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Fast atom bombardment mass spectrometry as a tool for the rapid determination of enantioselective binding of methylated cyclodextrins

Simon N. Davey [b,1], David A. Leigh [a,*], John P. Smart [a], Lee W. Tetler [b], Ada M. Truscello [a,2]

[a] *Department of Chemistry, University of Manchester Institute of Science and Technology, Sackville Street, Manchester M60 1QD, UK*

[b] *Michael Barber Centre for Mass Spectrometry, Department of Chemistry, University of Manchester Institute of Science and Technology, Sackville Street, Manchester M60 1QD, UK*

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Abstract

The first FABMS study of the enantioselectivity shown during complex formation between *per*-methylated cyclodextrins and pairs of enantiomeric guest molecules is described. The 1:1 mixtures of the cyclodextrins, both α- and β-, with the guests, the methyl esters of the amino acids tryptophan and phenylalanine, were studied in a 100:50:1 glycerol–thioglycerol–trifluoroacetic acid matrix. The uncomplexed cyclodextrin peaks were then used as internal standards to determine the preference of the cavity for one or other of the enantiomers. A clear trend for the preferential binding, greater than 5:1 in each case, of the D-enantiomers of the amino acid esters was observed in agreement with literature ^{1}H NMR experiments. This methodology provides a rapid route to assessing the enantioselectivity shown by the widely used cyclodextrins towards pairs of enantiomeric guests. © 1996 Elsevier Science Ltd.

Keywords: FABMS; MS–MS; α- and β-Cyclodextrins; Per-methylated cyclodextrins

* Corresponding author.

[1] Current address: BARC, Department of Biological Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK.

[2] Current address: Dipartimento di Chimica, Politecnico di Milano, via Mancinelli, 7, 20133 Milan, Italy.

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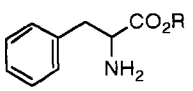
1. Introduction

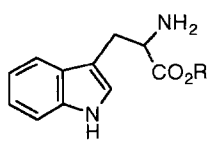
The determination of the enantioselectivity shown by chiral hosts towards pairs of enantiomeric guests is of the upmost importance, particularly with regard to cyclodextrins. Readily obtained by the action of an enzyme of *Bacillus macerans* on starch [1], cyclodextrins and their derivatives are able to bind to a wide range of small, neutral and charged organic molecules [2], and have been commercialised in the pharmaceutical and food industries where complex formation is used to modify the physical [3], chemical [4] or biological [5] properties of included guests. Because of the well expressed chirality of their C_n symmetric cavities, the cyclodextrins are often particularly efficacious in distinguishing between pairs of enantiomers when forming complexes, resulting in their widespread use as stationary phases in chiral HPLC columns [6] and more recently for the active separation of pairs of enantiomers in prodrugs [7]. These and other applications have made cyclodextrins and their derivatives the most studied and commercially successful chiral molecular receptors developed thus far.

Currently there are several time- and labour-intensive methods for the determination of the relative binding preference of cyclodextrin hosts to enantiomeric guests, the most common of which is NMR titration [8]. This requires the preparation of a series of samples and the correlation of proton signals affected by the non-covalent interactions. Problems can arise due to solubility or when the signals of the cyclodextrin host overlap with the signals of the guest making accurate chemical shift determination impossible. Furthermore, significant shifts in the NMR spectrum are generally only observed when aromatic groups are involved making this technique often inappropriate for non-aromatic substrates. Other procedures that can be used to determine chiral discrimination include the use of HPLC [9], where the host is fixed as the stationary phase and the retention time of the guest is used to calculate the relative strength of binding, and the use of UV [10] which requires that the guest has UV activity, again a technique often inappropriate for non-aromatic guest species.

These techniques have been applied to the study of the binding of cyclodextrins to the aromatic amino acids phenylalanine (**1**) and tryptophan (**2**), and the relative preference for complexation with one enantiomer or the other is therefore well documented [11].



