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Carbon–carbon bond ligation between β-cyclodextrin and peptide by photo-irradiation

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Abstract—The formation of a stable covalent bond between β-cyclodextrin and different diphenyl-ketones (*para*-benzoylphenyl-alanine (*p*Bz)Phe, *para*-benzoylbenzoic acid, *p*BzBz, and ketoprofen, KPF, under photo-irradiation is reported. These photo-probes have been incorporated into five peptides PEP1 = Ac-Arg-Lys-Asp-Val-(pBz)Phe-NH_2; PEP2 = Ac-Arg-Lys-Val-(pBz)Phe-NH_2; PEP3 = Arg-Pro-(KPF)Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2; PEP4 = N-(KPF)Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2; PEP5 = Biot(O_2)-Apa-Arg-Arg-Pro-(pBzBz)Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2. The carbon–carbon ligation occurs both in solution and in solid state and whatever the position of the diphenyl ketone, the ketyl radical is able to recombinate with β-cyclodextrin radical even in solid state. Photochemistry of diphenylketone is a complementary and orthogonal approach to chemical modifications of primary hydroxyl functions of β-cyclodextrin.

β-Cyclodextrins (β-CD) are described as truncated cones, the narrow rims bearing primary hydroxyl groups and the wide rims secondary hydroxyl groups.[1,2] Selective modifications of one primary hydroxyl group have allowed to graft various molecules with new recognition functions. Recently, efficient methods have been reported to access to large quantities of regioselectively disusbtituted β-CD at 6-position[3] or at 2-position.[4] These selective chemical modifications of hydroxyl groups permit the arrangement of multiple functional groups at appropriate positions, leading for example to biomimetic catalysts.[5] A large panel of photochemical reactions has been performed inside the hydrophobic cavity, which affect the behaviour of radicals. β-Cyclodextrins induce intramolecular charge transfer, proton transfer, and phosphorescence and fluorescence emission leading to enhancements of reaction rates, modifications of regioselectivity and stereoselectivity and protection from undesired photoprocesses.[6] In all these cases, the β-CD wall is chemically inert and thus β-CD can be considered as a 'nano-reactor vessel'.

To our knowledge, only four photochemical reactions have been reported that modified the chemical structure of β-CD with either β-naphtyldiazomethane[7] (NDM), *p*-nitroacetophenone,[8] benzophenone[9,10] (BP) or ketoprofen (KPF).[11] The inclusion complexes of *para*-nitroacetophenone led to oxidised β-CD, whereas photoadducts of NDM gave an O-ligation on a primary hydroxyl group. The (ketoprofen)/(β-CD) and (benzophenone)/(β-CD) photoadducts were identified only from their absorption spectra that revealed an intense band at 205 nm. Benzophenones and related aryl ketone photophores have been now established as one of the most efficient photoactivatable groups for covalent modification of hydrophobic regions of enzymes and receptors.[12,13]

The formation of a triplet radical and the ketyl radical of benzophenone and ketoprofen into the β-CD cavity have been clearly established by absorption spectroscopy after laser flash photolysis in either water or solid state.[9–11] However, the photoadducts between diphenyl ketones and β-CD have never been isolated. Photoirradiation of phenyl ketones containing peptides is complicated by their intrinsic stability upon irradiation. Benzophenone triplet radical can abstract an intramolecular proton from the peptide side chains. For example, photolysis of Arg^1-Lys^2-Asp^3-Val^4-(*p*-Bz)Phe^5-COOH in water led to complete photodestruction of the peptide into more than 15 photolysis products, corresponding to intramolecular insertion or unidentified rearrangement products.[14]

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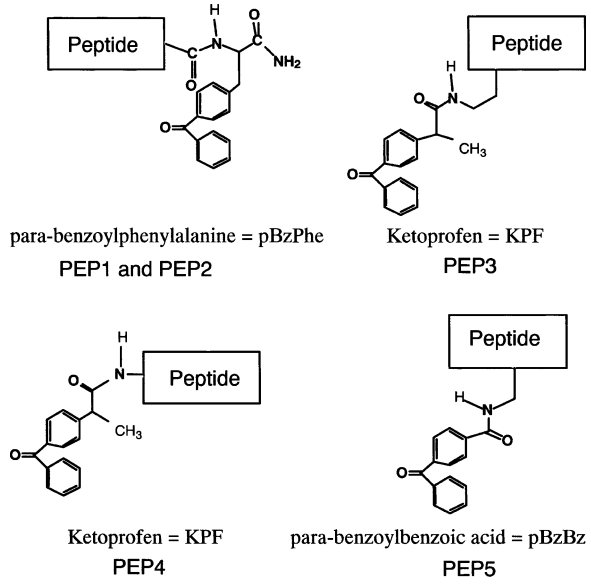
Our goal was to determinate if a cross-linking (O- or C-ligation) can occur in solution and in solid state whatever the position and the nature of diphenyl ketones between β-cyclodextrins and substituted peptides. The photoactivable peptides used in that study are schematised in Figure 1.

