SYNTHESIS OF β-CYCLODEXTRIN DIMERS AS CARRIER SYSTEMS FOR PHOTODYNAMIC THERAPY OF CANCER

A. RUEBNER, J. G. MOSER, D. KIRSCH, B. SPENGLER,
S. ANDREES, S. ROEHRS
Institute of Laser Medicine, University of Duesseldorf,
Universitaetsstr. 1, D-40225 Duesseldorf (Germany)

ABSTRACT

The aim of our investigation was the development of carrier systems for an application of inert drugs in polyphasic photodynamic tumor therapy. As carrier systems, β-cyclodextrin dimers linked at their primary and secondary faces by spacers of varying lengths were synthesized. Cyclodextrins are known to form stable inclusion complexes with porphyrinoïd photosensitizers. The influence of spacer length on the β-cyclodextrin dimer inclusion complexes with porphyrinoïd photosensitizers was studied.

1. INTRODUCTION

Photodynamic therapy (PDT) is a cancer treatment that uses a combination of light-activated drugs (photosensitizers, e.g. porphyrins) and laser light to create highly reactive forms of oxygen that destroy tumor cells. Photosensitizers, however, do not stain tumor tissue exclusively. This is the main drawback in PDT. Antibody-directed targeting of photosensitizers have produced promising results only in *in-vitro* systems [1,2], but have failed to work *in-vivo*. Only a recombination of photosensitizers with specific antibodies into a new class of drugs might fulfill the desired specificity of photodynamic therapy [3]. The first prerequisite is a stable inclusion complex of the cyclodextrin dimer and porphyrinoïd photosensitizers, which prevents photosensitizer transport on the lipoprotein pathway. This would avoid any unwanted targeting of organs except the tumor, provided that a bond between drug and tumor specific antibodies can be established. Since β-cyclodextrins are known to form stable inclusion complexes with porphyrinoïd photosensitizers, we have used them as carrier systems.

2. MATERIALS AND METHODS

2.1. Synthesis

The synthesis of mono-2-(ω-aminopropylamino)-2-deoxy-ß-cyclodextrin **5a**, mono-2-(ω-aminobutylamino)-2-deoxy-ß-cyclodextrin **5b**, mono-6-(2-aminoethylthio)-6-deoxy-β-cyclodextrin **6**, 2,2′N-β-cyclodextrin dimers **7a-c** and 6,6′S-β-cyclodextrin dimer **8a** were described elsewhere [4].

Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **25**: 35–38, 1996.

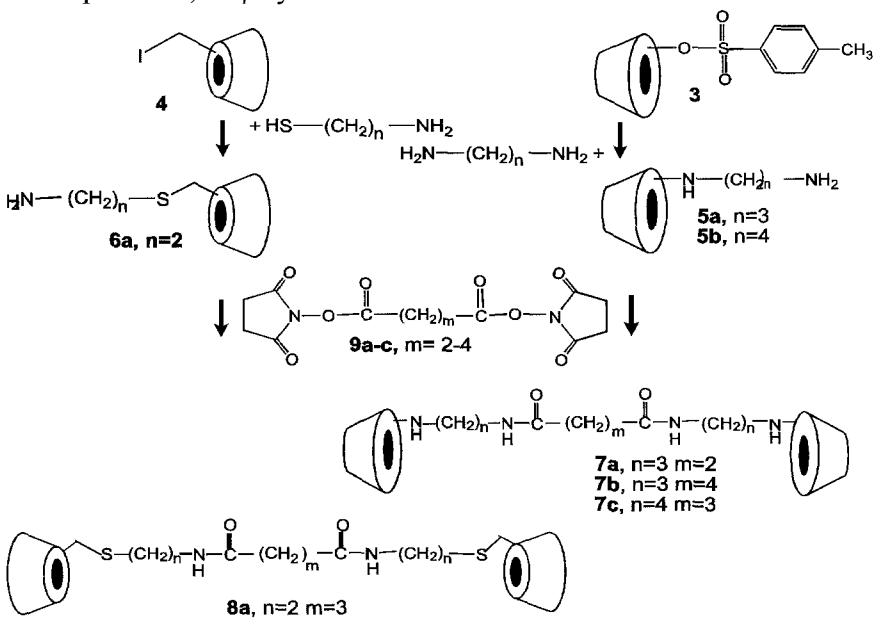
2.2. Determination of binding constants

Binding constants of porphyrinoïds with CD-dimers were studied by means of competitive spectrofluorometry and determined as described elsewhere [4].