1 R = H
3 R = Me



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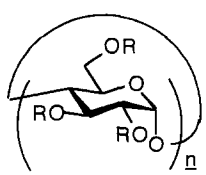

5 R = Me, n = 6,
6 R = Me, n = 7,

These two readily available compounds possess an aromatic portion which fits snugly into the CD cavity and a chiral centre close enough to interact with the inherent chirality of the cyclodextrin ring, and are therefore excellent substrates for enantiodiscriminatory binding. The mass spectrometric detection of host–guest complexes, two or more molecules held together by weak non-covalent interactions, has become possible over the last few years with the development of softer ionisation techniques such as fast atom bombardment (FAB) and electrospray. The first reported complexes detected by FABMS were between crown ethers and metal ions and the results used to probe crown-ether metal cation selectivity [12]. The first carbohydrate complexes studied by FABMS were carried out by Ballou and Dell [13]. Organic cation (RNH_3^+) complexes of both crown ether and cyclodextrin derivatives have been studied by Stoddart and ourselves [14,15], showing that the intensities of the peaks in the mass spectrum were a close approximation to the abundance of the different species in solution. Ohashi et al. have shown that inclusion complexes of underivatised cyclodextrins can also be detected and can be seen in either the positive or negative modes depending upon the nature of the guest [16]. Recently, Hofmeister and Leary [17] and Sawada et al. [18] have independently described the first uses of FABMS for studying enantioselective complex formation. Hofmeister and Leary investigated the chiral recognition of lithium co-ordinated diols, whilst Sawada et al. studied a carbohydrate-based crown ether molecule known to bind enantioselectively to different enantiomeric ammonium ion guests. To date, however, no study of the enantioselectivity shown by cyclodextrins, the most important class of chiral host molecules, towards pairs of enantiomers has been performed. Here we describe the use of FABMS as a general tool for the rapid determination of enantioselectivity by studying the binding of the methyl esters of two amino acids (**3** and **4**) by *per*-methylated α- and β-cyclodextrins (**5** and **6**).

2. Experimental

Complexes were prepared as 1:1 mixtures of amino acid ester to cyclodextrin. The compounds were dissolved in methanol and then evaporated to dryness and studied in a 100:50:1 glycerol–thioglycerol–trifluoroacetic acid matrix. All FABMS data were acquired in positive ion mode on a Kratos Concept IIHH four sector tandem mass spectrometer under FAB ionisation conditions, employing xenon atoms at 8 keV accelerating energy as the bombarding particle. The MS/MS data were acquired using the same instrument with an electro-optical array at the end of MS2. In order to compare directly the peak intensities of the diastereomeric complexes it was assumed that the complexes were equally soluble in the FAB matrix and produce ion currents relative to their solution concentration, and that no suppression effects occur between the D- and L-enantiomers.

3. Results and discussion

The spectrum of the (1:1) complex of D-phenylalanine methyl ester (**3-*R***) and *per*-methyl α-cyclodextrin (**5**) is shown in Fig. 1. The peak at m/z 1404 (100%,

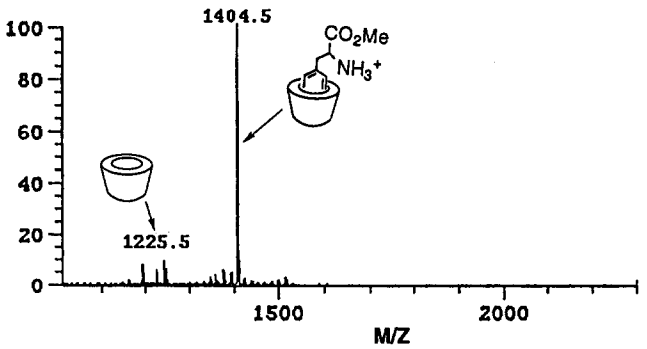

Fig. 1. The FAB mass spectrum of the 1:1 complex of *per*-methyl α-cyclodextrin (**6**) and D-phenylalanine methyl ester (**3**-*R*).

corresponding to the protonated molecular ion of the host–guest complex), is more abundant than the peak at m/z 1225 (8%, which corresponds to the protonated molecular ion of the unbound cyclodextrin) in a ratio of 25:2. In the mass spectrum of the complex of the L-enantiomer of phenylalanine methyl ester (**3**-*S*) with the same cyclodextrin (**5**, Fig. 2), however, this ratio falls to 5:3. Assuming that both diastereomeric complexes fragment to the same degree under the mass spectral experimental conditions (confirmed by MS/MS experiments, see later) then the two complex signals can be compared by standardising the free cyclodextrin signal. When this is done it implies a $> 7{:}1$ preference for binding of the D-enantiomer of phenylalanine into the cyclodextrin cavity relative to that of the L-enantiomer, in close agreement with the general trend for D-enantiomers of amino acids to be preferentially bound by both derivatised and underivatised cyclodextrins [19].