Peptide syntheses were carried out on a 0.1 mmol scale (ABI Model 431A synthesizer), starting from an α-*para*-methyl benzhydrylamine (MBHA) resin (typical substitution, 0.68 mmol/g of resin). All *N*-α-*t*-butyloxycarbonyl (Boc) amino acids were assembled using dicyclohexyl-carbodiimide (DCC) and 1-hydroxy benzotriazole (HOBt) as coupling reagents. The three photoprobes *para*-benzoylphenylalanine, *para*-benzoylbenzoic acid and ketoprofen have been incorporated into five peptides (PEP1, PEP2, PEP3, PEP4 and PEP5). (pBz)Phe was directly introduced during the assemblage of peptide, as previously described.[15] The *para*-benzoylphenylalanine and ketoprofen were introduced at the end of synthesis using HBTU (O-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexa-fluorophosphate) as the activator reagent. After hydrogen fluoride cleavage, the peptides were purified by HPLC. The millimolar aqueous solution of peptides was irradiated for 40 min using an ultra-violet light at 365 nm (HPR 125-W lamp) with or without β-cyclodextrins at a distance of 6–10 cm. For the solid state irradiation experiments the host–guest complex between cyclodextrin and peptides preformed in aqueous solution was quickly frozen in liquid nitrogen and lyophylised. The resulting powder was then irradiated in a quartz vat.



PEP1 = Ac-Arg -Lys-Asp-Val-(pBz)Phe-NH_2
PEP2 = Ac-Arg-Lys-Val-(pBz)Phe-NH_2
PEP3 = Arg-Pro-(ε-N-KPF)Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
PEP4 = (α-N-KPF)Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
PEP5 = Biot(O_2)-Apa-Arg-Arg-Pro-(ε-N-pBzBz)Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)NH_2
(pBz)Phe = parabenzoylphenylalanine ; pBzBz = para-benzoylbenzoic acid ; KPF = Ketoprofen ; Apa = aminopentanoic acid ; Met(O_2) = methionine sulfone ; Biot(O_2) = biotine sulfone.

Figure 1.

Photoirradiation of the peptide in the absence of β-CD

Photolysis for 40 min of the peptides in water or in solid state without CD was followed by reverse phase HPLC. The phototolysis products of PEP2 were analysed by Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF).[16] At pH 7, five major products were identified corresponding to the starting peptide (14%, $m/z = 694.35$), the reduced peptide (5%), identical to the product of reduction prepared by sodium borohydride, three rearrangement products (16%, $m/z = 694.50$; 25%, $m/z = 694.35$ and 11%, $m/z = 692.95$ the deamination product of Lys side chain leading to the aldehyde) and many other minor unidentified compounds (30%). The photolysis products of the other peptides were not analysed in detail. In solid state, all peptides were stable under irradiation.

Photoirradiation of the peptides in the presence of β-CD

The analyses of the crude mixtures were performed by HPLC after photoirradiation in presence of 50 equivalents of β-CD for each of the five different peptides. The relative percentages of each product are reported in Table 1. Two effects were observed on the HPLC

Table 1. HPLC and MALDI-TOF analyses after photoirradiation of peptides (PEP) bearing different diphenyl ketone photoprobes

	Experimental conditions	m/z SP*	M/z PA*	Number of PA (a)	PA (b) (%)	PP *(c) (%)	SP (d) (%)	Ratio PA/PP
PEP1	Solution	579.0	1713.1	4	44	54	2	0.81
PEP2	Solution	694.35	1828.7	6	54	44	2	1.22
PEP2	Solid	694.35	1828.7	6	63.5	31	5.5	2.03
PEP3	Solid	1583.9	2717.7	4	67	23	10	2.90
PEP4	Solid	1583.8	2717.7	4	40	17	43	2.35
PEP5	Solid	2004.3	3137.7	5	85 (e)	10	5	8.50

