New separation principle of proteins by liquid chromatography resulting in ultrafast high-resolution separation

The purpose of separation science is to isolate some useful substances from natural and/or synthesized products to serve for human society. An ideal separation method is to have the feature of “three high” of high resolution (quality), high speed, and mass recovery of target proteins. Liquid chromatography (LC) is referred to be the most powerful tool for carrying out this task. By statistics up to 1973, over 14 Nobel Prizes are awarded to the achievements involving in LC, two of them are especially for chromatography in 1947 and 1952.

The principle of chromatographic separation is to base on the various migration velocities of substances on chromatographic column which is dominated by only one variable of the partition coefficient of substances in stationary and mobile phases, i.e., it follows plate theory and, the longer the column length is, the better the substance resolution will be. In 1984, Dr Menet *et al* used to employ a column length of 22 m with 1 mm diameter to separate a light gasoline sample by taking 5 hours, due to high column limited fast flow rate.

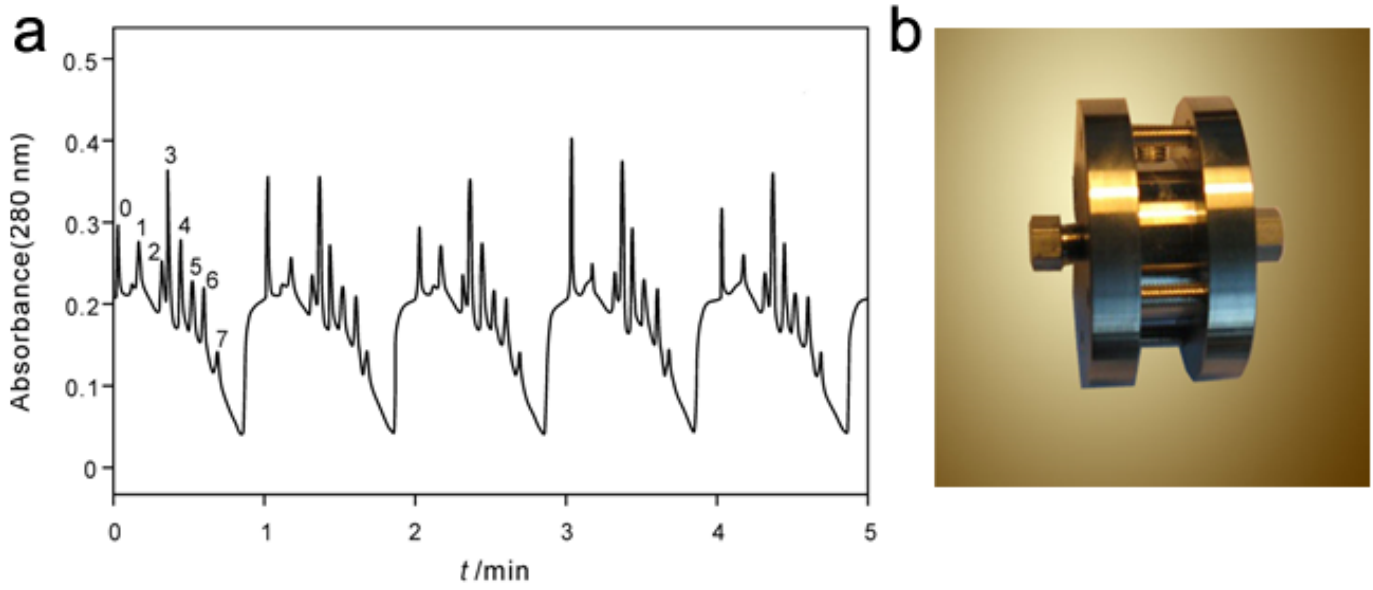

Fig.1 **a**. Fivesuccessive separations of seven proteins under gradient elution in five minutes 0-solvent 1-7-proteins. **b**.chromatographic cake

The authors recently found that the principle of protein separation is dominated by two variables of “migration region, MR” in which protein separation follows plate theory and of “steady region, SR

". The latter does not follow plate theory, but mainly contributes to protein retention. Since 1903 LC was invented, the traditional partition principle is actually accounted in a half of whole LC.

