, PI: 0.11]	P500 [536nm, PI: 0.06]	PSF500 [481nm, PI: 0.15]	PF500 [481nm, PI: 0.16]	—

Note: The actual particle size and the polydispersity index (PI) measured by PCS are added in brackets.

$$PQ = \frac{\text{amount Rose Bengal bound on surface}}{\text{amount Rose Bengal in dispersion medium}}$$

and plotted versus the increasing surface area. The slopes of the obtained straight lines are a measure of the degree of surface hydrophobicity. The slope increases with increasing surface hydrophobicity.

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was used to determine plasma protein adsorption patterns on the particles as described by Blunk (9,10). To analyze the plasma protein adsorption, particle suspensions containing constant surface areas were incubated in citrate stabilized human plasma for 5 minutes at 37°C. After separation and washing of the particles the adsorbed proteins were washed off the surface by a protein solubilizing solution (22), applied to the 2-D PAGE and processed like normal human plasma according to the procedure described by Hochstrasser et al. (22). In the first dimension (isoelectric focusing) the proteins were separated only according to their isoelectric points, in the second dimension (SDS-PAGE) the separation was solely based on the molecular weights. After SDS-PAGE the gels were silver-stained and scanned with a laser densitometer (Personal Densitometer, Molecular Dynamics, Krefeld, Germany). Data was processed with the help of an automated computer system (23). Experimental details of 2-D PAGE analysis can be found in previous publications (10,22). As each protein from human plasma appearing on a gel has its own well defined coordinates, the proteins can be identified by matching the obtained gels to the master map of human plasma (24,25).

RESULTS

The phagocytic uptake by HL60 cells of commercial labelled and non-labelled particles purchased from Polysciences is plotted in Figure 1. Particles labelled with the fluorescence marker showed only about 10% of the phagocytic uptake of unlabelled particles. The AUC values of the labelled particles were still distinctly higher (appr. factor 5) than for the non-incubated control (= CL-intensity originating from cell suspension in the absence of particles). This proves a weak phagocytic uptake of these particles.

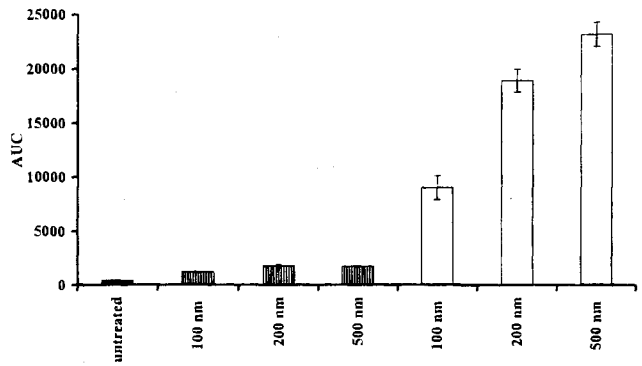

Fig. 1. Phagocytic uptake of polystyrene particles (Polysciences) monitored as the AUC of the chemiluminescence intensity/time curve (Rhodamine B labelled particles (hatched bars) and non-labelled polystyrene particles (open bars) of 100 nm, 200 nm and 500 nm diameter, control: AUC of untreated differentiated HL60 cells, i.e. not incubated with particles (full bar)).

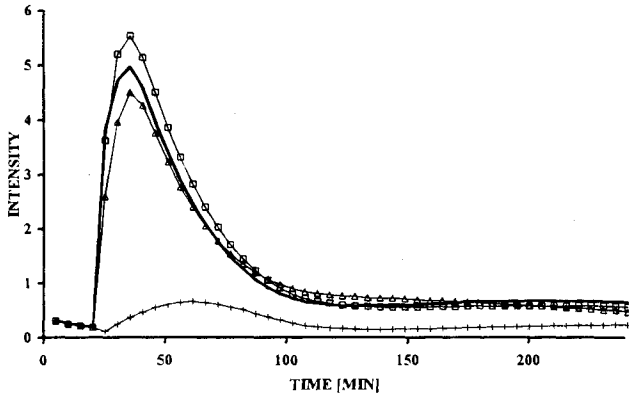

Fig. 2. Phagocytic uptake: Chemiluminescence intensity versus incubation time of 500 nm polystyrene particles, non-labelled (PS500—Polysciences (−) and synthesised by Paulke P500 (□)) and fluorescently labelled with Rhodamine B (PSF500—Polysciences (+) and synthesised by Paulke PF500 (△)).

