Biochemical Pharmacology, Vol. 42, No. 7, pp. 1502–1503, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Sulfation and hemolytic activity of cyclodextrin

(*Received* 18 *January* 1991; *accepted* 12 *June* 1991)

Knowledge of the hemolytic activity of cyclodextrins is important in view of the growing number of potential applications in pharmacology and therapy. Cyclodextrins are cyclic, doughnut-shaped oligosaccharides of 6, 7, or 8 glucopyranose units (α-, β- or γ-cyclodextrins, respectively) [1, 2]. The large number of hydroxyl groups (3 per glucose unit) located peripherally provide water solubility. The central cavities are hydrophobic and can, by inclusion, complex hydrophobic molecular entities. This combination of properties has been utilized to "solubilize" pharmaceutically active agents of limited solubilities at much larger effective (free plus complexed) concentrations [1–4]. Some substitutions of hydroxyl groups on the parent cyclodextrin can increase their solubility. Thereby, a larger quantity of pharmaceutical can be complexed and "solubilized". Examples are (O-methyl) [2–4], (O-2-hydroxypropyl) [5] and (O-2-hydroxyethyl) [6] substituted cyclodextrins.

Recently, sulfated cyclodextrins have been shown to have unusual biological activities: (1) anti-angiogenic activity in combination with suitable "angiostatic" steroids [7, 8], (2) strong binding affinity for fibroblast growth factor [9], and (3) a capability of inhibiting cellular infection by HIV retrovirus.* In view of the spectrum of broadening interest in cyclodextrins administered with or as therapeutic agents, it is of interest to examine their hemolytic activity.

Materials and Methods

β-Cyclodextrin (β-CD), 2-hydroxyethyl-β-cyclodextrin (β-CD-4Et), 2-hydroxypropyl-β-cyclodextrin (β-CD-4Pr) as well as β-cyclodextrin-tetradecasulfate sodium (β-CD-14S) were supplied by G. Reed, American Maize Products, Hammond, IN. Heptakis-(2-6-di-*O*-methyl)-β-cyclodextrin (β-CD-14M), also known as DIMEB, was supplied by J. Sejtli of Chinon Pharmaceutical and Chemical Works, Budapest. Heparin was purchased from the Sigma Chemical Co., St. Louis, MO.

Hemolytic activity was measured by a procedure patterned after that of Jodal *et al.* [10]. Sodium citrate was added (0.47%) to fresh human blood collected from healthy laboratory personnel. The supernatant erythrocyte fraction obtained after centrifugation (10 min at 400 *g*) was washed twice with isotonic phosphate buffer and diluted with the same buffer to 10% of the hematocrit value. Test material solution (3.6, 1.8, and 0.9 mL) was mixed with 0.4, 0.2, and 0.1 mL, respectively, of erythrocyte suspension, incubated at 37° and then centrifuged twice for 2 min each at 400 *g*. The supernatant was measured for absorbance at 543 nm using a Gilford spectrophotometer. The degree of hemolysis was determined by reference to that observed in samples containing saline solution only, and to that observed after sonicating the erythrocyte suspension to provide an absorbance value for 100% hemoglobin content.

Results and Discussion

β-Cyclodextrin is the most frequently studied cyclodextrin. Appreciable hemolytic activity at 37° in isotonic solution has been reported to occur [10, 11] at about 3–5 mg/mL of β-cyclodextrin, and at or below 1 mg/mL for β-cyclodextrin with 14 methyl group substituents (DIMEB). Other substitutions have been shown to decrease hemolytic activity [10–12]. We have now measured and compared hemolytic activity of the hydroxyalkyl- and the highly sulfated β-cyclodextrins with that of β-cyclodextrin and DIMEB (β-CD-14M).

Figure 1 shows the data obtained for β-cyclodextrin and β-CD-14M. The onset of hemolysis was near 6 mg/mL for β-cyclodextrin and near 2 mg/mL for β-CD-14M. [Data for β-cyclodextrin became unreliable (generally too low) near 12 mg/mL as it approached its solubility limit (18 mg/mL at 25°)]. Figure 2 shows the results obtained for 2-hydroxyethyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and β-cyclodextrin-tetradecasulfate sodium. Table 1 presents data obtained for heparin and for β-cyclodextrin-tetradecasulfate sodium in an attempt to explore hemolysis behavior at very high concentrations.