Similar results were obtained when the complexes were formed between the same two enantiomers (**3**-*R* and **3**-*S*) and the larger *per*-methyl β-cyclodextrin (**6**, Figs. 3 and 4). Normalising the free cyclodextrin peaks shows an even stronger 10:1 preference for

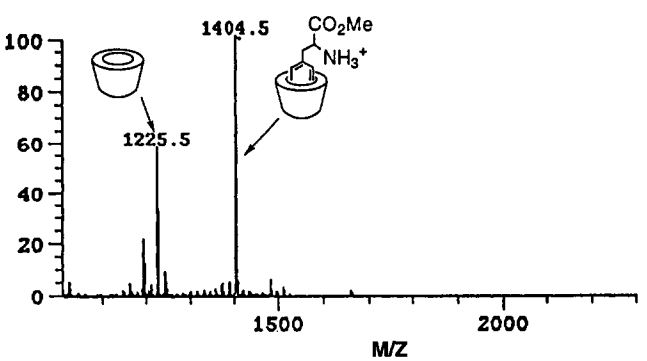

Fig. 2. The FAB mass spectrum of the 1:1 complex of *per*-methyl α-cyclodextrin (**6**) and L-phenylalanine methyl ester (**3**-*S*).

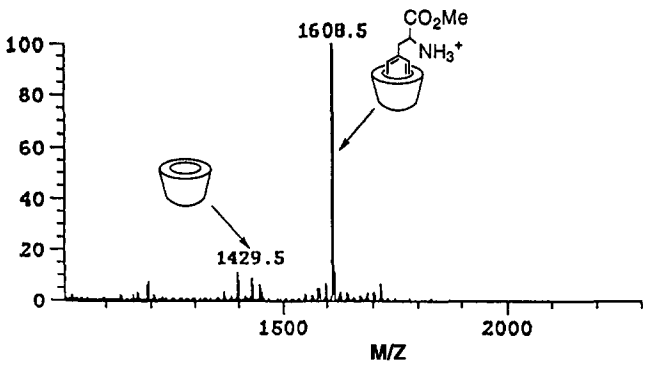

Fig. 3. The FAB mass spectrum of the 1:1 complex of *per*-methyl β-cyclodextrin (**7**) and D-phenylalanine methyl ester (**3**-*R*).

the D-enantiomer. In this case the free cyclodextrin appears at *m/z* 1429 and the signal for the complex appears at *m/z* 1609. The peaks flanking the cyclodextrin molecular ion peak in this spectrum are identifiable as *m/z* 1451 $[CD + Na]^+$, *m/z* 1397 $[CD–MeOH]^+$ and *m/z* 1193 [CD–one glucose residue–MeOH]$^+$. Of the six complexes examined, the complex formed between *per*-methyl β-cyclodextrin and L-phenylalanine is by far the weakest. The relative intensities of the peaks bordering the cyclodextrin molecular ion to the cyclodextrin molecular ion peak itself are consistent throughout the range of complexes.

The same D-enantioselectivity is also seen for the complexation of the methyl esters of D- and L-tryptophan (**4**-*R* and **4**-*S*) with *per*-methyl β-cyclodextrin (**5**) showing a fivefold preference for the D-enantiomer (**4**-*R*). In this case the free cyclodextrin signal appears at *m/z* 1429 and the complex signal at *m/z* 1648 (Figs. 5 and 6).

In order to ascertain the effect of fragmentation on the results, the host–guest complexes of *per*-methyl-β-cyclodextrin (**5**) with **4**-*S* and **4**-*R* were analysed by MS/MS in which the protonated molecular ions of interest (*m/z* 1648) were subjected

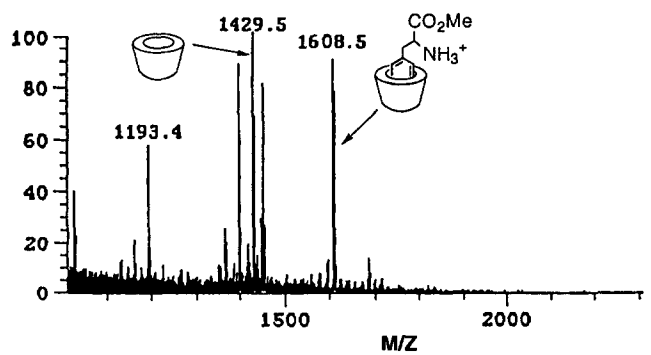

Fig. 4. The FAB mass spectrum of the 1:1 complex of *per*-methyl β-cyclodextrin (**7**) and L-phenylalanine methyl ester (**3**-*S*).

