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Carrier systems in PDT: On the way to novel anti-tumor drugs

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Abstract

A novel mode to apply photosensitizing drugs specifically to tumor tissue using the principles of polyphasic tumor therapies is lined out. Key compounds are tumor specific functionalized antibodies with reduced immunogenicity. These bind to drug inclusion complexes in a multiplicative manner. Drug inclusion complexes are designed on the basis of tethered functionalized ß-cyclodextrin dimers with maximum affinity to porphyrinoid photosensitizers forming monomeric chlathrates. To enhance porphyrin-cyclodextrin interaction peripheral groups of the porphyrin have to be chemically modified. The development of the method is not yet completed. First results are demonstrated.

Introduction

In a tumor-bearing organism there are two general routes by which photosensitizers find their target tissue: water soluble sensitizers (e.g. the polysulphonated m-tetraphenyl porphines or phthalocyanines) enrich in tumor tissue according to pH-equilibria of their differently ionized forms (1). Apolar photosensitizers use the lipoprotein pathway to be taken up as complexes via the lipoprotein receptors of the tumor cells (2). Both these pathways allow for targeting of healthy organs and lead to the wellknown side effects of PDT as well as of general chemotherapy.

To avoid these side effects we started with the simple consideration to construct a porphyrinoid -containing *inert drug which cannot be taken up by any living cell.* Such a drug should be functionalized in a manner that it can be bound exclusively to tumor cells. The stability of such a drug should be sufficiently high to prevent interaction with the known distribution pathways.

Water soluble complexes: cyclodextrins

Cyclodextrins are wellknown to form soluble complexes with several water-insoluble drugs (3). For example, α-cyclodextrin is admitted as an aid to solubilize prostaglandin E 1 for injections, γ-cyclodextrin has been used successfully to solubilize tin etio-purpurin for PDT purposes (4). In general, aggregates of apolar photosensitizers dissolve in cyclodextrin solutions into monomeric host-guest complexes (fig. 1). Highly symmetrical apolar phthalocyanins and porphycenes achieve an orientation towards the substructures of the cell membrane, and this results in a greatly enhanced uptake velocity by the cell (fig. 2). Despite the considerable stability of the 1:1 host-guest complexes (fig. 3) an interaction with the lipoprotein pathway is pre-programmed (4).

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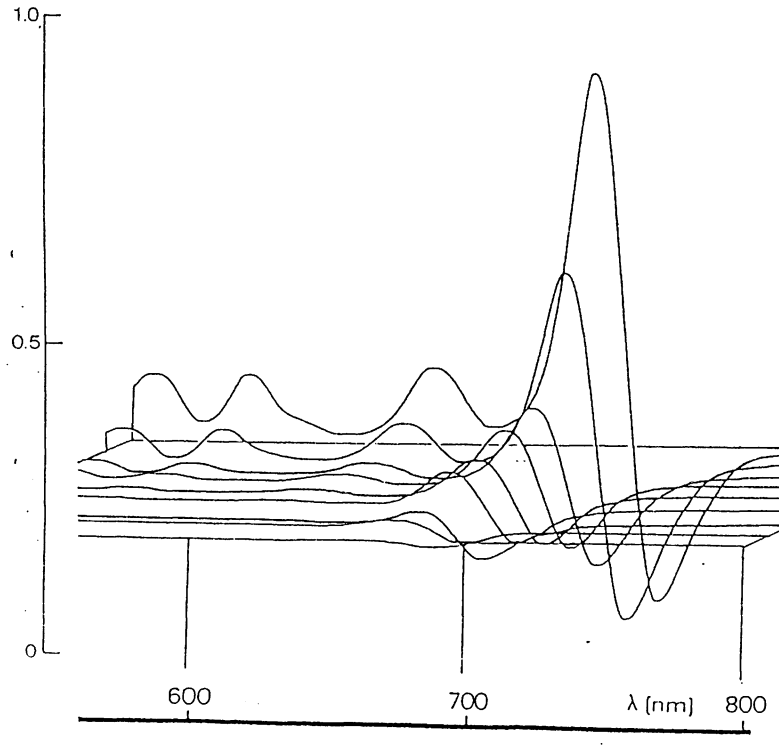
Fig.1 Disaggregation equilibria of pheophorbide a at rising CD concentrations.