The uptake of the commercial particles was compared with the internalization of particles synthesised by Paulke-both non-labelled and labelled. As example, the intensity/time profiles are shown for the 500 nm particles (Fig. 2, P500 and PF500). The particles synthesised by Paulke showed no differences with respect to the kinetics of phagocytic uptake by HL60 cells and only a minor difference in the resulting AUC. The phagocytic uptake of the labelled particles was slightly less (PF500).

In contrast, the two commercial particle types showed clear differences. The non-labelled particles (PS500) behaved similar to the polystyrene particles produced at the MPI. However, the fluorescent particles PSF500 showed a delayed and extremely reduced phagocytosis by HL60 cells. An intensity maximum of phagocytic uptake could hardly be defined. Similar results were obtained for the other sizes (data not shown).

Surface hydrophobicity was measured using the Rose Bengal partitioning method. The slope of each straight line is a parameter for the surface hydrophobicity: The steeper the slope, the more hydrophobic is the surface (26). The non-labelled particles (PS500, P500) and PF500 were relatively hydrophobic as indicated by steep slopes (Fig. 3). In contrast, the commercial

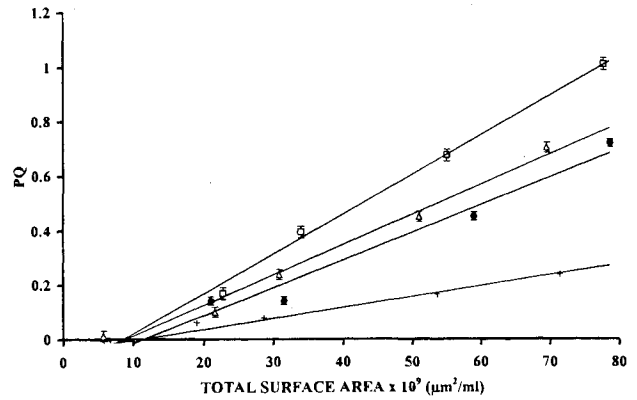

Fig. 3. Plot of partitioning coefficient (PQ) of Rose Bengal at increasing surface area of 500 nm polystyrene particles, non-labelled (PS500—Polysciences (●) and synthesised by Paulke P500 (□)) and fluorescently labelled with Rhodamine B (PSF500—Polysciences (+) and synthesised by Paulke PF500 (△)).

fluorescent latex (PSF500) showed a very low slope (Fig. 3). Its surface must be very hydrophilic resulting in weak adsorption of Rose Bengal. Similar results were also obtained for the 100 nm and 200 nm particles.

The particles with polysorbate 20 added were considered as very hydrophilic due to adsorption of the surfactant onto the surface of the particles (6). Determination of the surface hydrophobicity using the Rose Bengal partitioning method is not reasonable in this case, because a displacement of adsorbed surfactant by the hydrophobic dye is likely to occur leading to distortion of the measured degree of hydrophobicity (6).

Investigation of the phagocytic uptake of the particles synthesised by Paulke by HL60 cells yielded an increasing uptake with increasing particle size from 100 nm to 500 nm. The data proved that no differences existed between non-labelled and labelled ones of identical size (P, PF, Fig. 4). However, particles produced in the presence of polysorbate 20 (PFP, Fig. 4) showed no uptake at all—independent of the size. The AUC values were not significantly different from the AUC of the control cells not incubated with particles (Fig. 4).

The adsorption of plasma proteins was studied using 2-D PAGE. Quantitative assessment of the total protein amount is expressed in arbitrary units, the values for the single proteins as percentage of the overall amount of detected proteins (%).

