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Inclusion Complex of Acetohexamide with β-Cyclodextrin and Its Hypoglycemic Activity in Rabbit[1]

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Inclusion complex formation of acetohexamide with β-cyclodextrin in water and in solid state was ascertained by solubility method, circular dichroism spectroscopy, and powder X-ray diffractometry. A solid complex of acetohexamide with β-cyclodextrin in 1:2 molar ratio was prepared, and its dissolution behavior in water and hypoglycemic activity in rabbit were examined. Improved dissolution characteristic of acetohexamide by inclusion complexation resulted in potentiation of the reduction in blood glucose levels in rabbit, which may be due to the increase in absorption of the drug.

Keywords——inclusion complex; acetohexamide; β-cyclodextrin; phase solubility diagram; induced circular dichroism; powder X-ray diffraction pattern; dissolution profile; hypoglycemic activity; blood glucose level in rabbit

Acetohexamide [3-cyclohexyl-1-(*p*-acetylphenylsulfonyl)-urea], one of the hypoglycemic sulfonylureas, is widely used orally to lower the blood glucose level in diabetic patients. However, the compound is slightly water soluble (solubility at 25°=0.03 mg/ml) and, may result in poor absorption characteristics. Molecular complexes of drug with other chemicals have frequently proposed for inclusion in dosage form to improve the dispensing of medication.[3] Cyclodextrin complexation has been extensively applied to enhance the solubility,[4] dissolution rate,[5] membrane permeability,[6] and bioavailability[7] of slightly soluble drugs. Thus, this investigation was undertaken for obtaining information on inclusion complexation of acetohexamide with β-cyclodextrin, anticipating an improved dissolution characteristic and bioavailability of the drug. Study on the hypoglycemic activity of acetohexamide and its β-cyclodextrin complex was conducted by oral administration in rabbit, measuring the blood glucose levels.

Experimental

Materials——Acetohexamide and β-cyclodextrin were kindly supplied from Shionogi Pharmaceutical Co., Ltd., and Teijin Ltd., respectively. All other chemicals and solvents were analytical reagent grade. Deionized double-distilled water was used throughout the study.

Solubility Studies——Acetohexamide, 7 mg (4.3×10^{-3} M) was added to water or β-cyclodextrin solution (varied from 0.1 to 2.0×10^{-2} M) in glass stoppered tube and then sealed and shaken at 25°. After equilibration

1) A part of this study was presented at 98th Annual Meeting of Pharmaceutical Society of Japan, Okayama, April 1978.
2) Location: *5-1, Oe-honmachi, Kumamoto 862, Japan.*
3) M.C. Meyer, "Dispensing of Medication," 7th ed., E.W. Martin, Ed., Mack Publishing Co., Easton, Pa., 1971, pp. 558—591.
4) *a*) J.L. Lach and J. Cohen, *J. Pharm. Sci.*, **52**, 137 (1963); *b*) A.L. Thakkar, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *ibid.*, **61**, 1841 (1972); *c*) K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, *ibid.*, **66**, 706 (1977).
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6) M. Nakano, K. Juni, and T. Arita, *J. Pharm. Sci.*, **65**, 709 (1976).
7) K.H. Frömming and I. Weyerman, *Arzneim-Forsh.*, **23**, 424 (1973); K. Koizumi and Y. Kidera, *Yakugaku Zasshi*, **97**, 705 (1977).

was attained (about 2 weeks), an aliquot was centrifuged and sampled through a cotton filter attached pipette. A 0.5 ml of sample solution was diluted with 0.1 M phosphate buffer (pH 7.0) and assayed by ultra-violet (UV) spectrophotometry at 248 nm.

Preparation of Complex——According to phase solubility diagram (see Fig. 1), 0.21 g of acetohexamide and 2.5 g of β-cyclodextrin were added in 150 ml water and then sealed and shaken at 25° for 2 weeks. The complex precipitated as a microcrystalline powder was filtered, washed with a little amount of water, and then dried under vacuum at room temperature for 24 hr. This powder corresponded to 1: 2 acetohexamide-β-cyclodextrin which has a molecular weight of 2592 g±3%.

