^{1}H-Nuclear Magnetic Resonance (NMR) Studies on the Inclusion Complex of Prostaglandin E_1 (PGE_1) with α-Cyclodextrin

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Prostaglandin E_1 is currently marketed as a freeze-dried injectable inclusion complex with α-cyclodextrin for the treatment of peripheral arterial diseases. α-Cyclodextrin is used as a stabilizing agent and to improve the dissolution characteristics of prostaglandin E_1. Upon dilution with the infusion medium, the inclusion complex dissociates almost completely as shown by NMR chemical shift measurements of the complexed and uncomplexed prostaglandin E_1. Nuclear Overhauser effect (NOE) measurements of the interacting atoms of α-cyclodextrin and prostaglandin E_1 provide insight into the structure of the complex.

KEY WORDS: prostaglandin E_1; α-cyclodextrin; ^{1}H-nuclear magnetic resonance (NMR); nuclear Overhauser effect (NOE) difference measurement; complex formation.

INTRODUCTION

Cyclodextrins (CDs) are routinely used in drug formulations to improve the stability, solubility, or bioavailability of drugs. Cyclodextrins are cyclic oligosaccharides, having the capability to form complexes in the presence of water with guest molecules that are inserted with their hydrophobic moiety into the inner CD core. These features make CDs especially useful in pharmaceutical formulations, without influencing pharmacological effects.

Whereas the applications of drug CD complexes and the methods for their formulation have been described extensively (1,2), only a few papers discuss the driving forces behind complex formation (3–8,10). Entropy gain seems to be the main force for the association of CD and guest molecules. Lewis and Hansen (7) and Hardee *et al.* (3) studied the complexes of a number of guest molecules by microcalorimetry and found a compensation effect, i.e., an approximately linear correlation between the enthalpy changes and the entropy changes, a phenomenon which is frequently observed in highly ordered liquids like water. According to Lewis and Hansen a reaction in which the solvent becomes more disordered usually results in a favorable entropy change, however, this process is accompanied by the loss of some solvent–solvent bonds, causing an unfavorable enthalpy change. In the case of the relatively unspecific bindings of CDs to guest molecules, the authors found an increase in enthalpy, probably because of the gain in more ordered water molecules and, as a consequence, more hydrogen bonds after complexation. Thus the driving force for complex formation is probably due to different solvation states of water itself rather than direct interactions between CD and guest molecules. This view is discrepant from that of Gelb *et al.* (6). On the basis of thermodynamic and ^{13}C-NMR measurements, they evaluate the enthalpy change as a result of dipolar interactions between guest and host molecules.

The main analytic methods for investigation of geometry, stoichiometry, binding enthalpy, and entropy of complexes involve pH potentiometric methods (6), microcalorimetry (3), circular dichroism (1), ^{13}C-NMR and ^{1}H-NMR methods (1), HPLC (8), X-ray diffractometry, differential scanning calorimetry, and solubility measurements (1). From most techniques only indirect conclusions can be drawn, as they disturb the drug–CD complex equilibrium and the spatial geometry of the complex cannot be determined.

X-ray analysis of the structure of a monocrystal of a substance shows a three-dimensional but static picture of the molecule, which may not resemble the real dynamic conditions in solution. Pulse-NMR spectroscopy gives a clear picture of the analyzing substance—especially under near-physiological conditions. Until recently the limitating factor was the relatively high substance quantity required (0.2–0.5 mg/ml). Spectrometers with field strengths up to 600 MHz now allow measurements at concentrations of about 200 ng/ml, which may be encountered under physiological conditions. Chemical shift and coupling constants between binding atomic nuclei define a two-dimensional picture, whereas the nuclear Overhauser effect (NOE) establishes a three-dimensional relation of interacting atomic nuclei by means of space-reciprocal action if the nuclei sufficiently approach each other (the distance should be max. 4 Å). NOE measurements applied for two- and three-dimensional characteristics of peptides and proteins in solutions have been very successful (11,12).

Interesting questions arise in the case of therapeutically used α-cyclodextrin/PGE_1 preparations. Normally PGE_1 has a very limited stability even if cooled. However, in a mixture with α-cyclodextrin, PGE_1 shows an almost indefinite stability. We asked, first, whether complex formation can account for the described effect, for example, with a host–guest geometry [as proposed by Uekama and Hirayama (8)] and, second, whether the drug is completely released under infusion conditions at a concentration of 400 ng/ml. Thermodynamic and cyrstallographic data do not provide an exact answer.