* Abbrevations: SP = starting peptide; PP = photolysis products; PA = photoadducts; (a) number of HPLC fractions containing photoadducts, (b) percentage of photoadducts corresponding to the sum of the PA fraction areas measured after HPLC separation, (c) percentage of photolysis products PLP corresponding to the sum of unidentified compound areas, (d) percentage of starting peptide (e) 15% of PA fractions correspond to a (2/1: β-CD/peptide) photoadduct with mass $m/z = 4271.8$.

profile when β-CD was added to these peptides. The formation of photolysis products was partially prevented and new products were generated. These new products were purified by preparative reverse phase HPLC and analysed by MALDI-TOF to identify the fractions containing photoadducts. Using PEP2 to optimise the experimental conditions, we found that the reaction performed in solid state limited the number of products. The percentage of photolysis products (PP) is higher in solution in agreement with triplet radical and ketyl escaping from the β-CD cavity. In solid state, this escape must be blocked and nevertheless photodestruction products are still present. Photoirradiation of PEP3, PEP4 and PEP5 carried out in the solid state showed that the amount of photodestruction products (PP) depends on the peptide primary sequence. This result might be partially related to the relative stability (affinity) of the performed host–guest complexes during the freezing step. However, the origin of these photolysis products in solid state remains unclear since these peptides without β-CD are stable. Probably, transfers of radical should occur between β-CD radicals and peptide side chains. Except for PEP4, the position and the nature of photoprobes have no effect on the yield of cross-linking, the lower yield observed with PEP4 could be due to the N-terminal position of the photoprobe. The steric hindrance close to the photoprobe might decrease the affinity of PEP4 for β-CD. The better yield of cross-linking (85%) was obtained for PEP5, indicating that the best position to introduce the photoprobe is on an amino acid side chain.

For each peptide, four to six HPLC fractions contained photoadducts with the expected mass (PEP1 $m/z=$ 1713.1; PEP2 $m/z=1828.19$; PEP3 and PEP4 $m/z=$ 2717.7; PEP5 $m/z=3137.7$). These results indicated that different stable cross-linking occurs with a 1:1 stoichiometry between β-CD and a peptide leading to cyclodextrin-peptide hybrids. The O-ligation would have yielded a reversible hemiketal that should give back in aqueous solution the starting peptide. But the proportions of C- versus O-ligation vary from one peptide to the other from 2 to 43%.

The photoadducts of PEP2 and the low quantity of starting peptide observed after solid state and also solution irradiation indicated that the triplet radical abstracts preferentially a proton from a CH bond, even in the solid state. For PEP4, proton abstraction from O–H bond cannot be excluded since 43% of starting peptide are recovered after photoirradiation. All stable photoadducts (PA) correspond to a C-ligation between one peptide and one β-CD. Surprisingly, one fraction (15%) of PEP5 corresponds to the ligation of one peptide with two cyclodextrins. In this complex, the side chain of Lys should be able to cross the hydrophobic cavity of a first β-CD and the benzophenone must be linked to a second one, the triplet radical of benzophenone abstracts one proton of the second β-CD. This formation implies that benzophenone crossed the β-CD cavity during the formation of the host–guest complex. This type of complex is impossible to form with PEP1 and PEP2 since the peptide backbone is

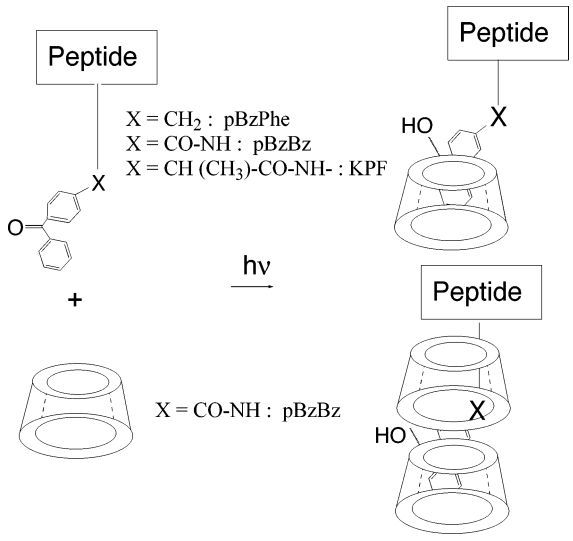


Figure 2.

unable to cross the β-CD cavity for interacting with a second β-CD. Surprisingly, the 2/1 complex is not observed when benzophenone was replaced by ketoprofen probably due to steric hindrance (Fig. 2).