The protein adsorption on the particles with polysorbate 20 added differed very much from the other labelled particle types. The overall detected amounts of proteins on the 2-D gels of PFP100 and PFP200 (1896 and 1901 arbitrary units, respectively) were markedly reduced when compared to the amounts on the particle types which were polymerized without any addition of surfactant (P and PF, mean amount of detected proteins: 4278 ± 348 arbitrary units). Fibrinogen was the dominant protein on all particles produced without addition of surfactant. It accounted for almost 50% of the overall detected amounts of proteins. On the contrary, it was found to be strongly reduced on the particle types stabilized by polysorbate 20, i.e. PFP100 and PFP200 (2.4% and 2.3%, respectively). The adsorption patterns of these particle types were dominated mostly by the immunoglobulins (IgG and Ig light chains) and the apolipoproteins, especially apoA-I, apoA-IV and apoJ were preferentially adsorbed. The apolipoproteins accounted for more than 50% of the overall detected amount of proteins.

To assess the influence of the fluorescent labelling on the plasma protein adsorption on particle surfaces, commercially labelled particles were compared with unlabelled particles of the same manufacturer. Additionally, labelled and unlabelled particles synthesised by Paulke were investigated. The size of the different particles were in the same order of magnitude (appr. 500 nm). Table II lists the data resulting from these protein adsorption studies.

In Figure 5 close-ups on the 2-D gels (pI 4–8 and MW 40–120 kDa) resulting from the analysis of commercial particles are compared. The labelled particles PSF500 showed a markedly different adsorption pattern as compared to that of the unlabelled commercially available particles PS500. Fibrinogen was the dominant protein on the unlabelled particles as on all other unmodified polystyrene particles being analysed in this study. It accounted for 34.7% of the overall amount of adsorbed proteins. In contrast to that, the fraction of fibrinogen on the labelled commercial particles was strongly reduced to 9.9% of the detected protein amount. PLS:6 (a yet unidentified protein named after (25)) was also reduced from 14.1% on the unlabelled to 5.3% on the labelled particles. Furthermore, on the gel of the commercially labelled particles some spots appeared which were hardly or not present on the gel of the unlabelled particles. For example, a chain of unidentified spots (no. 5, Fig. 5B) accounted after all for 8% of the overall detected amount of proteins. These spots were also detected by 2-D PAGE on various types of fat emulsions (27, T. Blunk. unpublished data). The coordinates of spot no. 6 (Fig. 5B) were identical to those of an unknown protein named U2 (24). The spots no. 7 and no. 8 (Fig. 5B) could not be identified by matching the gel to the master map of human plasma proteins (24,25). To assure that these spots did not result from compounds of the particle suspension, the particles were analysed by 2-D PAGE without incubation in plasma. No spots could be detected on the resulting gels. Thus, it is extremely likely that the unknown as well as the unidentified protein spots were

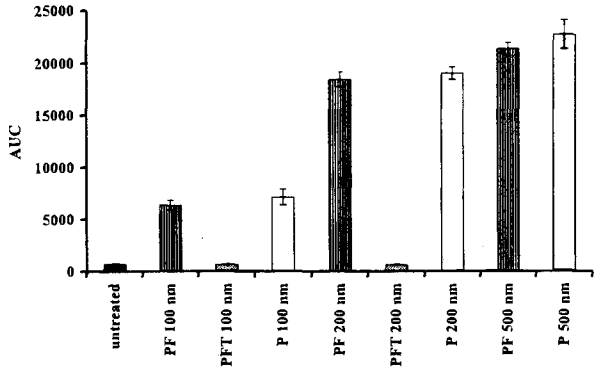

Fig. 4. Phagocytic uptake of particles synthesised by Paulke: AUC of the chemiluminescence intensity/time profiles of differently sized non-labelled (P100, P200, P500, white bars) and Rhodamine B labelled particles produced without polysorbate 20 (PF100, PF200 and PF500, hatched bars) and in the presence of polysorbate 20 (PFP100 and PFP200, dotted bars), control: AUC of untreated differentiated HL60 cells, i.e. not incubated with particles (full bar).

