SUPPLEMENTAL INFORMATION.

EXPERIMENTAL METHODS

Light scattering studies of PEG-avidin. The experimental pI's of avidin and PEG-avidin were obtained as described earlier with agarose gels. Avidin, 2P-avidin and 1P-avidin was mixed with pGL3 at different N/P ratios: 3, 5, 7, and 10. Samples were formed by adding equal volumes of pGL3 to protein at the respective N/P in water and incubated at RT for 15 min. Salt (1 mL, 20 mM HEPES, 150 mM NaCl, pH 7.4) was added and incubated for 10 min with each sample. Dynamic light scattering was used to measure particle diameters.

Cell transfection studies of PEG-avidin. MDA-MB-231 cells were plated at 300 000 cell/well in 6-well plates. Particles were formed at N/P 3, 5, 7 and 10, each formulated with 2 µg of pGL3/well. Particles were incubated with regular DMEM (10% FBS, 1% penicillin/streptomycin) and as needed, 100 µM chloroquine (CQ) per well was added to the media after adding the complexes. The transfection media was aspirated after 4 hrs and replaced with fresh DMEM. Plates were assayed the next day with a luminometer and readings were normalized to total protein content.

RESULTS AND DISCUSSION

Synthesis and characterization of PEG-avidin. 1 and 2PEG chains were randomly conjugated to chicken avidin (1). We confirm that avidin can condense pGL3 into nanosized particles (Figure 1), as has been previously reported before (2). Avidin, 2P-

avidin or 1P-avidin was formulated with pGL3 at N/P 3, 5, 7 and 10 and its aggregation behavior was measured with DLS after 10 min of incubation under salt conditions (Figure 2). Avidin nanoparticles tended to aggregate from N/P 3 to 10, with average intensity-weighted diameters of 178 nm to 335 nm. On the other hand, the diameters of 2P and 1P-avidin conjugates stayed below 200 nm throughout the N/P range tested (1P-avidin began at 147 nm and increased to 163 nm; 2P-avidin went from 105 nm to 123 nm). As comparison, for PEI at N/P 3 the diameter was 388 nm and decreased to 245 nm at N/P 10. Both PEG-avidin conjugates did not aggregate in salt solutions and could still form nanometer-sized particles suitable for transfecting cells.

Chicken avidin is a glycosylated tetrameric protein with an isoelectric point pI 10.6 (3). Therefore, at physiologic pH, this protein is highly charged. Morpurgo et al. have reported that the avidin-DNA interaction might involve more than electrostatics (2). Their study demonstrated that avidin could interact with pDNA in a specific manner, even under extremely high salt conditions. They found that lysozyme (pI 11) could also condense pDNA electrostatically, but that the interaction fell apart under high salt conditions. It was therefore important to investigate the potential for these particles to mediate gene expression.

Luciferase expression with PEG-avidin nanoparticles. Although avidin can interact with pGL3 to form salt stable nanoparticles, complexes could not mediate gene expression in the absence (Figure 3a) of PEI (250 ng, N/P<1) and chloroquine (CQ). When CQ alone was added to wells after transfecting with PEG-avidin particles, gene expression was inconsistent from well to well. 2P-avidin appears to transfect cells better than the other

conjugates, but these results were not consistent from well to well or repeatable (Figure 3c). When PEI (250 ng, N/P <1) alone was added following formulation of avidin nanoparticles, low gene expression was still observed (Figure 3d). As shown, note that only upon addition of PEI and CQ did we obtain consistently higher levels of luciferase expression.

PEI's mechanism of toxicity to cells has been attributed to its cell membrane-disrupting properties (4, 5). The small amount of PEI added to pre-formed avidin particles might have been sufficient to cause membrane disruptions, leading to increased cellular uptake of avidin particles and possibly facilitating escape into the cytoplasm. Avidin nanoparticles alone cannot buffer endosomes and are rapidly degraded by the acidic conditions and enzymes present in the cell. CQ is an endosomal buffering agent which is often used to neutralize the acidification of endolysosomes. Endosomal fate is believed to be pH-dependent and this can therefore affect cargo trafficking in the cell (6-8). In the absence of acidification or membrane disrupting PEI, avidin nanoparticles could not mediate gene expression (Figure 3a).