Dissolution Rate Studies——Rotating Disk Method:[8] The sample powder (100 mesh) was compressed into cylindrical tablet (diameter, 20 mm; thickness, 1.4 mm; and weight, 500 mg±2%) under vacuum at high pressure (about 150 kg/cm²). Release of acetohexamide was measured using a rotating disk apparatus in 150 ml of water at 58 rpm and at 25°. Corrections were applied for cumulative dilution caused by replacement of sample by equal volume of the original medium in same temperature. The tablets maintained a constant shape throughout the measurements. Dispersed Amount Method:[8] The equivalent of 10 mg of acetohexamide as a 100 mesh powder was weighed and put in dissolution cell. The dissolution medium (25 ml) was maintained at 25° and stirred at 150 rpm. At an appropriate interval, 0.5 ml of solution was sampled out by a cotton filter attached pipette, and diluted with 0.1 M phosphate buffer (pH 7.0). Concentration of acetohexamide was assayed by UV spectrophotometry.

Spectral Measurements——The circular dichroism (CD) and UV spectra were taken by a Jasco J-40 AS recording spectropolarimeter and a Shimadzu UV-200 spectrophotometer, respectively, in 0.1 M phosphate buffer (pH 7.0) at 25°. The CD spectra were expressed in terms of molar ellipticity, $[\theta]$.

X-Ray Diffraction Studies——Powder X-ray diffractometry was carried out using a Geiger Flex 2012 X-Ray Diffraction Analyzer (Rigaku Denki Co., Ltd.).

Measurements of Blood Glucose in Rabbit——Male rabbits weighing 2.5—3.0 kg were kept on a standard diet and made to fast for about 24 hr prior to experiments. The equivalent of 30 mg/kg of acetohexamide (100 mesh powder) was administered orally as a suspension in 80 ml water. At least 7 days were allowed to elapse between the blood glucose estimation in each case to allow the animal to recover from the effect of the previous drug. The blood glucose levels were measured by Somogi-Nelson method.[9]

Results and Discussion

Phase Solubility Diagram

Complex formation of acetohexamide with β-cyclodextrin was studied by solubility method.[10] Figure 1 shows an equilibrium phase solubility diagram obtained for acetohexamide-β-cyclodextrin system in water. The solubility of acetohexamide increased by the addition of β-cyclodextrin, showing a feature of B_S type phase diagram.[10] In higher concentration range of β-cyclodextrin, a solid complex was precipitated. Stoichiometry of the complex was then analyzed chemically, and found to be 1: 2 (acetohexamide: β-cyclodextrin). The result was in good accordance with that analyzed from the data in plateau region of solubility diagram. Apparent stability constant, K', of the complex was estimated ($K'=700\ \mathrm{M}^{-1}$) from initial straight line portion of the solubility diagram. This value was the largest among the previously reported sulfonylurea-β-cyclodextrin complexes.[11] Carbon 13 NMR study suggested that β-cyclodextrin is capable to interact with not only phenyl ring but also cyclohexyl moiety of acetohexamide.[12] This

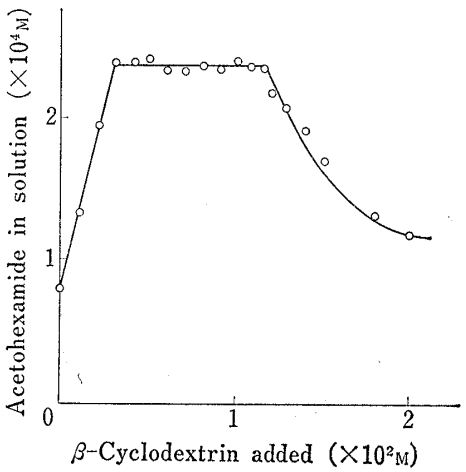

Fig. 1. Solubility of Acetohexamide as a Function of β-Cyclodextrin Concentration in Water at 25°