Table II. Adsorption of Main Proteins on Polystyrene Nanoparticles Purchased from Polysciences (PS) and Synthesised by Paulke (P)

	PS500		PSF500		P500		PF500	
		±		±		±		±
total amount	1901	78	932	66	3915	187	3459	209
Albumin	6.5	0.38	5.3	0.63	4.7	0.31	6.9	0.79
Fibrinogen	34.7	1.50	9.9	0.69	39.4	1.26	43.7	2.52
PLS:6	14.1	0.54	5.3	0.09	9.6	0.17	9.6	0.71
IgG	7.5	0.93	13.2	1.05	13.7	0.70	9.5	0.42
lg light chains	9.4	0.7	5.9	0.50	5.9	0.45	4.3	0.43
U2	0.2	0.03	4.1	0.08	2.9	0.16	2.4	0.12
unidentified 1	0	0	8.0	0.69	0	0	0	0
unidentified 2	0	0	3.7	0.03	0	0	0	0
unidentified 3	0	0	5.8	0.32	0	0	0	0

Note: The particles were non-labelled (PS500 and P500) and Rhodamine B labelled (PSF500 and PF500). The total protein amount is expressed as arbitrary units, the value of a single proteins as percentage of the overall detected protein amount. The values are the means of two experiments.

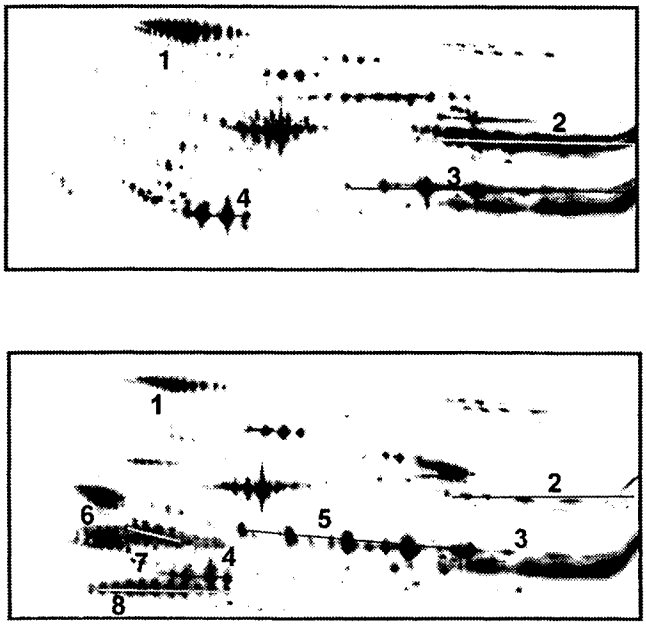

Fig. 5. Protein adsorption on commercial polystyrene particles (appr. 500 nm): (A—unlabelled PS500, upper; B—fluorescently labelled PSF500, lower). Close-ups of the part of the gels which ranges from pI 4,0 to 8,0 (from left to right, not linear), and approximately from MW 40 kDa to 120 kDa (from bottom to top, not linear). (1) PLS:6, (2) Fibrinogen α, (3) Fibrinogen β, (4) Fibrinogen γ, (5) unidentified 1, (6) U2, (7) unidentified 2, (8) unidentified 3.

plasma proteins adsorbed onto the particle surface. Further studies including electroblotting and N-terminal microsequencing are necessary to identify these protein spots.

In Figure 6 close ups (scales according to Fig. 5) of the relevant parts of the 2-D gels of 500 nm non-labelled (P500) and labelled particles (PF500) are shown. Both particle types were synthesised by Paulke. The adsorption patterns of the two particle types were found to be very similar. The data for the main proteins listed in table II underline this observation. For all other particle types produced at the MPI similar results were obtained except for the particle suspensions containing polysorbate 20 which have already been described.