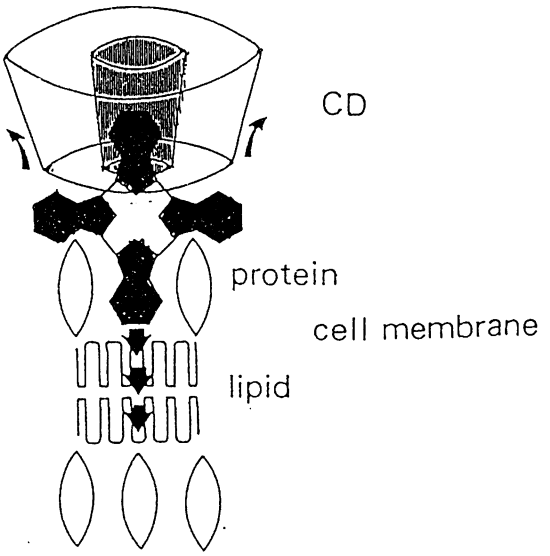


fig.2 Acquiring polarity by binding to CD of an apolar sensitizer, phthalocyanine.

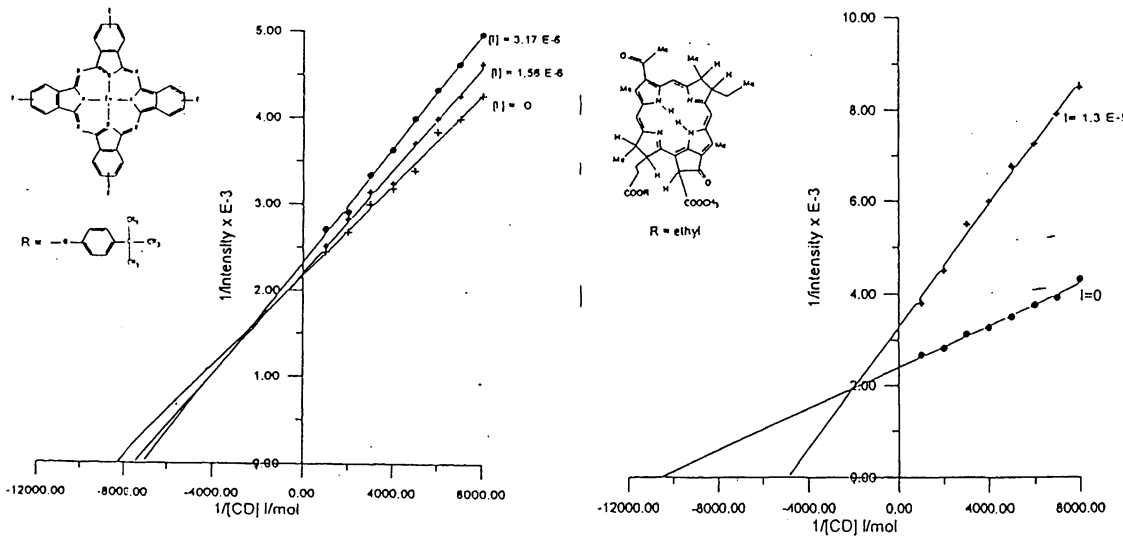


fig. 3 Association equilibria of tetra-t-butyl-phenoxyphthalocyanine and BPEE as revealed by TNS-exclusion fluorescence technique. K_D (TTBP) = 14.8 x 10^{-6}M
K_D (BPEE) = 8.3 x 10^{-6}M

Stabilization of the complex: cyclodextrin oligomers

As a signpost towards stable complexes, BRESLOW and CHUNG (5) investigated the complexation of a very simple symmetrical diester, propane diol p-tert.butyl benzoic acid diester, with a dimeric "clamshell" ß-cyclodextrin. The stability of the complex exceeded the expected value by two orders of magnitude and resulted in a $K_D = 10^{-10}M$ comparable with the complex stability between antibodies and their antigens. In the mean time several other groups constructed cyclodextrin dimers mainly to study and to mimick enzyme-like activities. Our first model of a more flexible cyclodextrin dimer to complex porphyrinoid compounds (6) has been reconstructed to a box-of-bricks principle (fig. 4): starting from tosylated ß-cyclodextrin (at positions 2 or 6) we substitute by nucleophilic attack with alkyl diamines of short chain length and connect the resulting N-alkyl amines with homobifunctional reagents to form ß-cyclodextrin dimers with variable extension including the possibility to introduce preformed break-points to finally break the complex at the target site.