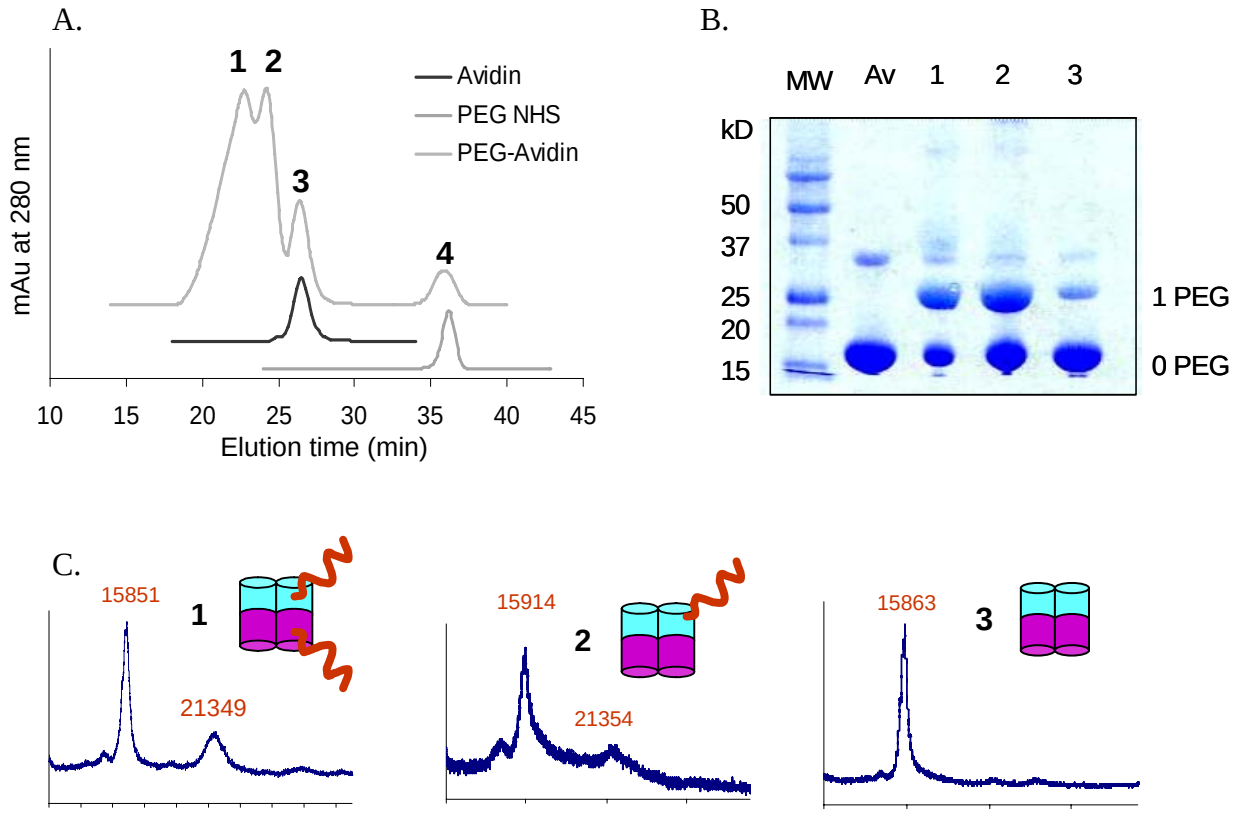

Figure 1. Purification and characterization of 2P and 1P-avidin. (a) SEC elution profile for PEG-avidin reaction, where peak 1 = 2P-avidin, peak 2 = 1P-avidin, peak 3 = avidin and peak 4 = PEG NHS groups. (b) SDS-PAGE gel of each corresponding peak reveal presence of major and minor bands either at ~18 kDa for avidin subunits or ~25 kDa corresponding to 1PEG chain conjugated to an avidin subunit. (c) MALDI-TOF MS analysis confirm conjugation of 1PEG chain on subunits of avidin for the fractions collected for 2P and 1P-avidin.

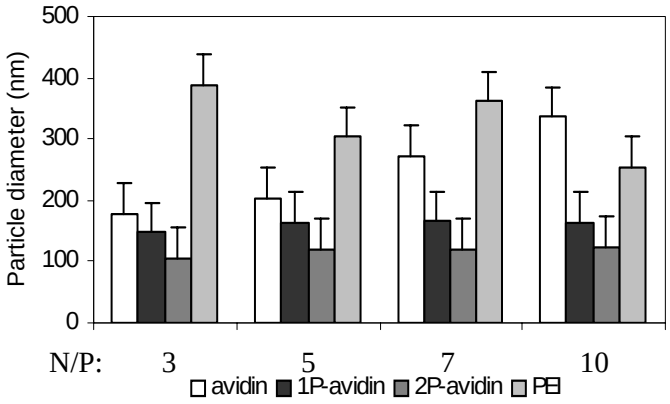

Figure 2. Investigating the salt stability of avidin and PEG-avidin nanoparticles. Protein conjugates were mixed with 1 µg of pGL3 in water at each respective N/P 3, 5, 7, 10. Diameters were measured after 10 min incubation in salt (20 mM PBS, pH 7.2) (n = 3).

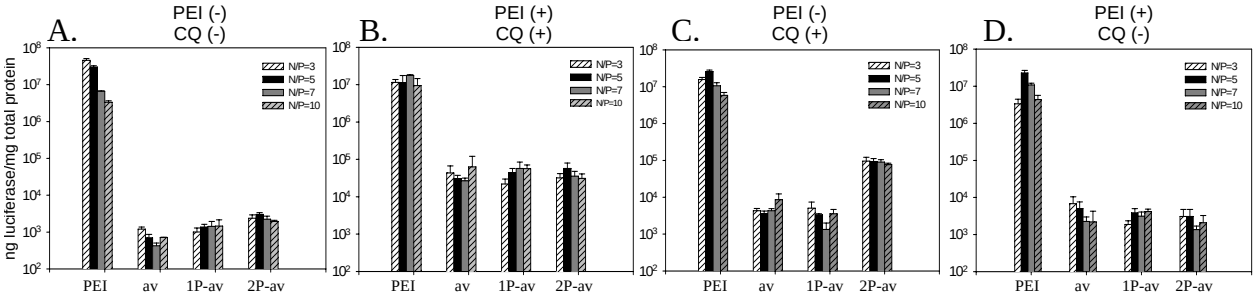

Figure 3. Transfection for MDA-MB-231 cells with PEG-avidin conjugates in serum-media. 250 ng PEI and 100 µM CQ presence in the media are represented, where (-) is absence and (+) is presence. (A) No PEI, no CQ (B) PEI present, CQ present (C) No PEI, CQ present (D) PEI present, no CQ. Conjugates were formed at N/P 3, 5, 7 and 10 using 2 µg DNA/well; n=3. Key: av = avidin, 1P-av = 1P-avidin, 2P-av = 2P-avidin

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