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Binding of Vitamin A by β-Cyclodextrin and Heptakis(2,6-*O*-dimethyl)-β-Cyclodextrin

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Abstract. Inclusion complexation of all-*trans*-retinol, retinal and retinoic acid with β-cyclodextrin (β-CD) and heptakis(2,6-*O*-dimethyl)-β-cyclodextrin (DM-β-CD) were investigated by means of UV-vis spectroscopy. The association constants (K_a) obtained for vitamin A with DM-β-CD is greater than with β-CD. On the other hand, for the same host compound K_a values of retinol, retinal and retinoic acid are very close to each other.

Key words: Association constant, DM-β-CD, β-CD, vitamin A.

1. Introduction

The importance of vitamin A for human health has been stressed in recent studies [1]. In the proper doses it is crucial for fetal development, for vision, and for cell proliferation and differentiation throughout life [2]. However, as vitamin A is a lipophilic compound, the question raised is how it and its derivatives are processed in the gut, transported through the intestinal lymph into the general circulation, stored in cells, and mobilized for use; this is an area of great current interest [3]. In our previous paper [4] we reported on the interaction of vitamin A with anionic, cationic and neutral micelles in water.

The inclusion complexes of β-cyclodextrin (β-CD) with retinyl acetate [5] and retinoic acid [6] in aqueous solution have been reported. An NMR study [7] indicated that in the complex the cyclohexyl ring of retinyl acetate was embedded in the cavity of heptakis(2,6-*O*-dimethyl)-β-cyclodextrin (DM-β-CD). The solubility of DM-β-CD in water is greater than that of β-CD [8], therefore, the solubility of fat-soluble vitamin A is increased in water in the presence of DM-β-CD.

Vitamin A can exist in alcoholic (retinol), aldehydic (retinal) and acidic (retinoic acid) forms. As mentioned above, vitamin A and its derivatives can form inclusion complexes with β-CD and DM-β-CD. Cyclodextrins are water-soluble host compounds which are widely used as enzymatic models. In this paper we report a

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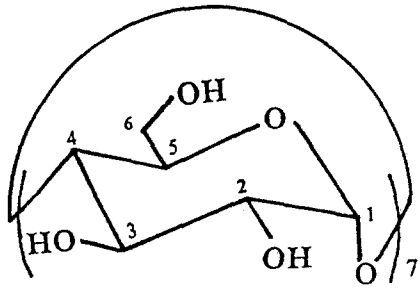
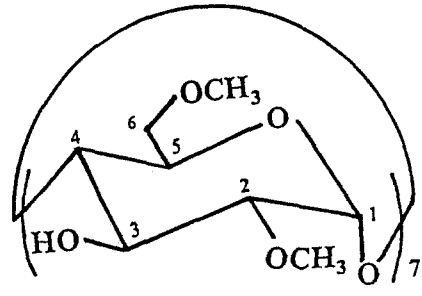
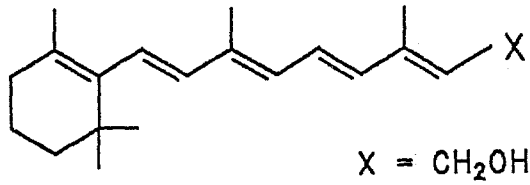


β–CD



DM–β–CD



X = CH_2OH, CHO, COOH

quantitative study on the binding of all-*trans*-retinol, retinal and retinoic acid by β-CD and DM-β-CD in MeOH/H_2O (1 : 10 v/v) binary solvents.

2. Experimental

2.1. INSTRUMENTATION

UV-vis spectra were measured with a Hitachi 557 UV-vis spectrophotometer. The cell compartments were connected to an external thermostat to control the temperature at 25 ± 0.1 °C.

2.2. MATERIALS

All-*trans*-retinol (Fluka), >99%, all-*trans*-retinal (Aldrich), 99%, all-*trans*-retinoic acid (Merk), >99%, and β-cyclodextrin (Tokyo Kasei), 99%, were used as received. Heptakis(2,6-*O*-dimethyl)-β-cyclodextrin was prepared according to the procedure described in the literature [9]. Triply distilled water was employed.

2.3. PREPARATION OF SAMPLES

The solutions of all-*trans*-retinol (7.2×10^{-6} mol/dm^3), all-*trans*-retinal (7.0×10^{-6} mol/dm^3), and all-*trans*-retinoic acid (7.0×10^{-6} mol/dm^3) containing 2.0×10^{-4} mol/dm^3 to 6.2×10^{-3} mol/dm^3 of β-CD or DM-β-CD were prepared by dissolving the weighed vitamin A and cyclodextrins in methanol–water (1 : 10 v/v)

binary solvents. After degassing with an argon stream, the solution was ultrasonicated for 10 min at room temperature, and allowed to stand for several hours in the dark.

3. Discussion

Vitamin A is almost insoluble in water; the solubility is improved somewhat in the presence of 10 vol-% of methanol in water. A previous study [10] showed that the presence of alcohol even up to 20 vol.-% does not influence the association constants (K_a) for the inclusion of cyclodextrins with isomeric chloronitrobenzenes in aqueous solution. The K_a value for inclusion of methanol with β-CD is only 0.32 dm^3/mol [11], which can reasonably be neglected compared with the K_a value for a suitable guest compound.