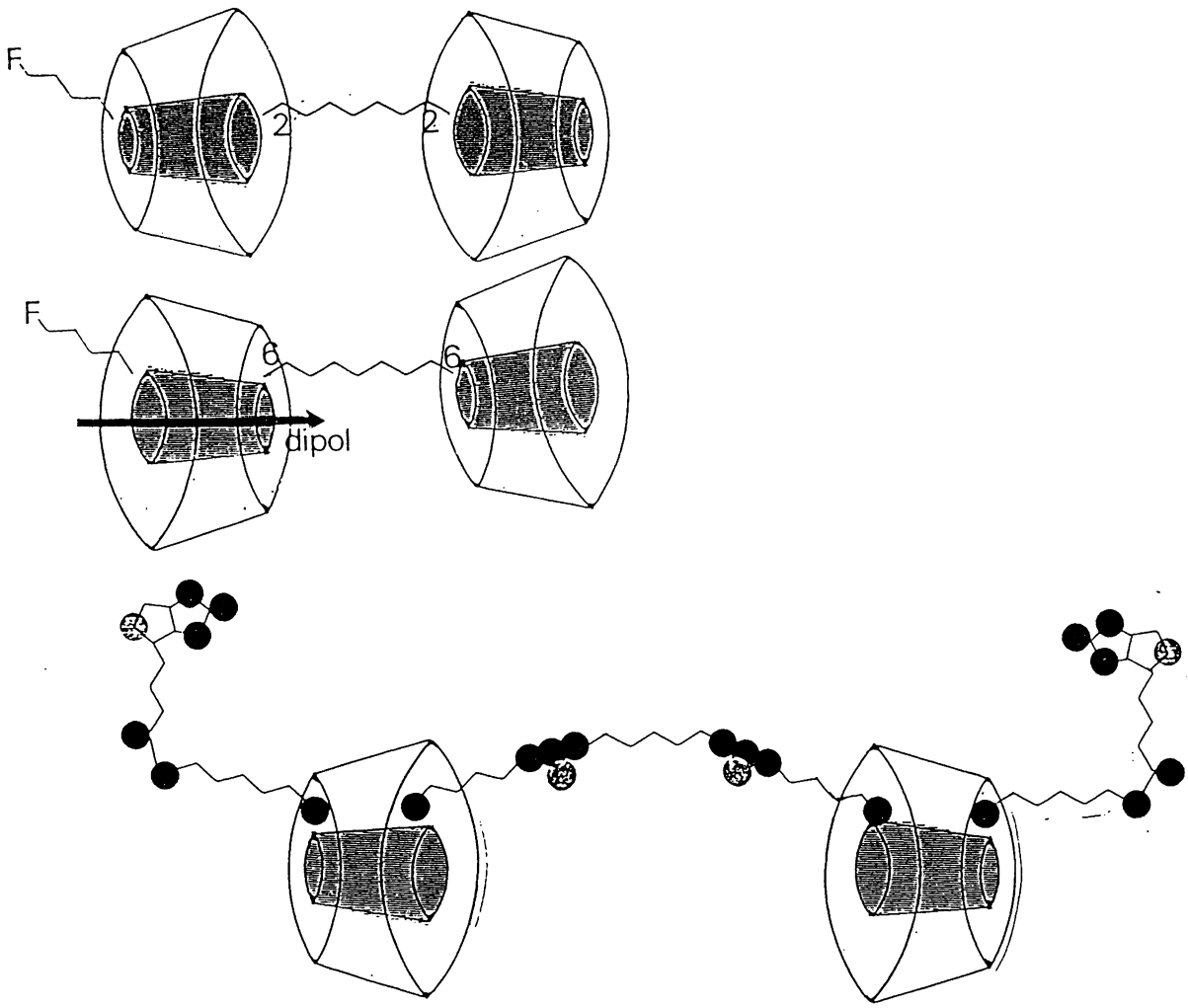


fig. 4 Orientation of CD's against guests and bridge forming modalities to achieve optimum binding