8) H. Nogami, T. Nagai, and T. Yotsuyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 499 (1969).
9) N. Nelson, *J. Biol. Chem.*, **153**, 375 (1944).
10) T. Higuchi and K.A. Connors, "Advance in Analytical Chemistry and Instrumentation," C.N. Reilly, Ed., Interscience, New York, N.Y., 1965, pp. 197—212.
11) K. Uekama, F. Hirayama, S. Nasu, N. Matsuo, and T. Irie, *Chem. Pharm. Bull.* (Tokyo), **26**, 3477 (1978).
12) K. Uekama, F. Hirayama, N. Matsuo, and H. Koinuma, *Chem. Lett.*, **1978**, 703.

may substantially result in 1:2 inclusion complex formation with large stability constant.

Further Evidence of Inclusion Complexation

Solubility study suggested that acetohexamide forms soluble complex with β-cyclodextrin in water. This interaction was further examined by circular dichroism (CD) and X-ray diffractometry.

When optically inactive compounds form inclusion complexes with cyclodextrins, these compounds are known to exhibit optical activity.[4b,13] In acetohexamide-β-cyclodextrin system, new CD band was induced with a positive sign peak at 242 nm in UV absorption region of acetohexamide, as shown in Fig. 2, where distinct UV spectral change was also accompanied. This may indicate that the drug chromophore was located within an asymmetric cavity of β-cyclodextrin.

Figure 3 shows the powder X-ray diffraction pattern of the complex in comparison with that of physical mixture in the same molar ratio. Diffraction pattern of the physical mixture was found to

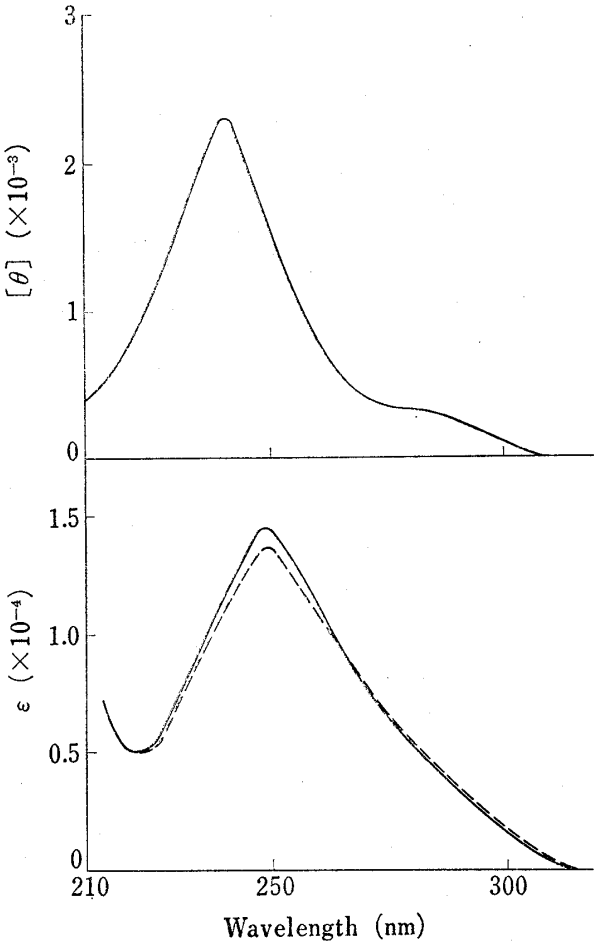

Fig. 2. Circular Dichroism (upper) and UV Absorption Spectra (lower) of Acetohexamide-β-Cyclodextrin System in 0.1 M Phosphate Buffer (pH 7.0)

——: acetohexamide (6.15×10^{-5} M) alone,
——: acetohexamide (6.15×10^{-5} M) + β-cyclodextrin (1.0×10^{-2} M).