This paper describes our work on the interaction of α-cyclodextrin (α-CD) with prostaglandin E_1 (PGE_1). With the use of NMR measurements and molecular modeling we studied the extent of complex formation and the complex geometry of PGE_1/α-CD in solutions.

MATERIALS AND METHODS

Prostavasin resp. Prostandin as a prostaglandin E_1 (PGE_1) formulation is currently marketed in Germany by

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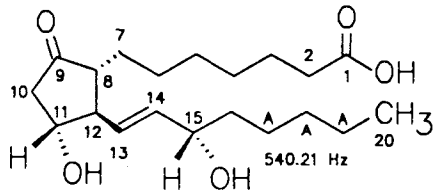

Fig. 1. Prostaglandin E_1 (PGE_1).

Schwarz Pharma AG and in Japan by Ono Pharmaceutical Co. One ampoule of the product—obtained by lyophilization (9)—contains 20 μg PGE_1 and 646.7 μg α-CD. α-CD and PGE_1, for reasons of comparison, were obtained from Schwarz Pharma, Monheim, and Sigma, München; D_2O was from Merck, Darmstadt.

The ^{1}H-NMR spectra were taken at room temperature using D_2O as solvent and internal standard (δ = 4.6 ppm) on the high-field NMR spectrometers AM 400, 500, and 600 of Bruker, Karlsruhe. Bruker standard software was used for measurements and analysis of the NMR spectra. The assignment of the PGE_1 and α-CD protons resulted from two-dimensional (2D) ^{1}H,^{1}H COSY experiments and the respective proton shift values were related to D_2O as internal standard.

Proton spectra were obtained from PGE_1 and from the mixture PGE_1/α-CD (in the following, called PCD) within the concentration range of 400 ng to 200 μg PGE_1/ml D_2O. The effect of dilution of PCD on the shift values (Hz) of proton group A were determined. The percentage of the dissociation of PCD was calculated as follows: the value of complete dissociation (100%) was obtained from the proton shift value of group A in a reference solution of 400 ng PGE_1 in 1 ml D_2O (540.21 Hz); extrapolation of the dilution to zero gave the shift value of proton group A at an infinite concentration of PCD (623 Hz), which corresponds to zero dissociation of PCD. NOE difference measurements concerning PCD were taken from a PGE_1 concentration of 200 μg/ml.

RESULTS

The proton spectra of PGE_1 and α-CD taken independently in D_2O at 200 μg to 400 ng show only minimal shift differences of individual protons: <2 Hz. However, the ^{1}H-NMR spectrum of PCD (200 μg PGE_1 and 6.46 mg α-CD/ml D_2O) shows considerable shift differences of the PGE_1 protons in comparison to the solution of PGE_1 without α-CD. In particular, proton group A (corresponds to protons 17/18/19 in PGE_1; Fig. 1) shows the greatest shift difference (71 Hz) in comparison to the solution of PGE_1 without α-CD. When the achieved δ values of proton group A at different PCD concentrations (Table I) are plotted against PCD dilution (Fig. 2), a straight line results, which changes to an asymptote at high dilutions, reaching the shift value of the free PGE_1 solution (540.21 Hz). If the curve at low dilutions is extrapolated to zero, the shift value of proton group A at an infinite concentration is obtained (623 Hz). From these two values, the percentage of the dissociation of PCD at the respective concentrations can be calculated. The corresponding values are shown in Fig. 2 against the dilution of PCD.

NOE difference measurements of α-CD protons 3 and 5 in PCD (200 μg PGE_1 and 6.46 mg α-CD/ml D_2O) show difference signals for proton group A, H-20, and H-15 in the PGE_1 component of PCD (Fig. 3).

DISCUSSION

In the ^{1}H-NMR spectrum of PCD proton group A shows the greatest shift difference compared with the free PGE_1 solution. There can be different reasons for this effect, for example, changes in the molecule geometry even in distant centers, intermolecular interactions by association, or formation of an inclusion compound. An exact description is successful only if it is possible to attribute the observed effect to the α-CD cavity or to the α-CD outer surface and to use the respective protons as probes. This is possible by measurement of the nuclear Overhauser effect (NOE). Indeed, NOE difference signals are observed between the inner protons 3 and 5 of α-CD and proton group A of PGE_1 in PCD, so that in an inclusion complex the ω side chain of PGE_1 must be located in the cavity of α-CD. One can find in α-CD the inner proton 3 in the wide opening of the truncated cone and proton 5 at the narrower side (Fig. 3). Now H-3 (α-CD) shows a NOE effect to H-15 (PGE_1), which is not detectable for H-5 (α-CD). On the other hand, H-5 (α-CD) shows a NOE effect to proton group A and H-20. Thus it follows the position of α-CD on the side chain of PGE_1 as