High affinity complexes: anchor structures

Porphyrins normally contain very short side chains which allow only poor interactions with the extended lipophilic cavity of ß-cyclodextrin. More voluminous side chains, like the p-tert.butyl phenyl group, may allow for much higher stability of the host-guest complex, and by the same time may enhance the dipole momentum of the included drug (7). At the moment we try to substitute pheophorbide a in C-3[1] with a p-tert.butyl phenyl ether according to the general method given by PANDEY et al (8) in order to better understand anchor functions (fig.5).

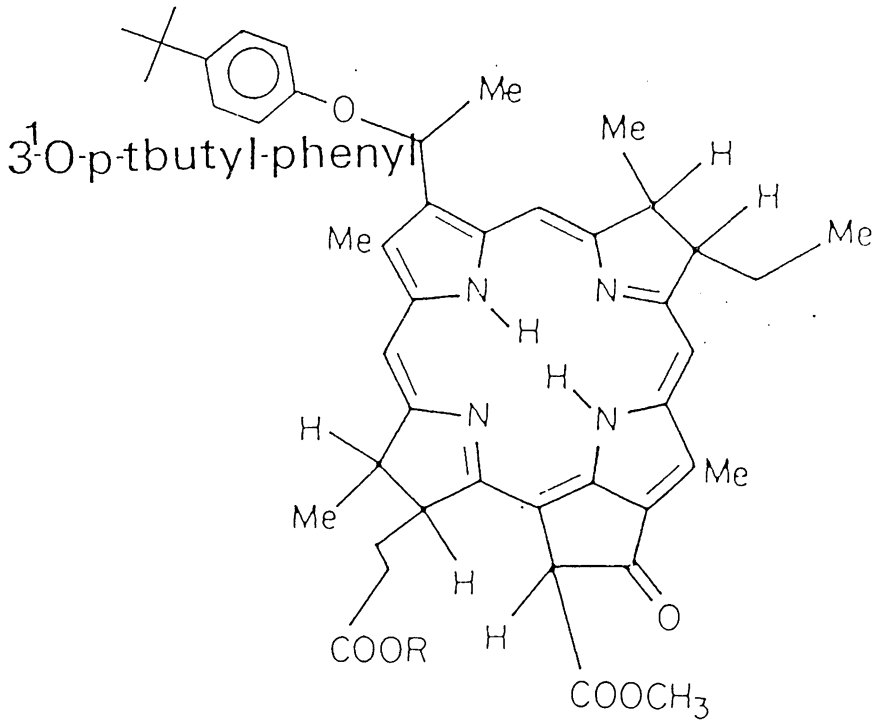


fig. 5 Substitution of apolar side chains may enhance complex stability

Functionalization of the complex: biotin-avidin complexes

The biotin-avidin system is wellknown to improve considerably the tumor localization by radioscintigraphic methods (9).Thus, biotinylation of the drug-cyclodextrin complexes as well as of monoclonal tumor-localizing antibodies is investigated in our group. An anti-CEA antibody, BW 431/26, has been biotinylated successfully with up to 7 biotin molecules without loosing its affinity to the antigen localized on the surface of LoVo adenocarcinoma cells. Biotinylation of ß-cyclodextrins in position 6 over a C_6-spacer was easily achieved using the methods yet described.

Immunological problems: immunogenicity of foreign proteins

The introduction of foreign proteins, avidin from hen's egg white and mouse monoclonal antibodies, into the system raises immunological problems. BOSSLET et al.(10) circumwent the problem by constructing an ingenious fusion protein to be applied with the Antibody Directed Enzyme Prodrug Therapy (ADEPT) splitting an adriamycin glucuronide at the target site by a humanized antibody-ß-glucuronidase complex. This is beyond our today facilities.
So we study the reduction of immunogenicity by coupling our proteins with unifunctional m-polyethylene glycols (11) which in a modified form may be used as additional centers to accumulate the drug complexes.