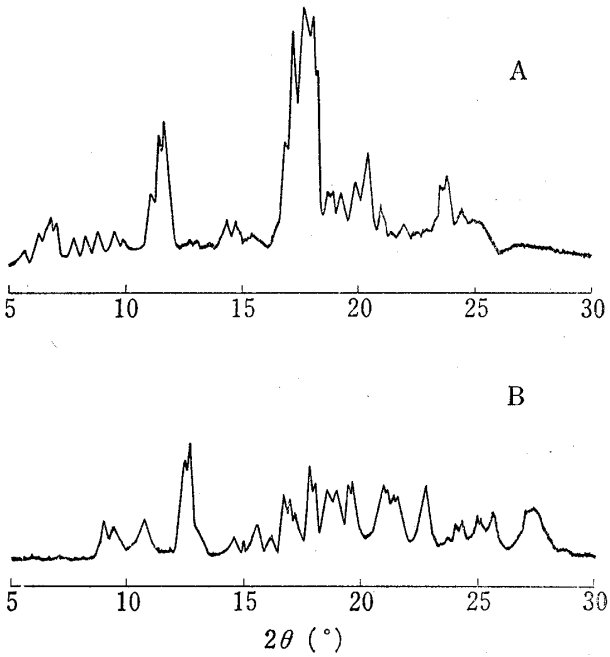

Fig. 3. Powder X-Ray Diffraction Patterns of Acetohexamide-β-Cyclodextrin Complex (A) and Physical Mixture (B)

be simply made up by the superposition of each component, while that of the complex was apparently different from the constituents to give new solid phase.

Above results indicate that acetohexamide interacts with β-cyclodextrin both in solution and in solid state to form inclusion complex.

13) K. Ikeda, K. Uekama, M. Otagiri, and M. Hatano, *J. Pharm. Sci.*, **63**, 1618 (1974).

Dissolution Behavior of the Complex

The relative rates of dissolution of acetohexamide and acetohexamide-β-cyclodextrin complex in powder and in compressed tablet are shown in Fig. 4 and 5, respectively. It is

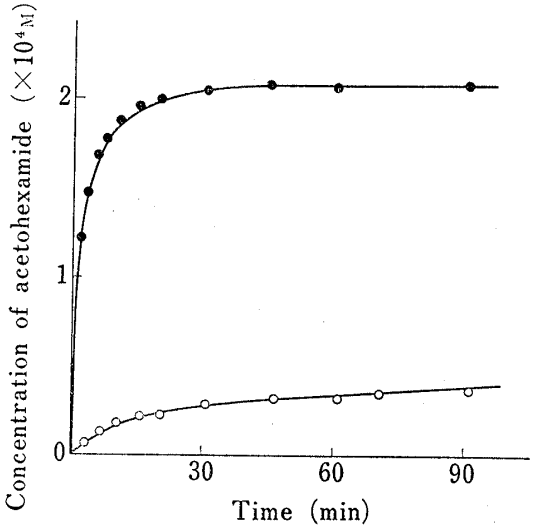

Fig. 4. Dissolution Behaviors of Acetohexamide (○) and Its β-Cyclodextrin Complex (●) in Water at 25° by Dispersed Amount Method

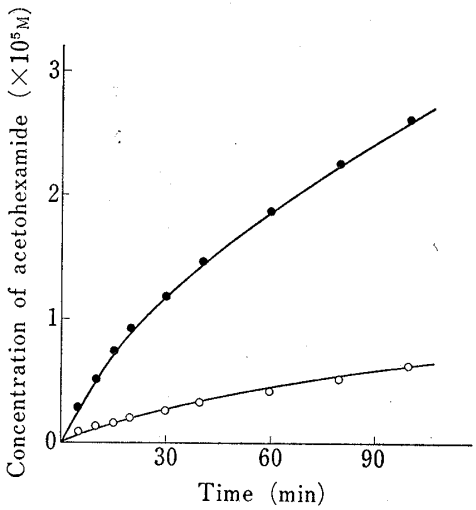

Fig. 5. Dissolution Behaviors of Acetohexamide (○) and Its β-Cyclodextrin Complex (●) in Water at 25° by Rotating Disk Method

evident that the complexed form of acetohexamide dissolved much more rapidly than acetohexamide itself. Improved dissolution characteristic of acetohexamide by inclusion complexation may be due to the enhanced solubility, as expected from Fig. 1. In the present system, however, various factors such as diffusion coefficient, wettability of the complexing agent, and dissociation of the complex in dissolution medium could also be responsible for dissolution rate of the complex.