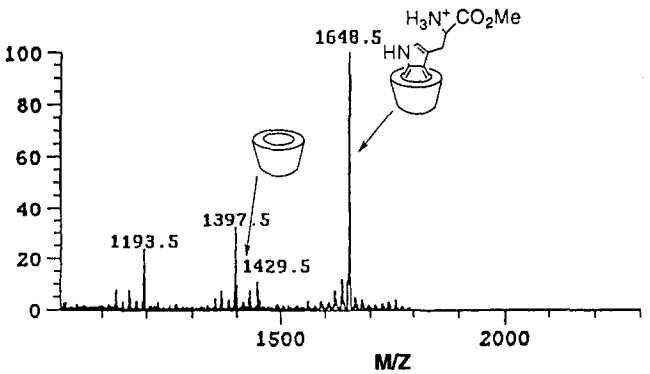

Fig. 5. The FAB mass spectrum of the 1:1 complex of *per*-methyl β-cyclodextrin (**7**) and D-tryptophan methyl ester (**4**-*R*).

to collision induced decomposition using helium gas. The fragmentation patterns of the diastereomeric complexes were very similar. Remarkably, in neither case were peaks observed in the MS/MS corresponding to 'free' cyclodextrin (m/z 1429), clearly demonstrating that fragmentation of the host–guest complex does not result in a fragment of protonated 'free' cyclodextrin. The major observed fragmentation of the complexes involves the loss of methanol from one component of the host–guest complex; other fragmentation losses are of the derivatised glucose rings (204 Da) from the cyclodextrin unit of the intact complex. An experiment where the d_3-methyl ester of D-tryptophan was similarly complexed with *per*-methyl β-cyclodextrin and the MS/MS of the complex was studied, showed that the loss of methanol was occurring from the cyclodextrin, i.e. M − 31 rather than from the amino acid methyl ester i.e. M − 34. The lack of cyclodextrin signal implies that the proton is carried, as expected, on the nitrogen of the amino acid ester, such that on complex breakdown almost no protonated cyclodextrin molecule is observed. Similar observations were reported by Ohashi et al.

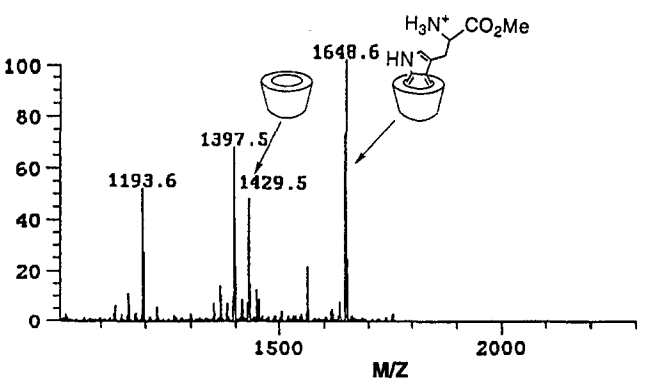

Fig. 6. The FAB mass spectrum of the 1:1 complex of *per*-methyl β-cyclodextrin (**7**) and L-tryptophan methyl ester (**4**-*S*).

during their investigation into the study of underivatised cyclodextrin complexes [16]. The unbound cyclodextrin observed in the FABMS spectra, therefore, does not arise from decomposition of the complex so that the relative peak intensities of complex to free cyclodextrin are likely to be a good indication of the degree of binding.

In conclusion, the FABMS results for both *per*-methyl α- and *per*-methyl β-cyclodextrin mirror closely the trend determined by solution ^{1}H NMR, demonstrating that chiral discrimination shown by methylated cyclodextrins can be rapidly and easily studied by FABMS. Studies are currently underway to apply this technique to other cyclodextrin derivatives and non-aromatic guest molecules.

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