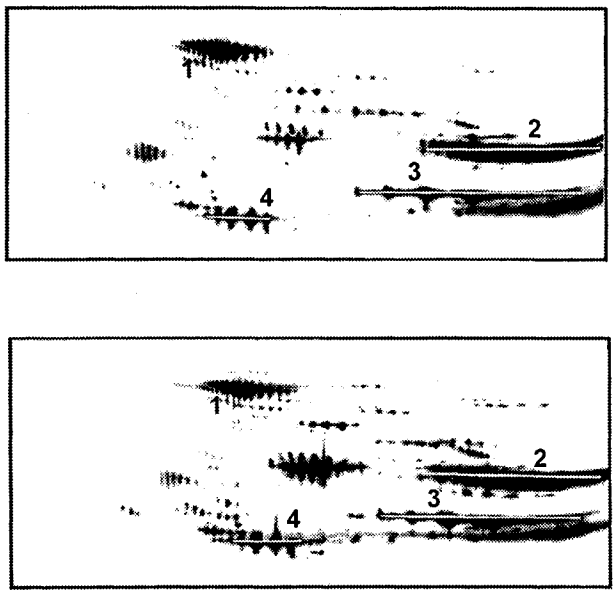

Fig. 6. Protein adsorption on polystyrene particles synthesised by Paulke (appr. 500 nm): (A—unlabelled P500, upper; B—fluorescently labelled PF500, lower). Scales of gel are according to figure 5. (1) PLS:6, (2) Fibrinogen α, (3) Fibrinogen β, (4) Fibrinogen γ.

DISCUSSION

The phagocytic uptake of particles in vitro and in vivo has been reported to depend on surface properties, surface hydrophobicity previously being identified as a very important parameter (28,29). An explanation for the marked differences in total uptake (Fig. 1) by HL60 cells and uptake kinetics of commercial non-labelled and labelled particles (Fig. 2) are, therefore, possible changes in their surface properties due to the attachment of the fluorescent label.

The aim of the synthesis by Paulke was to create particles with identical or at least very similar surface properties. From the similar uptake of these particles—labelled and non-labelled—it appears likely that this goal has been achieved. A possible explanation for the obtained results are differences in the methods of particle synthesis. One approach is the polymerization of the plain polystyrene particles, in a second production step the surface is modified, e.g. by introduction of functional groups on the surface or by attachment of a fluorescent label. Marked differences in the surface hydrophobicity of non-labelled and fluorescently labelled commercial particles were reported previously including also batch to batch variations (6). The production method of the particles synthesised by Paulke was optimized to include as much as possible of the fluorescent label inside the particle core, the surface contamination by the fluorescent marker was reduced by intensive purification. Assuming identical production methods, the observed differences in surface properties might be due to inefficient purification of the particle suspension.

The differences in surface hydrophobicity expected from the uptake behaviour could be confirmed by the Rose Bengal measurements. The first step in phagocytosis of particles is the adsorption onto the cell surface by hydrophobic interactions. Only after this adsorption the phagocytes begin to form pseudopods around the particle (30). Compared to the non-labelled commercial particles (PS500), the labelling creates a less hydrophobic surface and subsequently leads to reduced attachment to the cell membrane and reduced phagocytic uptake (Fig. 1). The particles synthesised by Paulke (P500 and PF500) were more hydrophobic than the commercial ones. However, PF500 particles were slightly less hydrophobic than P500 particles which might be possibly explained by remaining Rhodamine B anchored into the surface during the polymerization process which could not be removed by cleaning. Despite the differences in surface hydrophobicity between PS500, P500 and PF500, the phagocytic uptake by HL60 cells showed only little difference (Fig. 1 and 3). This can be explained by a "threshold" surface hydrophobicity, above which particles are strongly taken up and differences in hydrophobicity have no detectable effect (19). Less hydrophobic particles showed a reduction in uptake, pronounced when approaching very low slopes (Fig. 2, PSF500). Similar large differences between relatively hydrophobic particles (e.g. P500 and PF500) have less effect

on the uptake. This seems to confirm the existence of a threshold of hydrophobicity (6). A minimum hydrophobicity is required for a strong uptake. A similar effect has been reported for the adsorption of proteins. Above a contact angle of 60°, proteins bind to surfaces to the same extent (31).

In general, it could be observed that phagocytic uptake by HL60 cells increased with increasing particle size. This is consistent with results obtained in other studies (7,8). Measured similar surface hydrophobicities are well in agreement with similar or identical phagocytic uptake of particles of identical size measured *in vitro* (Fig. 1 and 3). A charge effect appeared less likely, all particles possessed a zeta potential of about −35 mV (measured in the cell culture medium). It confirms the importance of the surface hydrophobicity as a determining factor for phagocytosis. Consequently, it highlights the importance that particles of identical surface properties are used for such studies.

