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Note

Chromatographic detergent exchange in the preparation of membrane receptor protein

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A common problem that is encountered in the study of detergent-solubilized membranes is the removal of the initial solubilizing detergent for further studies on the membrane protein or alternatively the replacement of the initial detergent with a more suitable detergent for the next phase of experiments. Unfortunately for most intrinsic membrane proteins, no single detergent is well suited for all aspects of a study. Furthermore, the high cost of many versatile detergents makes the rapid exchange between less costly detergents an attractive economical proposition.

Previous methods for detergent exchange have been cumbersome and time-consuming. For example, deoxycholate can be exchanged for Triton X-100 by gel permeation chromatography [1], but this method can only be used if the size of of the protein–detergent complex is larger than the size of the mixed micelles to facilitate separation. Detergent exchange chromatography has been shown to be a useful method for a wide range of detergents and proteins using phenyl-Sepharose CL-4B® [2] and Extracti-Gel D® [3]. The following method utilizes the very rapid separation provided by fast protein liquid chromatography (FPLC) to exchange detergents bound to a human hepatic membrane protein, the ferritin receptor.

EXPERIMENTAL

Phenyl-Superose® FPLC 5/5 column, pump P-500, gradient programmer GP-250 and polyacrylamide gradient gels 4AA/30 were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). A Gilson 111B UV spectrophotometer was

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used to monitor the elution of protein from the FPLC column. Teric 12A9 (non-ionic polyoxyethylene) was obtained from ICI Chemicals (Sydney, Australia). Matrexpel 102® was obtained from Amicon (Lexington, MA, U.S.A.). Ferritin receptor protein was purified by affinity chromatography as previously described [4,5]. Human ferritin was purified as previously described [6] and iodinated with 1 mCi of ^{125}I according to the method of Bolton and Hunter [7]. All chemicals were of reagent grade.

The purification of the detergent-solubilized ferritin receptor protein from human liver results in a membrane protein in a concentration of approximately 10% Teric 12A9. The protein solution was passed through a 0.2-μm filter prior to application to the FPLC column. The column was equilibrated with a buffer of low ionic strength containing 20 m*M* Tris–HCl, 1 m*M* EDTA, 1% sodium dodecyl sulfate (SDS), pH 9.0 (elution buffer). The buffer was filtered through a 0.2-μm Millipore filter prior to application to the column. A 500-μl sample of a 10% Teric 12A9 solution in elution buffer was initially run through the column. A 500-μl sample of the ferritin receptor (350 μg/ml) in approximately 10% Teric 12A9 was applied to the column and the protein was eluted with the elution buffer with a flow-rate of 0.4 ml/min. The eluent was collected in 0.5-ml fractions with the absorbance at 280 nm being constantly monitored. The fraction containing the protein corresponding to the initial peak on the graph was used for further studies.

A pooled sample of the receptor protein from four chromatography runs of 500 μl was adjusted to pH 8.0 and then bound to 0.2 ml of Matrexpel 102, a microparticulate support as previously described [4]. Binding studies of ^{125}I-labelled human ferritin to the ferritin receptor–Matrexpel 102 complex were carried out before and after the removal of Teric 12A9 as previously described [4]. SDS polyacrylamide gel electrophoresis (PAGE) was carried out on the ferritin receptor protein before and after removal of Teric 12A9. Protein concentrations were determined according to the method of Lowry et al. [8] before and after the removal of Teric 12A9.

RESULTS

The separation of ferritin receptor protein from Teric 12A9 along with a control injection of a 10% Teric 12A9 solution is shown in Fig. 1.

The receptor–Matrexpel complex bound 6967 ± 1969 cpm of [^{125}I]ferritin before the removal of the Teric 12A9 and 7599 ± 462 cpm after the FPLC detergent exchange. Non-specific binding was approximately 8%.

SDS-PAGE confirmed that the receptor protein was present in the fraction corresponding to the first absorbance peak at 280 nm. Entry into the gel was greatly facilitated by the removal of the Teric 12A9, and the distortion associated with the presence of Teric 12A9 could be removed by the detergent exchange.

Protein determination before and after the detergent exchange demonstrated a 95% recovery of receptor protein following detergent exchange.

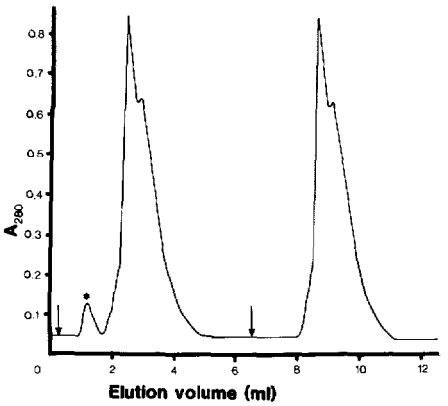

Fig. 1. Absorbance at 280 nm of 175 μg of receptor protein in 10% Teric 12A9 versus the elution volume (ml) followed by a control sample of 10% Teric 12A9. (*) Ferritin receptor peak. Arrow indicates injection of sample on the column. The flow-rate of the column was 0.4 ml/min, the paper-speed was 1.0 cm/min.

DISCUSSION

We have described a rapid and simple FPLC method for exchanging a high concentration of non-dialyzable detergent for a low concentration of dialyzable detergent. The technique allows for a high recovery of receptor protein and the specific binding characteristics of the receptor with its ligand are retained. The detergent exchange facilitates the application of receptor proteins into SDS-PAGE systems to determine receptor purity and molecular mass as well as for use in Western blotting. Previous studies have determined that the detergent may interfere with the determination of molecular mass [3].

The technique also allows the use of inexpensive detergents in the initial solubilization steps which can later be exchanged for SDS. The advantage of this method over previous approaches is the very rapid separation of receptor protein from detergent that the FPLC methodology permits.

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