Table I. Chemical Shifts of Proton Group A upon PCD Dilution and PCD Dissociation

PCD[a] in D_2O (ml)	Conc. of PGE_1 (μg/ml)	Shift values[b] of A (Hz)	Dissociation of PCD (%)
0.1	200	611.28	16.1
0.2	100	600.08	29.3
0.3	66.67	594.30	36.1
0.7	28.5	577.72	55.7
1	20	570.40	64.3
2	10	556.96	80.2
4	5	547.81	91.0
8	2.5	544.15	95.3
16	1.25	542.71	97.0
32	0.62	541.71	98.2
50	0.40	541.21	98.8

[a] 20 μg PGE_1 and 646.7 μg α-CD.

[b] For comparison, the shift value of proton group A of a PGE_1 solution (0.4 μg/ml) is 540.21 Hz.

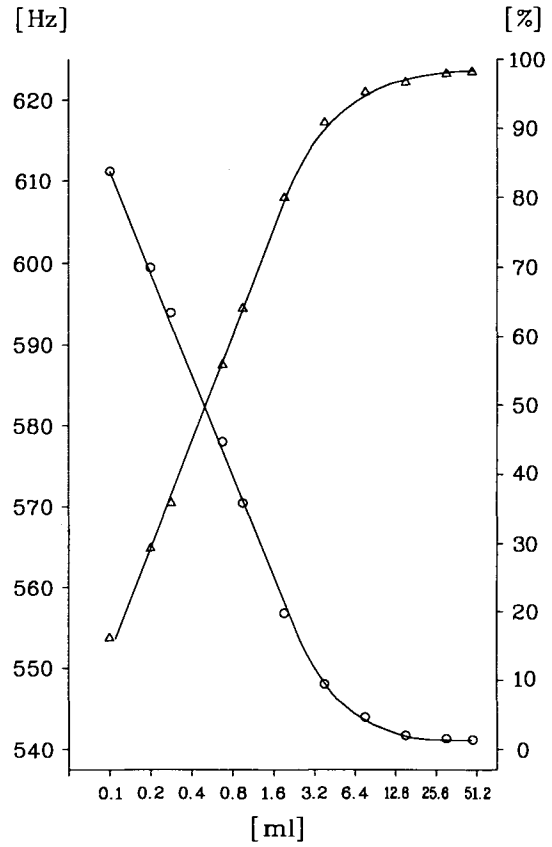

Fig. 2. Shift values (Hz) of protons A in PCD as a function of dilution (ml) (○); calculated percentage of the dissociation of PCD (△).

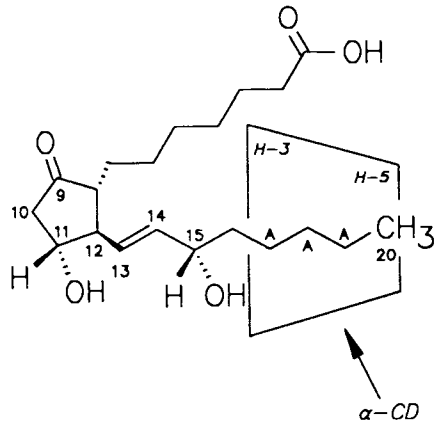

Fig. 3. Prostaglandin E_1/α-cyclodextrin inclusion complex. (A) Proton group A of PGE_1.

shown in Fig. 3; hence, the wide opening of the α-CD truncated cone points into the direction of the cyclopentanone ring of PGE_1.

The 1:1-complex between α-CD and PGE_1 at high concentrations, as determined by NOE measurements, accounts for the observed shift differences of proton group A in PCD. It is traced back to anisotropy effects which the inner atoms of the α-CD cavity have on the ω side chain of PGE_1. The observed decrease in the shift difference upon dilution, approaching the value of signal A in the free PGE_1, corresponds in this case to the dissociation of the α-CD/PGE_1 complex, so that at a concentration of 400 ng PGE_1 and 12.93 μg α-CD/ml D_2O (this means a solution of 1 ampoule Prostavasin in 50 ml isotonic salt solution), PGE_1 is uncomplexed—this means freely available—to an amount of 98.8%.

In summary, NOE effects and shift differences in the ^{1}H-NMR spectra of PCD and free PGE_1 show that α-CD and PGE_1 at high concentrations form a 1:1-complex in D_2O, which at low concentrations or high dilutions (for example, 400 ng PGE_1 and 12.93 μg α-CD/ml D_2O), dissociates completely into the components α-CD and PGE_1.

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