3. RESULTS AND DISCUSSION

3.1. Synthesis

Various primary and secondary face linked cyclodextrin dimers were successfully prepared by a multistep procedure, starting from monosubstituted β-cyclodextrin (Scheme 1). Alkylation of linear α,ω-diaminoalkanes with mono-2-tolylsulfonyl-2-deoxy-β-cyclodextrin **3** (route 1) resulted in ω-aminoalkylamino substituted β-cyclodextrins **5a, b**. These were reacted with N-hydroxysuccinimide esters **9a-c** to produce 2,2′N-β-cyclodextrin dimers **7a-c**. Ion exchange chromatography was used to purify crude dimers. Side products which eluted last, having only one β-cyclodextrin bound to the spacer were isolated, activated with N-hydroxysuccinimide and reacted with **5a** or **5b** respectively to produce the desired dimers. The three fractions of ion exchange chromatography were characterized by MALDI-MS and are shown in Fig. 1. Reaction of mono-6-iodo-6-deoxy-β-cyclodextrin **4** (route 2) with 2-amino-ethanthiol resulted in mono-6-(ω-aminoethylthio)-6-deoxy-β-cyclodextrin **6**. This was reacted with N-hydroxysuccinimide ester **9b** to produce 6,6′S-β-cyclodextrin dimer **8a**.



Scheme 1. Synthesis of β-cyclodextrin dimers.

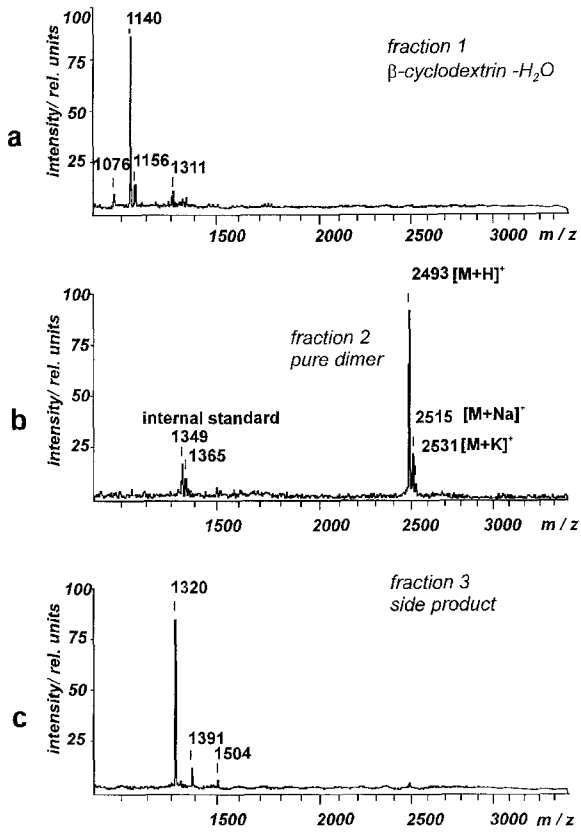


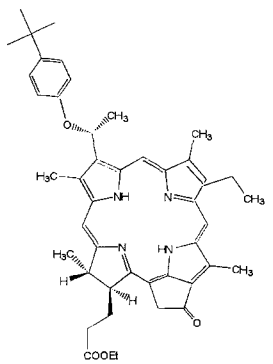
Fig.1. Fractions of ion exchange chromatography. a: β-cyclodextrin-H_2O [M+ Na], b: dimer **7b** [M+H], c: side products with only one β-cyclodextrin bound to the spacer [M+H].

