

Human serum lipoprotein-cyclodextrin interaction in agarose gel

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Abstract

Cyclodextrins (CDs) are cyclic oligosaccharides with low molecular weight. They have been known to bind to some serum lipoproteins and to form complexes. To elucidate whether each serum lipoprotein subclass could be separated by electrophoresis using CDs, we performed preliminary experiment, in which lipoprotein-CDs interaction was examined on electrophoresis with agarose gel. When the supporting agarose gel containing both α -CD and β -CD was prepared and was applied to isoelectric focusing for fractionating serum lipoproteins, apoB lipoproteins were found to be clearly separated into several fractions on this electrophoresis. This finding suggested that apoB lipoprotein may be detected as isolated form by arranging amounts of added CDs.

Key words : serum apoB lipoprotein, cyclodextrin, agarose gel, isoelectric focusing (IEF)

Introduction

Cyclodextrins (CDs) are neutral, cyclic glucose polymers with low-molecular weight. α -, β - and γ -CDs consist of 6, 7 and 8 monomers, respectively. Such water-soluble oligosaccharides have hydrophobic cavities, so that they can interact with various substances and form inclusion complexes. When the CD was added to serum, lipoproteins would be captured and greater concentration of the CD would cause insoluble precipitation as a result of interactions between lipoproteins and the CD. These interactions, however, present soluble complexes under the appropriate experimental conditions^{1,2)}. We noted that the surface charge of serum lipoproteins altered with the addition of CDs, and applied these interactions between lipoproteins and CDs. To develop a new electrophoretic isolation technique for serum lipoproteins, we performed fundamental experi-

ments of interactions between lipoproteins and CDs in agarose gel.

Materials and methods

1. Samples

Blood samples collected from normal and hyperlipidemic subjects were centrifuged and fresh non-frozen sera were applied.

Human plasma lipoprotein standards of low density lipoprotein (LDL; 5.0 mg protein/ml), very low density lipoprotein (VLDL; 1.0 mg protein/ml) and high density lipoprotein (HDL; 10.0 mg protein/ml) were obtained from Sigma Chemical Company (USA).

2. Electrophoresis

Agarose gel for serum lipoprotein electrophoresis was obtained from Ciba Corning Diagnostics Corp., (MA, USA). 1.0 μ l aliquot of each sample was applied to the gel and electrophoresed at 90 V for 45 min. The lipoprotein frac-

tions were stained with Fat Red 7B.

3. Isoelectric focusing

CDs were added to 1 % agarose solution. Agarose gel plate containing 8.5 ml agarose solution, 1.4 ml ampholine (pH range 3.5-1.0; Pharmacia LKB Biotechnology, Uppsala, Sweden) and 0.4 ml ampholine (pH range 4.0-6.5; Pharmacia LKB Biotechnology) was molded. 2.0 μ l aliquot of each sample was applied and isoelectric focused at 100 V for 20 min, 200 V for 20 min and 300 V for 10 min with Multiphor (Pharmacia LKB Biotechnology).

4. Immunofixation

Immunofixation was performed according to the description by Ritchie and Smith³⁾. Briefly, after isoelectric focusing, agarose gel plate was incubated with anti human apolipoprotein antibody (Chemicon international INC.) for 60 min in a humid chamber. Excess protein and ions were removed, then the plate was dried and stained with Coomassie Brilliant Blue R-250.

Results

Electrophoretic studies

With the addition of α -CD and β -CD solution to human serum led to the immediate flocculation of its lipoproteins. Figure 1 represents the electrophoretic analysis of supernatant samples obtained from the mixture. The formation of soluble CD-lipoprotein complexes caused increased anodic mobility of apoB lipoprotein.

Isoelectric focusing on agarose gel containing CDs

For isoelectric focusing, two kinds of CDs (α -CD and β -CD) were directly added to agarose gel and each sample was applied to the supporting gel.

Figure 2A shows the isoelectric focusing patterns on agarose gel with the addition of 10.0 mmol/l α -CD. Both HDL and VLDL (Fig. 2A, lanes 1 and 2, respectively) show the extremely increased electrophoretic mobility to the anodal

