Folding-induced CO_2-soluble peptides†

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The first CO_2- and water-soluble peptide is reported, in which folding facilitates its solubility in CO_2.

The search for environmentally friendly processes is an active endeavour in current chemistry. With advances in technology, the widespread use of halogenated and organic solvents is accompanied by increasing environmental problems stemming from waste disposal and pollution. Recent research has promoted the use of CO_2 in place of organic solvents as a benign solution for these issues.[1] CO_2 is an attractive solvent due to its variable properties. The supercritical state, in which there is no separation of liquid and gaseous phases, is observed above 73.8 bar and 31 °C.[2] A range of densities can be explored through small variations in pressure and temperature, making it an attractive, tunable solvent. CO_2 has several additional advantages, in that it is non-flammable, inexpensive and easily removed from products. The majority of CO_2 sold for current processes is produced as a by-product of other industries,[3,4] so the use of CO_2 as a solvent does not increase the amount of CO_2 released to the atmosphere.

However, the non-polar environment of CO_2 is problematic for the solubilization of polar and hydrophilic materials. Many techniques have been investigated to improve solubility, including the use of surfactants, microemulsions and reverse micelles.[5,6] Fluorinated hydrocarbons are commonly used as surfactants,[7] but they are expensive and potentially toxic. Recently, the use of acetylated sugars as surfactants was reported.[8,9] It was observed that the acetylated sugars interact with CO_2 through CH···O hydrogen bonds with the acetate groups. The use of sugars as surfactants provides many advantages over fluorocarbons in that they are inexpensive, non-toxic and allow the exploration of numerous stereochemical configurations by the use of different sugars.

A novel approach for solubilizing polar molecules in CO_2 is to exploit folding to effectively block the polar groups and minimize their interactions with CO_2.[10] Peptides contain numerous polar groups, including the amide backbone, but many of these groups are involved in interactions that contribute to the stabilization of secondary structure, including hydrogen bonding and side-chain–side-chain interactions. Therefore, short, structured peptides provide an ideal model to investigate this approach.

To determine the effect of folding on CO_2 solubility, we have investigated a short peptide that favors a helical structure and compared its solubility to an unstructured peptide. Peptide **1a** includes four Ala residues, which have a high helical propensity in both aqueous[11] and non-polar[12] environments, and an N-terminal peracetylglucoserine, which should aid in CO_2 solubility (Fig. 1). The 5-residue sequence is long enough for the nucleation of alpha- or 3_{10}-helical structures in a non-polar solvent, which is expected to bury the polar backbone within a helix of methyl groups and aid solubilization. The peracetylglucoserine was placed at the N-terminus to minimize any structural effects associated with the large side-chain. The solubility of peptide **1a** was compared with **2a**, in which a ^{D}Ala is substituted at position 3, and **3a**, in which the glycosylated residue is replaced with Ser. ^{D}Ala has been shown to be helix-breaking,[13] so results from **2a** provide information about the importance of structure for solubilization, while comparison to **3a** elucidates the importance of the acetylated sugar as a solubilizing group for the peptide. Peptides **1b–3b**, which incorporate a Gly–Gly–Tyr sequence as a UV tag for accurate concentration determination, were used for circular dichroism (CD) studies (see below).

The solubility of pentapeptide **1a** was investigated in neat CO_2. Peptide solubility was observed over a range of temperatures and pressures *via* visualization of the sample in a high pressure CO_2 cell. Below the cloud point, the peptide was visible as solid particles floating in the CO_2. Above the cloud point, the solution was homogenous. The peptide was observed to be soluble in CO_2 at pressures over 80 bar at room temperature (Fig. 2). As the temperature was increased, the cloud point pressure also increased. Interestingly, peptide **1a** was also quite soluble in water.‡

In contrast, attempts to dissolve peptide **2a**, which contains ^{D}Ala, and peptide **3a**, which lacks the acylated sugar, in CO_2 were unsuccessful. ^{D}Ala peptide **2a** was not soluble over the temperature and pressure range studied. Unglycosylated peptide **3a** was not soluble above 24 °C, but appeared to be close to the cloud point below 24 °C at a pressure of 345 bar. Higher pressures could not be investigated due to the limits of the apparatus.

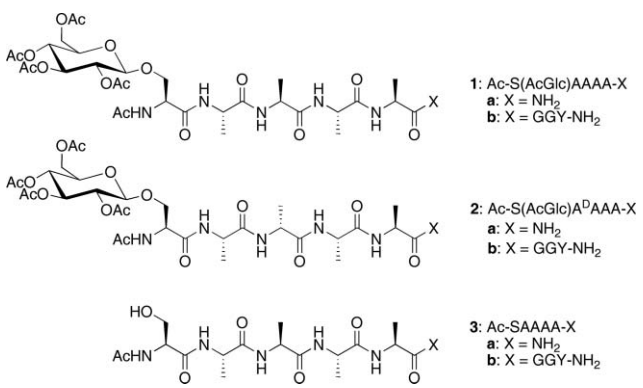

Fig. 1 Peptide structures and sequences for the investigation of CO_2 solubility.