Broad absorption bands at 295 nm for retinol, 373 nm for retinal and 326 nm for retinoic acid were recorded in MeOH/H_2O (1 : 10 v/v) binary solvents. When β-CD or DM-β-CD was added to the solution, the absorption band shifted from 295 to 328 nm, which is in agreement with that in n-octane solvent and in the micelles for retinol [4], from 373 nm to 384 nm for retinal, and 326 nm to 335 nm for retinoic acid, and the intensities increased markedly. The intensity increase was observed to vary as a function of the concentrations of host compounds. The variations of wavelength and absorbance of retinol in the absence and in the presence of β-CD with different concentrations are shown in Figure 1. Similar results were obtained for retinal and retinoic acid.

The changes in the absorption spectra were interpreted as a result of the inclusion complexation of vitamin A with β-CD and DM-β-CD. The inclusion process was considered and the absorption data were treated on the basis of 1 : 1 inclusion complex formation.

$$\mathrm{CD} + \mathrm{VA} \overset{K_a}{\rightleftharpoons} \mathrm{CD \cdot VA}$$

The association constant K_a was obtained by treating the absorbance measurements according to the Benesi–Hildebrand equation [12].

$$\frac{l[\mathrm{CD}][\mathrm{VA}]}{\Delta A} = \frac{[\mathrm{CD}] + [\mathrm{VA}]}{\Delta \epsilon} + \frac{1}{K_a \Delta \epsilon}$$

Where l is the optical path length of the cell used (l = 1 cm). [CD] and [VA] represent the concentrations (mol/dm^3) of cyclodextrin and vitamin A, respectively. In this work the [VA] in the first term on the right-hand side of the equation was retained, it is sometimes omitted in the literature [13]. ΔA is the change in the absorbance of vitamin A upon the addition of cyclodextrins, and $\Delta\epsilon$ is the difference in the molar absorptivities between complexed and free vitamin A ($\epsilon_{\mathrm{CD \cdot VA}} - \epsilon_{\mathrm{VA}}$).

Plotting [CD][VA]/ΔA against ([CD] + [VA]) gives a straight line with slope equal to $1/\Delta\epsilon$ and intercept equal to $1/K_a\Delta\epsilon$. The association constant K_a was obtained from the slope/intercept ratio (Figure 2).

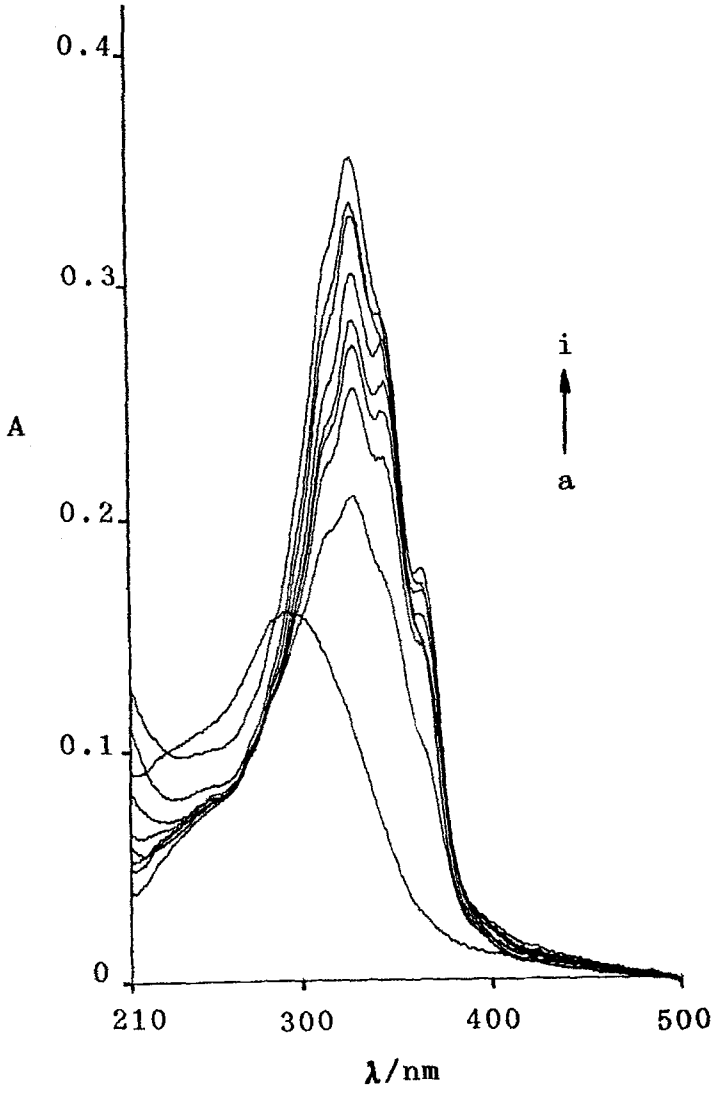


Fig. 1. Absorption spectra of retinol (7.2×10^{-6} mol/cm^3) recorded in the presence of 0 (a), 2.3×10^{-4} (b), 4.2×10^{-4} (c), 6.2×10^{-4} (d), 8.1×10^{-4} (e), 9.9×10^{-4} (f), 2.0×10^{-3} (g), 4.1×10^{-3} (h), and 6.0×10^{-3} (i) mol/dm^3 β-CD in MeOH/H_2O (1 : 10 v/v) binary solvents.