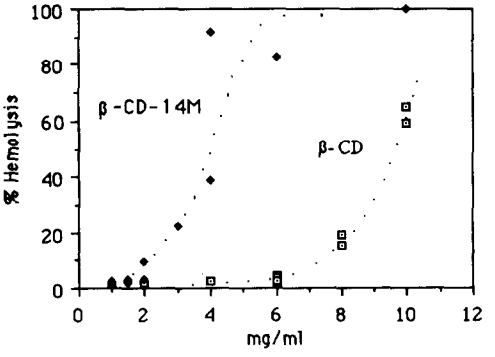

Fig. 1. Hemolysis of β-cyclodextrin (β-CD) and heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (β-CD-14M).

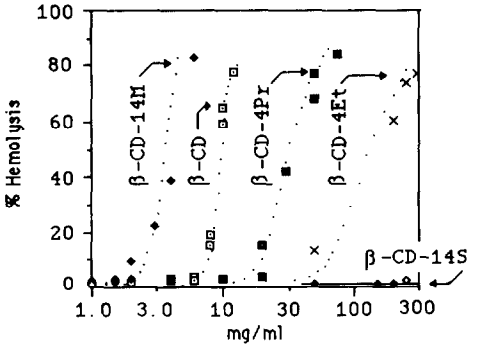

Fig. 2. Hemolysis of 2-hydroxypropyl-β-cyclodextrin (β-CD-4Pr, 4 substituents/molecule); 2-hydroxyethyl-β-cyclodextrin (β-CD-4Et, 4 substitutents/molecule); and of β-cyclodextrin-tetradecasulfate sodium (β-CD-14S, 14 substituents/molecule) in comparison to β-cyclodextrin (β-CD) and heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (β-CD-14M or DIMEB).

* Weiner DB, Williams WV, Weisz PB and Greene MI, Synthetic cyclodextrin derivatives inhibit HIV infection *in vitro*. Manuscript submitted for publication.

Table 1. Results of hemolytic activity tests of heparin and β-CD-14S at very high concentrations

Concn (mg/mL)	Percent hemolysis observed			
	β-CD-14S*		Heparin*	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
50	3.6	1.3		
100	2.7	0.0		
150	3.0	1.3		
200	2.7	0.0	2.5	2.0
300	1.4	2.0	3.8	2.8
400	2.4	2.6	3.8	12.0

* Data were obtained from two separate experiments.

The magnitudes of hemolysis for the reference materials β-cyclodextrin and β-CD-14M are in good agreement with previous reports [10–12]. The hydroxypropyl and especially the hydroxyethyl derivatives were significantly less active. The highly sulfated cyclodextrin, β-CD-14S, had no demonstrable hemolytic effect up to concentrations of hundreds of mg/mL. To demonstrate this quantitative comparison, we have presented the data in Fig. 2 using a logarithmic concentration scale on the abscissa.

It was also of interest to examine the hemolytic activity of heparin, in view of certain similarities in its properties and those of β-CD-14S. Table 1 indicates no demonstrable activity for physiologically reasonable concentrations. The small hemolytic effects found for very high concentrations are likely to be artifacts resulting from hydrodynamic effects (e.g. shear) due to the high viscosity of the fluids at these high saccharide concentrations (above 20 weight percent).

There appeared to be a total absence of hemolytic activity of the polysulfated cyclodextrin. This is consistent with the suggestion [13–15] that hemolytic activity involves interaction with and/or extraction of phospholipids, since the polysulfate would be expected to have the least, if any, lyophilic binding capability.

Acknowledgements—The authors thank Pamela S. Howard and Dr. Thomas Murray for collection of blood samples. This work was supported, in part, by NIH Grant HL34005 and by a grant for interdisciplinary research by the Mobil Foundation.

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