For each peptide, four to six HPLC fractions contain photoadducts indicating the formation of isomers. Laser flash photolysis/time-resolved EPR (electron paramagnetic resonance) spectroscopy of β-CD in acetone solution has revealed the possibility of different types of β-CD radicals (C1, C3 and C5 but not C2 and C6),[17] the C1 radical may also shift to a C5 radical by opening of glucose ring. If one considers that only the H1, H2 and H4 protons of glucose are clearly outside the cavity,[18,19] the triplet radical of diphenyl ketone in the inclusion complex could abstract the protons in position 6, 5 and 3. The radical recombination creates simultaneously two chiral centres: one on the glucose and the other one on the diphenyl methanol moiety, so twelve isomers can be expected. The ratio (data not shown) and the number of photoadducts vary for the different peptides. These data suggest that regioselectivity and (or) diastereoselectivity could be different for each peptide. Indeed, preliminary NMR and modelling studies agree with the presence of regio and diastereomers (Lequin to be published).

We have demonstrated that β-CD forms a stable covalent bond with diphenyl ketone containing peptide both in solution and in solid state. After irradiation, the ketyl radical is able to recombinate even in solid state with β-cyclodextrin radical leading to the formation of a carbon–carbon bond. These chimeric molecules made of peptide and glucose correspond to a new family of C-glycopeptides, opening new possibilities in different domains. For example, the ligation of β-CD to peptide might be useful to increase the solubility of a bioactive hydrophobic peptide and also its stability, as glycosylation decreases the degradation of proteins. The use of β-CD as a scaffold in protein engineering requires many selective chemical modifications, so photochem-

istry of diphenylketone appears as a complementary and orthogonal approach for chemical modifications of hydroxyl functions of β-CD. This photochemical reaction made easier the attachment of different peptides on a molecule of β-cyclodextrin.

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References

1. Wenz, G. *Angew. Chem., Int. Ed.* **1994**, *33*, 803–822.
2. Saenger, W.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S.; Takahaa, T. *Chem. Rev.* **1998**, *98*, 1787–1802.
3. Pearce, A. J.; Sinaÿ, P. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 3610–3612.
4. Teranishi, K. *Tetrahedron Lett.* **2000**, *41*, 7085–7088.
5. Breslow, R.; Dong, S. T. *Chem. Rev.* **1998**, *98*, 1997–2011.
6. Takahashi, K. *Chem. Rev.* **1998**, *98*, 2013–2033.
7. McAlpine, S. R.; Garcia-Garibay, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 2750–2751.
8. Chow, Y. L.; Michon, J.; Michon, P.; Morat, C.; Rassat, A. *Tetrahedron Lett.* **1992**, *33*, 3315–3318.
9. Monti, S.; Flamigni, L.; Martelli, A.; Bortolus, P. *J. Phys. Chem.* **1988**, *92*, 4447–4451.
10. Barra, M.; Scaiano, J. C. *Photochemistry and Photobiology* **1995**, *62*, 60–64.
11. Monti, S.; Sortino, S.; De Guidi, G.; Marconi, G. *New J. Chem.* **1998**, *22*, 599–604.
12. Dorrman, G.; Elliott, J. T.; Marecak, D. M.; Chaudhary, P. *Photochemistry and Photobiology* **1997**, *65*, 222–234.
13. Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64–77.
14. Weber, P. J. A.; Beck-Sickinger, A. G. *J. Peptide Res.* **1997**, *49*, 375–383.
15. Sachon, E.; Girault-Lagrange, S.; Chassaing, G.; Lavielle, S.; Sagan, S. *J. Peptide Res.* **2002**, *59*, 232–240.
16. Matrix-assisted laser desorption ionization and time-of-flight (MALDI-TOF) were obtained on a Voyager Elite mass spectrometry (PerSeptive Biosystems). α-Cyano-4-hydroxycinnaminic acid was used as matrix with 5×10^{-2} M concentrations in acetonitrile/water (4:1, v/v).
17. Lehmann, M. N.; Bakker, M. G. *J. Soc., Perkin Trans. 2* **1997**, 2131–2133.
18. Zubiaur, M.; Jaime, C. *J. Org. Chem.* **2000**, *65*, 8139–8145.
19. Brett, T. J.; Stezowski, J. J. *Chem. Commun.* **2000**, 857–858.