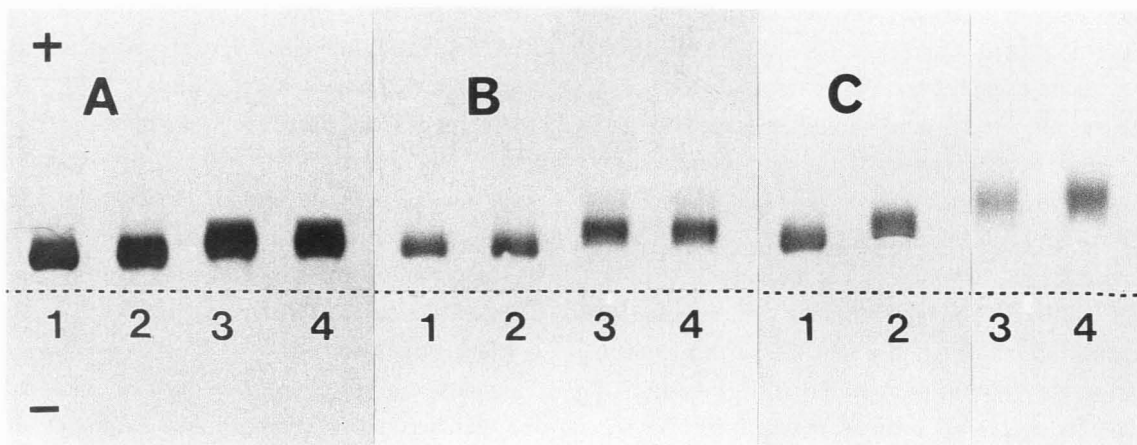


Fig. 1 ApoB immunofixation patterns of supernatant samples derived from the mixture of human serum and various concentrations of (A) α -CD, (B) β -CD and (C) α -CD + β -CD after electrophoresis. Final concentrations of α -CD are; (2) 5.0 mmol/l, (3) 10.0 mmol/l and (4) 15.0 mmol/l. Final concentrations of β -CD are; (2) 2.0 mmol/l, (3) 4.0 mmol/l and (4) 8.0 mmol/l. Final concentrations of α -CD + β -CD are; (2) 10.0 mmol/l + 2.0 mmol/l, (3) 10.0 mmol/l + 4.0 mmol/l and (4) 10.0 mmol/l + 8.0 mmol/l, respectively. Each of lane 1 shows control.

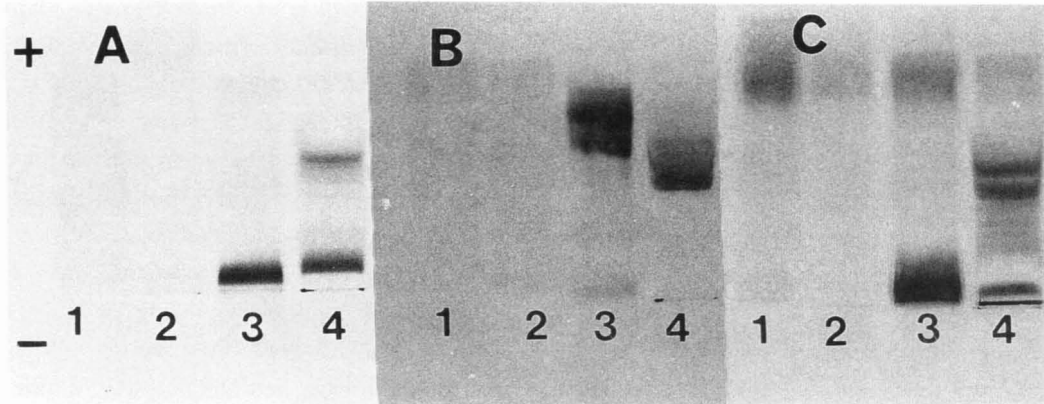


Fig. 2 ApoB immunofixation patterns after electric focusing using agarose gel with the addition of (A) α -CD, (B) β -CD or (C) α -CD + β -CD. Lane 1: HDL, lane 2: VLDL, lane 3: LDL and lane 4: human serum.

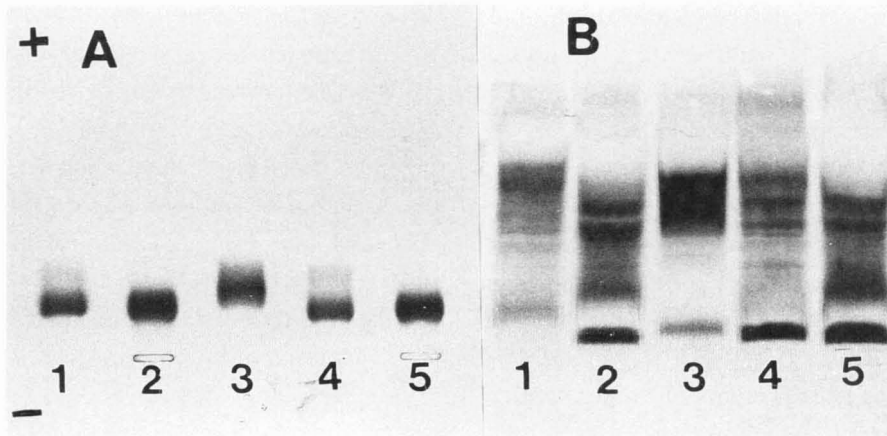


Fig. 3 ApoB immunofixation patterns after (A) electrophoresis or (B) isoelectric focusing. Lane 1: normolipidemic serum, lanes 2-5: hyperlipidemic sera.

side, while LDL (Fig. 2A, lane 3) shows the slight anodal mobility from origin. Human serum lipoprotein (Fig. 2A, lane 4) shows the similar mobility to LDL and another anodal fraction was also seen.