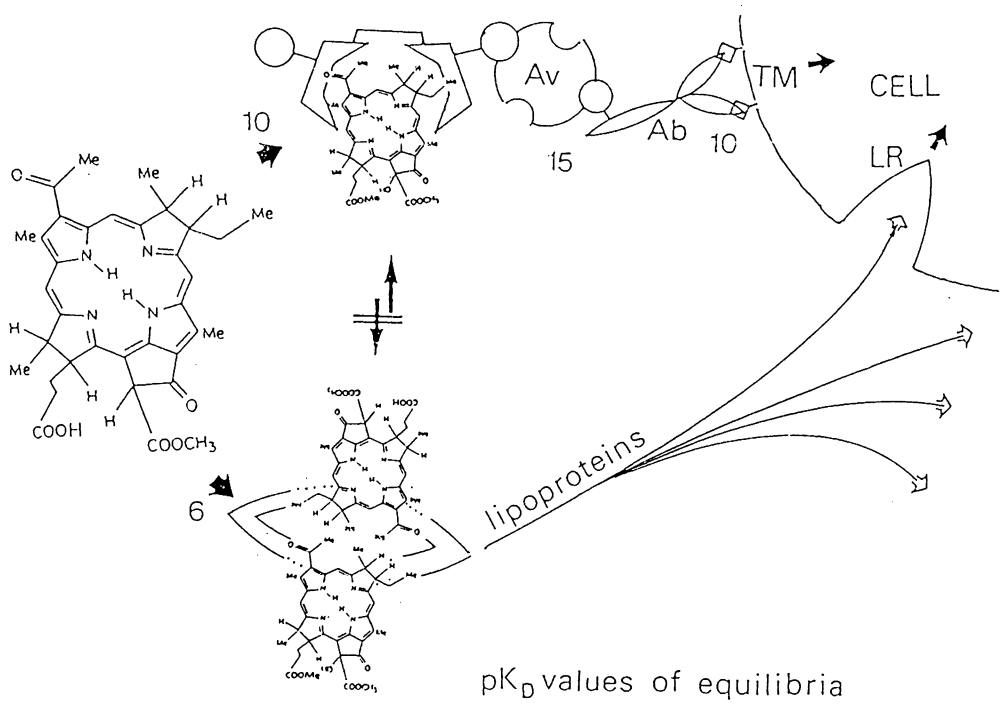


fig. 6 High stability of inclusion complexes prevents sensitizer transport on the lipoprotein pathway

Outline of the targeting system: polyphasic PDT

The intended targeting approach is closely related to the ADEPT concept just in phase II-studies in Great Britain and in Germany. In a first step, biotinylated antibodies with tumor-localizing properties are injected and then cleared for some days as controlled by scintigraphy. In a second step avidin or just avidin-sensitizer chlathrate complexes can be injected at very low concentrations due to the high affinity to biotin. We expect concentrations as low as 10^{-12}M to be able to saturate the antibody-bound biotins excluding any toxicity of ß-cyclodextrin. The multiplicative effect due to multiple binding sites of avidin and the polyvalency of the drug complexes should allow for an accumulation of 10^6 to 10^8 molecules of sensitizer per tumor cell assuming 5×10^4 antigenic binding sites. This is fairly in the range of phototoxic concentrations and should allow for irreversible damage by laser irradiation (fig. 6).