Table I lists the association constants of retinol, retinal and retinoic acid with β-CD and DM-β-CD. From Table I it can be seen that the K_a value for the different kinds of vitamin A molecules are very close to each other. This means that the influence of the terminal functional groups on the stabilities of inclusion complexes is very small. On the other hand, the association constants of vitamin A with DM-β-CD are always greater than with β-CD. This finding also indicates

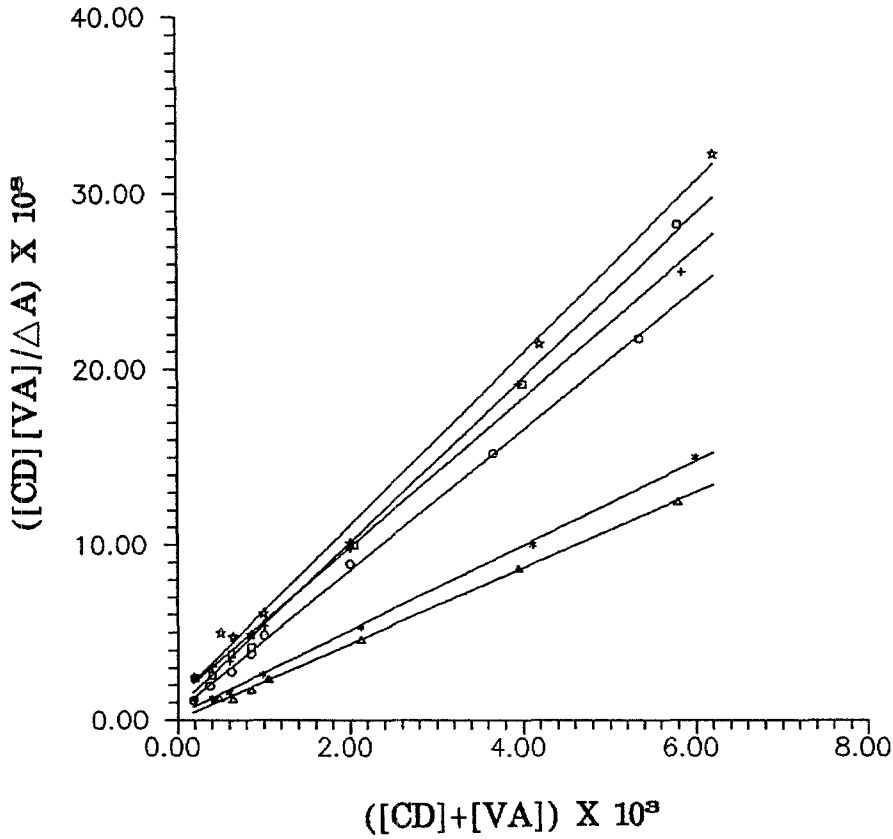


Fig. 2. Plots of [CD][VA]/ΔA against ([CD] + [VA]) for the inclusion complexation of all-*trans*-retinol with β-CD (○) and DM-β-CD (×), all-*trans*-retinal with β-CD (□) and DM-β-CD (☆), and all-*trans*-retinoic acid with β-CD (*), and DM-β-CD (△) in MeOH/H_2O (1 : 10 v/v) binary dolvents.

TABLE I. Association constant K_a (dm^3/mol) for the inclusion complexation of vitamin A with β-CD and DM-β-CD in MeOH/H_2O.

Compound	β-CD	DM-β-CD
Retinol	3.62×10^3	5.86×10^3
Retinal	3.64×10^3	5.63×10^3
Retinoic acid	3.35×10^3	5.57×10^3

that the cyclohexyl ring of the vitamin A molecule is included in the cavity of the CD. The stability of the inclusion complex is dependent on the contact area between host and guest [14]. DM-β-CD provides a deeper cavity than does β-CD, and therefore a large contact area is expected. About 10 kJ/mol of stability energy

was gained in forming the inclusion complexes of vitamin A with DM-β-CD than with β-CD.

4. Conclusion

UV-vis absorption measurements showed that stable inclusion complexes of vitamin A with β-CD and DM-β-CD were formed. The absorbance of vitamin A is dependent on the concentration of cyclodextrin. Little effect of the various functional groups (CH_2OH, CHO, and COOH) of vitamin A on the stabilities of the inclusion complexes was observed. On the other hand, since the cavity of DM-β-CD is deeper than that of β-CD, vitamin A can form more stable inclusion complexes with DM-β-CD.

Acknowledgements

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