Figure 2B represents the isoelectric focusing patterns with the addition of 4.0 mmol/l β -CD

to the supporting gel. Both HDL and VLDL (Fig. 2B, lanes 1 and 2, respectively) again show the extremely increased electrophoretic mobility to the anodal side, but LDL and serum lipoprotein (Fig. 2B, lanes 3 and 4, respectively) which have the anodal mobility, show the broad tailing patterns.

Both α -CD and β -CD were added to the same agarose gel and used for isoelectric focusing (Fig. 2C). HDL and VLDL show the anodal mobility (Fig. 2C, lanes 1 and 2, respectively), and LDL (Fig. 2C, lane 3) shows the slight anodal mobility from origin. In addition to the similar mobility to LDL, four fractions of serum lipoprotein were migrated to the anodal side (Fig. 2C, lane 4).

Based on the suitable amount of CDs added to agarose gel which supplied clear focusing patterns of HDL, VLDL, LDL and serum lipoproteins, further isoelectric focusing of serum lipoprotein was performed. Figure 3 shows serum apoB immunofixation patterns after usual electrophoresis (Fig. 3A) or isoelectric focusing (Fig. 3B). Lane 1 represents normolipidemic serum and the rest of them (lanes 2-5) are derived from hyperlipidemic patients. The usual electrophoretic patterns of apoB lipoprotein show a band or two at pre β - β migration. Isoelectric focusing patterns of apoB lipoprotein have 5 or 6 bands.

Discussion

Several kinds of lipoproteins are present soluble in human serum. Common isolation methods of lipoproteins are ultracentrifugation, precipitation method and electrophoresis. To obtain detailed lipoprotein subclasses, density gradient ultracentrifugation⁴⁾ was introduced. But this technique requires long complicated manipulation and a big apparatus, so that the assay is not suited for routine analysis. We therefore aimed at electrophoresis for lipoprotein analysis using interactions between lipoproteins and CDs.

Following interactions were observed in the previous reports; the surface charge of lipoprotein particle altered with the addition of

CDs to human serum, and greater concentration of CDs precipitated serum lipoproteins. We also demonstrated in the present study that apoB lipoprotein had anodal mobility, when supernatant which obtained from centrifugation of serum-CD mixture was applied to electrophoresis (Fig. 1).

The effect of anodal mobility on apoB lipoprotein was emphasized when both α -CD and β -CD were added to serum, but not enough to identify apoB lipoprotein subclass. So we performed isoelectric focusing because of its high resolution, using the supporting gel to which α -CD and β -CD were both added. With the addition of either α -CD or β -CD to the gel resulted in deficient resolution and it was impossible to identify apoB lipoprotein subclass (Fig. 2A and 2B) however, several fractions were detected on the gel containing both α -CD and β -CD (Fig. 2C). On the basis of the suitable conditions mentioned above, hyperlipidemic serum samples were applied and 5 or 6 fractions of apoB lipoprotein were isolated (Fig. 3B).

It suggested that the addition of CDs to agarose gel caused the interactions between each kind of serum apoB lipoprotein and every CD, and that each reaction complexes were differentiated and isolated. It seems that every CD has the different style of binding to serum apoB lipoprotein in the gel.

We performed fundamental experiments of interactions between serum apoB lipoprotein and CDs in agarose gel. It is possible to isolate lipoprotein subclasses if supporting gel for isoelectric focusing would contain the proper amount of CDs.

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ヒト血清リポタンパク質とシクロデキストリンの アガロースゲル内における相互作用

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要 約

シクロデキストリンは低分子量の環状オリゴ糖で、リポタンパク質と複合体を形成する。シクロデキストリンを使用した電気泳動による血清リポタンパク質サブクラスの分離の可能性を明らかにするために、リポタンパク質とシクロデキストリンとの泳動用ゲル内での相互作用について検討をおこなった。その結果、 α -CD と β -CD の 2 種類の CD をアガロースゲルに添加し、このゲルを泳動用支持体として等電点電気泳動をおこなったところ、apoB 含有リポタンパク質が数分画分離された。したがって、ゲル内へのシクロデキストリンの適正な添加条件が設定できるならば、apoB 含有リポタンパク質サブクラスの分離が可能となることが示唆された。

キーワード：血清 apoB 含有リポタンパク質、シクロデキストリン、等電点電気泳動

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