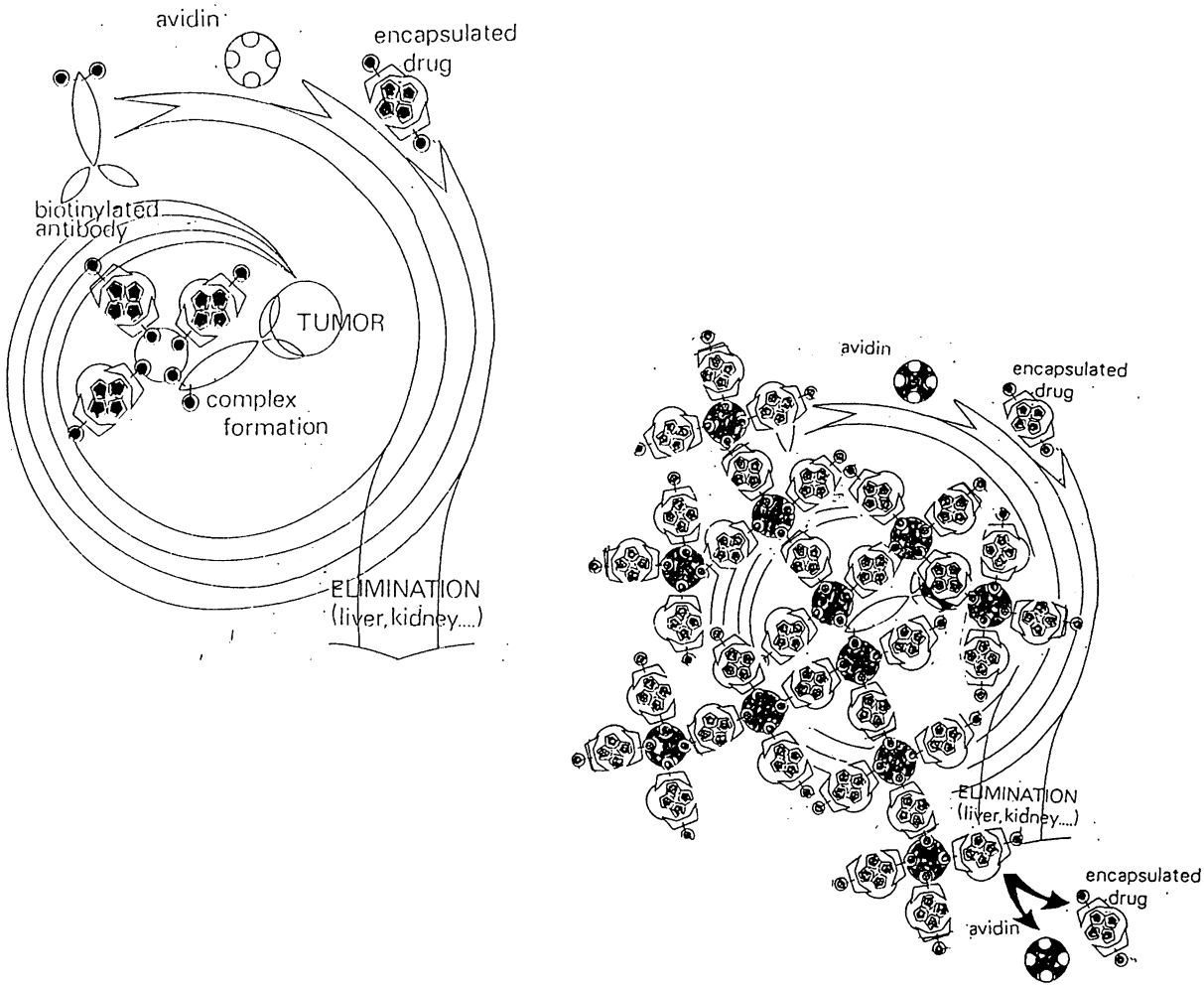


fig. 7 Sequential administration of antibodies and drug complexes leads to accumulation of sensitizer due to polyvalency of the single compounds

A glance over the plate's border: tumor chemotherapy

We should take in mind that complexation of drugs by cyclodextrins is originally invented to solubilize water-insoluble drugs in general. Porphyrinoid binding is only one of the possible applications. Looking over the manifold of today anti-cancer agents there are several potent but harmful drugs which could be detoxified by inclusion into cyclodextrin oligomers and, by chance, transferred to its target without considerable side effects. Moreover, there are several potent anti-cancer drugs which cannot be given to a patient because of their high general toxicity. Some of them are porphyrin derivatives. So, in future such a system may be helpful to revive and to modify general chemotherapy to a curative modality without its inherent side effects.

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