3.2. Binding constants

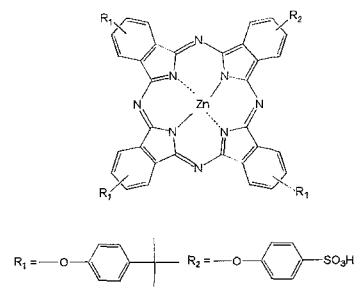
The binding constants of the inclusion complexes of β-cyclodextrin dimers with taylor-made porphyrinoïd derivatives are summarized in Table 1. We examined the porphyrinoïd derivatives **1** and **2** as guest molecules. They are similar in that they are substituted with one or more t.-butylphenyl group. Breslow and co-workers [5,6] showed that guest molecules with t.-butylphenyl groups bind tightly into the cavity of β-cyclodextrins. Our investigations yielded binding constants of β-cyclodextrin dimer inclusion complexes with porphyrinoïd derivatives ranging from 10^5 to 10^7 l/mol. It was shown that all secondary face linked dimers exhibit larger binding constants with porphyrinoïd derivatives as do primary face linked dimers. By comparing the two porphyrinoïd derivatives **1** and **2** (Fig. 2) it can be seen that the insertion of more than one t-butylphenyl residues enhanced the binding constant. The largest binding constant found was that of Zn-tri-tert.butylphenoxy-mono-sulfophenoxy-phthalocyanine **2** in combination with dimer **7c**.

TABLE 1. Binding constants [l/mol] of β-cyclodextrin dimers with porphyrinoïd derivatives. 3^1-tert.-butylphenoxy-ethyl-pyropheophorbide-ethylester **1**, Zn-tri-tert.-butylphenoxy-mono-sulfophenoxy-phthalocyanine **2**.

hosts	guests	porphyrinoïd derivatives	
cyclodextrins	TNS	1	2
7a	$3.6 \pm 0.2 \times 10^3$		$3.3 \pm 0.5 \times 10^5$
7b	$3.8 \pm 0.3 \times 10^3$	$2.9 \pm 0.4 \times 10^5$	$1.3 \pm 0.3 \times 10^5$
7c	$5.5 \pm 0.3 \times 10^3$	$5.7 \pm 0.5 \times 10^6$	$1.5 \pm 0.3 \times 10^7$
8a	$8.3 \pm 0.4 \times 10^3$		$4.0 \pm 0.7 \times 10^6$



1



2

Fig. 2. **1** = 3^1-tert-butylphenoxy-ethyl-pyropheophorbide-ethylester, **2**= Zn-tri-tert-butylphenoxy-mono-sulfophenoxy-phthalocyanine

4. CONCLUSION

As a result of synthesizing β-cyclodextrin dimers of varying spacer lengths, we were able to establish the optimal spacer length for a strong binding to taylor-made porphyrinoïd derivatives. Using these results, it was possible to outline a β-cyclodextrin dimer suitable for use as a carrier for photosensitizers in PDT.

ACKNOWLEDGEMENTS

Financial support by the BMBF (Bundesministerium fuer Bildung, Wissenschaft Forschung und Technologie, grant #13N6291) is gratefully acknowledged.

REFERENCES

[1] Mew, D., Wat, C.-K., Towers, G. H. N., Levy, J. G. *J. Immunol.*, **130**, 1473-1477 (1983)
[2] Oseroff, A. R., Ara, G., Ohuoha, D., Aprille, J., Bommer, J. C., Yarmush, M. L. *Photochem. Photobiol.*, **46**, 83-96 (1987)
[3] Moser, J. G., Ruebner, A., Vervoorts, A., Wagner, B., this issue.
[4] Ruebner, A., Kirsch, D., Andrees, S., Decker, W., Roeder, B., Spengler, B., Kaufmann, R., Moser, J. G., Dimeric cyclodextrin carriers with high binding affinity to porphyrinoïd photosensitizers. submitted to Tetrahedron.
[5] Breslow, R., Halfon, S., Zhang, B. *Tetrahedron*, **51**, 377-388 (1995)
[6] Breslow, R., Greenspoon, N., Guo, T., Zarzycki, R. *J. Am. Chem. Soc.*, **111**, 8296-8297 (1989)