The two batches stabilized by the addition of polysorbate 20 were not taken up by the cells. This surfactant adsorbs onto the surface of polymeric particles (e.g. polyhydroxybutyrate) leading to a more hydrophilic surface (6) and consequently to a reduction of phagocytic uptake. *In vivo* darlagin-loaded polysorbate 80-coated nanoparticles were found to show an increased analgesic effect, indicating enhanced transport of the drug through the blood-brain barrier and some avoidance of the liver macrophages (32). This was also attributed to the hydrophilic surface and a preferential adsorption of plasma proteins (publication in preparation).

Previously, preferential adsorption of plasma proteins on nanoparticles was shown (9,10), i.e. some proteins were enriched on the particle surface, others were diminished, as compared to bulk plasma. The same applied to the particles presented in this study. Addition of a surfactant after particle synthesis or introduction of a fluorescent label—in the case of commercial particles—led to distinct alterations of the protein adsorption patterns compared to non-labelled, polysorbate-free polystyrene particles.

The addition of polysorbate 20 after particle production lead to a coating of the particle surface resulting in completely altered surface characteristics, e.g. decreased surface hydrophobicity. Thus, the minimized hydrophobic interactions between surface and proteins contribute considerably to a decrease of the total amount of proteins adsorbed. In addition, the PEG chain of 20 units in the polysorbate molecules creates a steric barrier for protein adsorption. The "windscreen wiper" effect of the moving hydrated chains hinders the approach of protein molecules to the surfaces (33,34). This effect is less pronounced as compared to poloxamer polymers possessing longer chains (9,10) but still significant enough to reduce the total amount of adsorbed proteins by appr. 60%. Thus, the reduced interactions with plasma proteins were plausible.

Differences in surface hydrophobicity and in the adsorption behaviour of plasma components on these particles are likely to be of relevance for the observed reduction in phagocytic uptake by HL60 cells. In cell cultures containing serum a difference in the uptake was observed depending whether opsonic factors were present on the particles or not (e.g. comparison between active and inactivated serum in the culture medium (35)). The phagocytic uptake will be affected if opsonic factors are adsorbed (hydrophobic particles) or not adsorbed onto the surface (hydrophilic particles). The presence or absence of particular proteins adsorbed on the surface of PSF and PFP particles might also explain the observed phagocytosis of these particle types. In contrast to the PFP particles, the PSF particles were still considerably phagocytized by HL60 cells, although the PFP particles had the double amount of adsorbed proteins. Thus, the qualitative composition of the protein adsorption pattern seems to be more important with regard to phagocytosis than the total amount of adsorbed proteins. Probably, this is also valid for circumvention of MPS recognition *in vivo*.

Some commercial products contain surfactants as stabilizers, sometimes the manufacturers even refuse to disclose the chemical nature of the surfactant used. This is, of course, a possible source for the creation of artefacts in biological testing.

Further, it could be established that the surface properties of the commercial particles determining the plasma protein adsorption were strongly affected by the fluorescent labelling. This probably explains the completely different phagocytic uptake by HL60 cells and uptake kinetics of the commercial labelled and non-labelled particles (Fig. 1). It highlights the importance of the particle properties remaining unchanged during labelling. The polysorbate-free particles synthesised by Paulke did not differ in their surface properties hydrophobicity and zeta potential when compared to non-labelled particles. This resulted in very similar phagocytic uptake in the cell culture (Fig. 3). Obviously, the relevant properties determining the phagocytic uptake *in vitro* remained during labelling without distinct change. This was supported by the very similar patterns of adsorbed proteins—the determining factor for the *in vivo* fate of i.v. administered particles.

The characterization data provided by commercial manufacturers—in general mean size and width of size distribution, sometimes charge density—are not sufficient when employing such particles in biological studies.

The inclusion of an additional parameter, surface hydrophobicity, is suggested.The results exemplify the extent to which the protein adsorption pattern can be changed by pure chemical modification of the surface. Thus, it may be possible to create site-specific drug carriers by a chemical modification process instead of coating procedures.

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