

Hydration of cyclodextrins studied by neutron
and X-ray scattering

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Zusammenfassung

Cyclodextrine sind Oligosaccharide mit 6, 7, 8 $\alpha(1\rightarrow4)$ gebundenen D-Glukose Einheiten, genannt α -, β - und γ -CD; ihre Löslichkeit in Wasser steigt mit der Temperatur an. Derivate von Cyclodextrinen mit methylierten 2,6-OH oder 2,3,6-OH Gruppen (mCDs) zeigen einen negativen Temperaturkoeffizient der Löslichkeit, d. h. die sind im kaltem Wasser sehr löslich, kristallisieren aber wenn die Lösung geheizt wird. Wässrige Lösungen von normalen und methylierten Cyclodextrinen stellen ein Modell dar, das es erlaubt, die Hydratation von Biomolekülen in deren wässrigen Lösungen zu studieren und somit besser den hydrophoben Effekt zu verstehen.

Das Hauptziel dieser Arbeit war die Anwendung der quasielastische Neutronenstreuung (QENS) für die Untersuchung der Dynamik von Solute und Hydratationswasser Molekülen sowie der Struktur der Hydrathülle in wässrigen Lösungen der CDs und mCDs. Röntgen und Neutronen Kleinwinkel Streuung (RKS und NRS) waren als ergänzende Methoden eingesetzt.

RKS und NKS zeigten, dass es keine Oligomere bzw. Aggregate in Lösungen von β -CD, γ -CD, per(2,6-O-methyl)- β -CD (DIMEB) und per(2,3,6-O-methyl)- γ -CD (TRIMEG) im Wasser gibt. Im Gegensatz zu β -CD und γ -CD werden Solute-Solute Wechselwirkungen in Lösungen von DIMEB und TRIMEG attraktiver mit steigender Temperatur und/oder Solute Konzentration. Dieser Befund erklärt kleine Unterschiede zwischen den Werten der Diffusionskonstanten, die von DIMEB mit QENS und PFG-NMR bestimmt wurden.

Bei der Analyse der QENS Spektren von DIMEB Lösungen wurde eine Komponente entdeckt, die einer Bewegung mit der charakteristischen Zeit von etwa 5 ps entspricht. Diese Bewegung wurde der Rotation von $-\text{CH}_3$ und $-\text{CH}_2\text{-O-CH}_3$ Gruppen zugeordnet. Die aus QENS Spektren bestimmte Rotationsdiffusionskonstante von DIMEB stieg bis ca. 30 °C mit der Temperatur an, wurde aber kleiner bei weiterem Temperaturanstieg; dies wurde mit dem Entstehen kurz lebender Aggregate bei hohen Temperaturen erklärt (entsprechend stärkeren attraktiven Solute-Solute Wechselwirkungen).

Die QENS Intensität von DIMEB und TRIMEG Lösungen in D_2O stieg sehr stark bei Werten des elastischen Momentum Transfers unter 0.4 \AA^{-1} ; ein solcher Anstieg war dagegen bei D_2O Lösungen von β - und γ -CD nicht der Fall. Nach der hier vorgeschlagenen Erklärung sind die Residenzzeiten der Hydratwasser Moleküle in DIMEB und TRIMEG Lösungen länger (als die in CDs Lösungen) und führen zu dem zusätzlichen kohärenten Streuanteil. Die Streuung von der Hydrathülle wurde in einem speziell entwickelten Modell berücksichtigt. Anwendung des Modells führte zur Bestimmung der Zahl der Hydratwasser Moleküle pro Solute Molekül (N_{HYD}); diese Zahl betrug etwa 60 für DIMEB und 70 für TRIMEG. Für DIMEB nahm N_{HYD} leicht ab bei Temperaturen über 40 °C, möglicherweise wegen des allmählichen Auflösens der Hydrathülle bei hohen Temperaturen.

Zum Schluss: die Methodologie und die Modelle, die in dieser Arbeit entwickelt bzw. benutzt wurden, können auch bei Studien von Lösungen anderer Biomoleküle angewendet werden.

Abstract

Native cyclodextrins (CDs) are oligosaccharides consisting of 6, 7 and 8 $\alpha(1\rightarrow4)$ linked D-glucose units called α -, β - and γ -CD, respectively; their solubility in water increases with temperature. Cyclodextrin derivatives with 2,6-OH or 2,3,6-OH groups being methylated (mCDs) show a negative temperature coefficient of solubility, i.e. they are well soluble in cold water and crystallize (are less soluble) upon heating of the solution. Aqueous solutions of native and methylated cyclodextrins represent a well-defined model system for studying the hydration of biomolecules in aqueous solutions and further understanding of the hydrophobic effect.

The main topic of the present work was the application of quasielastic neutron scattering (QENS) for the investigation of the dynamics of the solute and hydration water molecules together with the structure of the hydration shell in aqueous solutions of CDs and mCDs. As complementary techniques small-angle X-ray and neutron scattering (SAXS and SANS, respectively) were employed.

SAXS and SANS showed that no oligomers and aggregates exist in solutions of β -CD, γ -CD, per(2,6-O-methyl)- β -CD (DIMEB) and per(2,3,6-O-methyl)- γ -CD (TRIMEG) in water. As opposed to β -CD and γ -CD, the solute-solute interactions in solutions of DIMEB and TRIMEG were found to become more attractive with increasing temperature and/or solute concentration. This finding allowed to rationalize the discrepancies between values of the translational diffusion coefficient of DIMEB as determined by QENS and PFG-NMR.

The analysis of the QENS spectra of DIMEB solutions revealed the presence of a motion with a characteristic time of about 5 ps; this motion was attributed to the rotation of $-\text{CH}_3$ and $-\text{CH}_2\text{-O-CH}_3$ groups. The value of the rotational diffusion coefficient of DIMEB increased with temperature up to 30 °C but fell slightly upon further temperature increase, probably corresponding to the formation of transient aggregates at higher temperatures (due to stronger attractive solute-solute interactions).

A substantial increase of the QENS intensity in D_2O solutions of DIMEB and TRIMEG was observed for the values of elastic momentum transfer below 0.4 \AA^{-1} ; such an increase was not seen in the QENS spectra of D_2O solutions of β - and γ -CD. This finding was tentatively explained by an additional coherent scattering contribution due to the hydration shell of mCDs (apparently, hydration water molecules have greater residence times in DIMEB and TRIMEG solutions than in solutions of CDs). A model accounting for the scattering by the hydration shell was developed; its application to the QENS spectra resulted in the number of hydration water molecules per one solute molecule (N_{HYD}) to be about 60 and 70 for DIMEB and TRIMEG, respectively. For DIMEB, N_{HYD} decreased slightly above 40 °C, possibly due to a gradual disruption of the hydration shell with temperature.

Finally, the methodology and models developed and/or used for the analysis of the QENS spectra of CDs and mCDs solutions can be further applied in studies of protein solutions.

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