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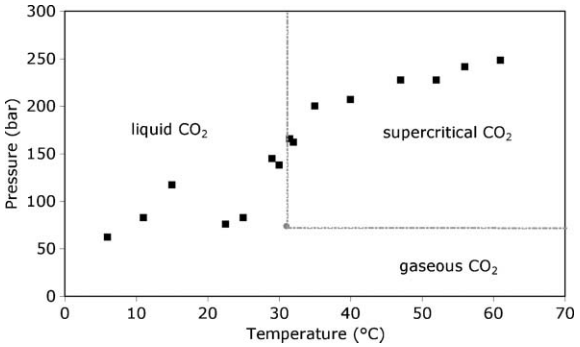

Fig. 2 Cloud point profile for **1a** in CO_2 (0.67 mM). At temperature/pressure combinations above the black data points, the peptide is soluble in CO_2. The data is the overlay of two duplicate experiments. The gray circle represents the critical point, and the gray dashed lines roughly indicate the boundaries of the supercritical phase. All data points are in the liquid or supercritical phase.

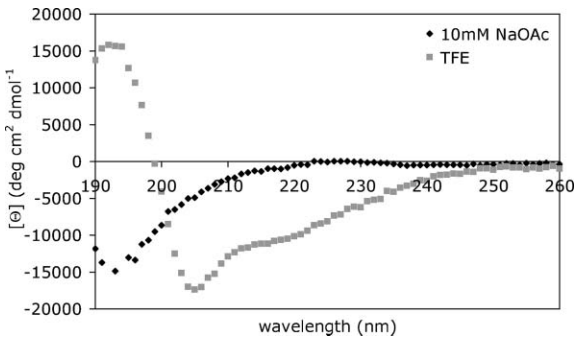

Fig. 3 CD spectra of **1b** in TFE (gray squares) or 10 mM sodium acetate buffer, pH 4.5 (black diamonds) at 273 K.

To characterize the secondary structure of peptide **1**, we investigated its CD spectra in water and trifluoroethanol (TFE) (Fig. 3). TFE was selected as a comparative solvent to CO_2, since a high pressure CD cuvette is not available to characterize secondary structures in CO_2. TFE was selected because it is typically used to promote α-helix formation, and the different CO_2 solubilities of peptides **1a** and **2a** suggested differences in helical structure.

The observed minimum for **1b** in aqueous buffer is at 195 nm, which is consistent with a random coil or possibly a polyproline (PPII) helix. This was expected, as peptides of 16 residues or more are typically required to form well-folded helices in water. In TFE, the minimum is red-shifted to 205 nm with a shoulder at ~219 nm, which is consistent with literature values for a 3_{10}-helix of 207 nm for the minimum and 222 nm for the shoulder.[14]

Peptides **1b–3b** were compared in TFE due to the solubility of all three peptides in this solvent. Peptide **2b**, containing a ^{D}Ala, is less structured than **1b**, in agreement with a report of the helix-breaking ability of ^{D}Ala (Fig. 4).[9] In contrast, peptide **3b** is more structured than **1b**, with an apparent α-helical structure and minima at 209 and 219 nm. This indicates that the AcGlc in peptide **1b** destabilizes the helix relative to Ser,[15] suggesting that both an acylated sugar and a helical structure are required for CO_2 solubility.

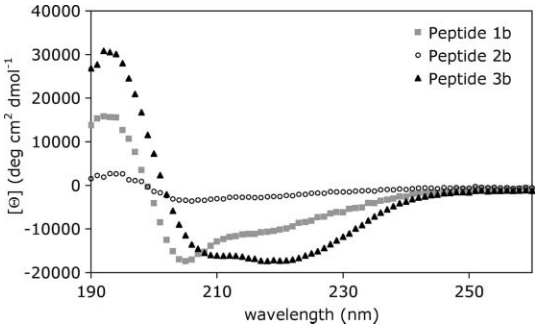

Fig. 4 CD spectra for peptides **1b** (gray squares), **2b** (open circles) and **3b** (black triangles) in TFE. Concentrations are 130–150 μM.

In conclusion, we have designed a short polyalanine peptide, **1**, that exhibits good solubility in both water and supercritical CO_2, which is quite unusual. 3_{10}-Helix formation of peptide **1** in CO_2 is suggested to play a large role in its solubility, as well as the incorporation of an acetylated sugar, whereas a switch to a random coil is likely to be responsible for its solubility in H_2O. These results indicate that the formation of non-covalent interactions that bury polar groups can greatly facilitate solubility in supercritical CO_2.

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Notes and references

‡ Solubility in aqueous buffer was investigated up to 1 mM.

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