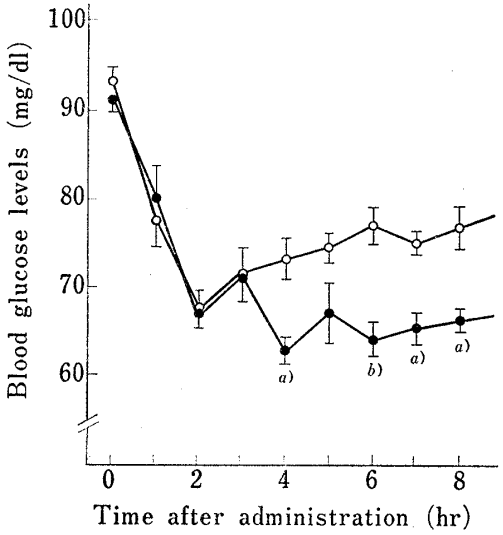

Fig. 6. Changes in Blood Glucose Levels after Oral Administration of Acetohexamide (○) and Its β-Cyclodextrin Complex (●)

Values represent the mean±S.E. of 5 rabbits.
a), *b*): significantly different from acetohexamide alone (paired-student *t*-test), *a*) $p<0.01$, *b*) $p<0.005$.

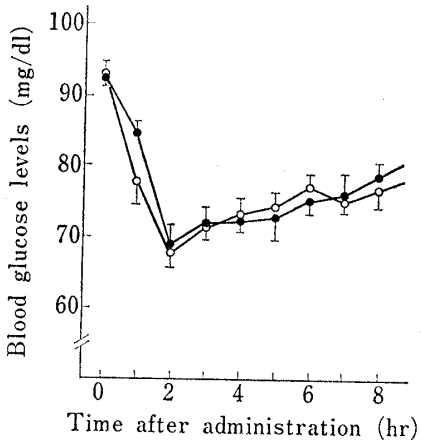

Fig. 7. Changes in Blood Glucose Levels after Oral Administration of Acetohexamide (○) and Its β-Cyclodextrin Physical Mixture (●)

Values represent the mean±S.E. of 5 rabbits.

Hypoglycemic Activity of the Complex

Hypoglycemic action of acetohexamide-β-cyclodextrin complex was compared with that of acetohexamide by oral administration in rabbit. As shown in Fig. 6, the reduction in blood glucose level was potentiated in the system containing the complex. Using paired-student t-test, the difference in each case was found to be statistically significant. On the other hand, no appreciable difference between physical mixture (acetohexamide and β-cyclodextrin in 1: 2 molar ratio) and acetohexamide itself was observed (Fig. 7). The potentiation caused by the complex may be due to the improved dissolution characteristic of the drug. However, it is interesting to note in Fig. 6 that the initial decrease in blood glucose levels observed for the complex appeared to be rather slow in spite of the rapid dissolving form of acetohexamide. This may be ascribed to smaller diffusibility of the complex because of the molecular weight increase (about eight-fold). Furthermore, decrease in membrane permeability of the complex due to poor lipophilic nature of cyclodextrin molecule will not be excluded in the present system. Although detailed study should be made to elucidate the absorption mechanism of cyclodextrin complex, the potentiation of hypoglycemic activity observed for the inclusion of acetohexamide with β-cyclodextrin suggested the decrease in dose in oral sulfonylurea therapy with decrease in side effect.

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