The groundbreaking discovery features can be used: (1) for ultrafast separation of proteins using very short columns under high flow rates to maintain a high resolution; (2) the SR as an operation space (OP) for all assisted operations in online two dimensional LC (2D-LC) to carry out high-throughput protein analysis and separations. Fig. 1-a shows the chromatograms of five successive complete separations of seven native proteins in less than 5 min under flow rate 10mL/min by a hydrophobic interaction chromatographic cake (4 mm for thickness and 10 mm for diameter) shown in Fig.1-b. Such an ultrafast high-resolution separation has been unprecedentedly seen in LC. It is expected that a thinner (

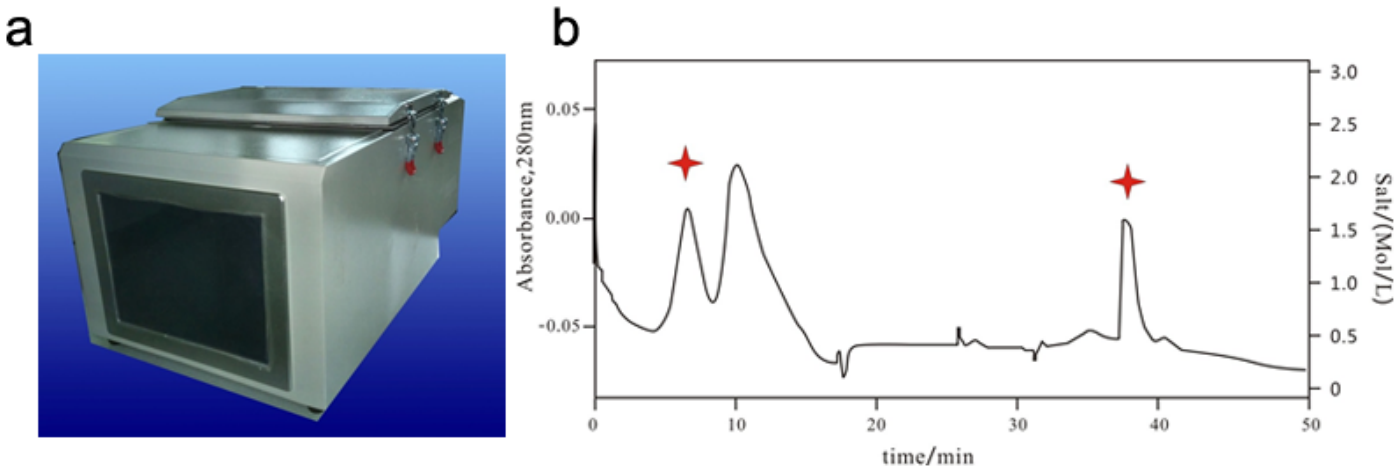

Fig. 2. **a**. ChromatoExper **b**. Ribonuclease A purification from the crude extraction of bovine pancreas by online 2D-LC.

Based on the found SR feature, a next-fully automation liquid chromatograph composed of the designed equipment, shown in Fig. 2-a, in conjunction to a commercial liquid chromatograph, was designed and employed for the fast purification of ribonuclease A from bovine pancreas by means of online 2D-LC (shown in Fig. 2-b) to have 95.8% purity with 93.2% mass recovery in 45 min. All operations from the first sampling to obtaining the final pure ribonuclease A were carried out in a closed system with positive pressure, resulting in not only a fast purification and high mass recovery, but also prevents contamination from the environment. In comparing with the conventional method which takes many steps and several days by handling, the presented "three high" method by fully automation operation really carries out the dream of separation scientists.

Theoretically, because the HIC environment of neutral salt aqueous solution is closed to human body, this investigation will provide some information of molecular mechanism about the

transportation, multiple molecular interactions among proteins, drugs, pharmacology, and so on. The SR is known to really exist and be important in life science, but it has been not fully know in detail, requiring scientists further deeply to investigate.

Publication

Two variables dominating the retention of intact proteins under gradient elution with simultaneous ultrafast high-resolution separation by hydrophobic interaction chromatography.
Geng X, Jia X, Liu P, Wang F, Yang X
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