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Note

Adaptable system for microdialysis

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Dialysis of microliter quantities of a sample can be a valuable technique in many laboratory situations. Several methods which are applicable to this problem have been proposed[1–4]. This paper describes a microdialysis method which overcomes some of the limitations encountered in other methods and utilizes common laboratory equipment. Extensive studies in our laboratory have shown that this system combines quantitative recovery of protein retentates and ease of sample manipulation into a relatively inexpensive microdialysis technique.

EXPERIMENTAL

Microdialysis chambers (3 cm long) were prepared from standard bore gel electrophoresis tubing (7 mm O.D. × 5 mm I.D., Bio-Rad) and were fitted with a Vacutainer tube top as illustrated in Fig. 1. Wet dialysis membranes (Spectrapor Nos. 2 and 3, Fisher Scientific Co.) were arranged as double thicknesses on one end of the tube and were held in place by an O-ring (No. 2-0.007 NC 74-7, Southern Rubber Co., Greensboro, NC, U.S.A.) which was positioned with an O-ring applicator supplied with a YSI Model 53 oxygen analyzer (Yellow Springs, OH, U.S.A.). After the dialysis chambers were assembled, they were placed into the top chamber of a Bio-Rad Model 150A electrophoresis cell, and the lower chamber, placed over a magnetic

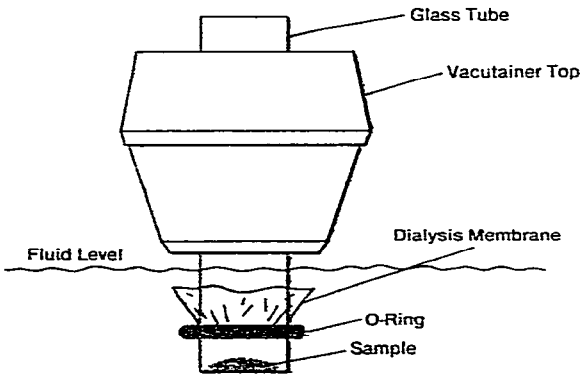

Fig. 1. Schematic diagram of microdialysis chamber.

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stirrer, was filled with distilled water. A Lauda K-2/R cooling circulator (Brinkmann Instruments) controlled the temperature of the electrophoresis unit.

The dialysis chambers were filled with 100 μl of [1-^{14}C]glycine or [^{3}H]acetic acid in distilled water, and samples were removed from the dialysis chambers at various times to measure the effectiveness of dialysis. To measure recovery of protein in the dialysate, 50 μl of [1-^{14}C]glycine and 50 μl of bovine serum albumin (BSA) (10 mg/ml) were added to duplicate chambers. At the various times, samples were removed and tested for protein concentration and remaining radiolabel.

The effect of sample volume on dialysis efficiency was determined by using [^{3}H]acetic acid. Different volumes of dilute radiolabel were dialyzed for 1 h, samples were removed, and the remaining radiolabel was determined.

All samples were counted in a Beckman LS-133 liquid scintillation system while using Bray's Solution as a fluor. Bovine serum albumin concentrations were determined by ultraviolet absorbance[5]. The radiolabels were chosen because of their small molecular weight and availability to our laboratory.

RESULTS

Figs. 2 and 3 illustrate the efficiency of dialysis with and without BSA at 5 and 25°C. Temperature and pore size had the greatest effects on the speed of dialysis, the apparent enhancement of dialysis by BSA in one case (Fig. 2) was not evident at 3 h. In all cases, the Spectrapor No. 2 (mol.wt. cutoff, 12,000–14,000) gave a more efficient dialysis than did Spectrapor No. 3 (mol.wt. cutoff, 3500). The experiments performed at 5°C indicated slower dialysis rates than did experiments at 25°C, regardless of the membrane used.

For samples with and without BSA, 3.5 and 5.0% of the radiolabel remained in the samples dialyzed with Spectrapor No. 2 at 25°C for 3 h. At 5°C, 12.5 and 11.5% of radiolabel remained in samples dialyzed with Spectrapor No. 2 (Fig. 2). For samples with and without BSA, 24.5 and 17.7% of radiolabel remained in the samples dialyzed at 25°C with Spectrapor No. 3, while 40.0 and 35.0% of radiolabel remained in samples dialyzed at 5°C (Fig. 3).

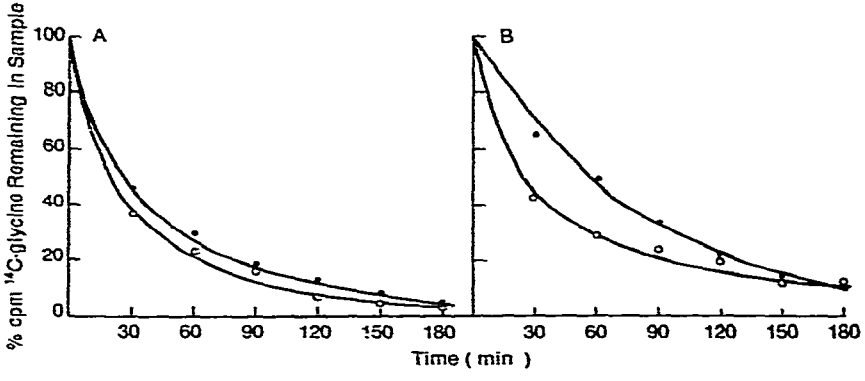

Fig. 2. Effect of protein on dialysis of [1-^{14}C]glycine at 25°C (A) and 5°C (B) utilizing Spectrapor No. 2 membranes. Samples contain 100 μl of [1-^{14}C]glycine (●) or 50 μl of [1-^{14}C]glycine and 50 μl BSA (10 mg/ml) (○).

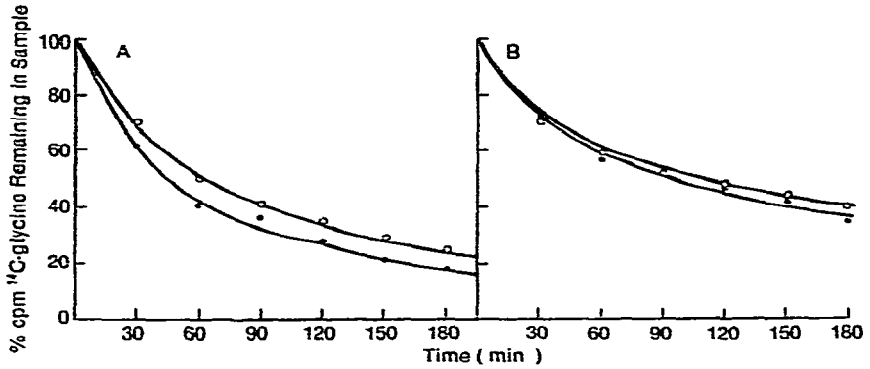

Fig. 3. Effect of protein on dialysis of [1-^{14}C]glycine at 25°C (A) and 5°C (B) utilizing Spectrapor No. 3 membranes. Samples contain 100 μl of [1-^{14}C]glycine (●) or 50 μl of [1-^{14}C]glycine and 50 μl BSA (10 mg/ml) (○).

When Spectrapor No. 2 was used for dialysis, the extent of dialysis at 3 h was not altered significantly when protein was present in the dialysand. However, the initial rates of dialysis were affected. Fig. 2 indicates that the presence of BSA increased the rate at which initial dialysis occurred, especially at 5°C. The presence of BSA in chambers in which Spectrapor No. 3 was used (Fig. 3) appeared to slow the rate of dialysis at 25°C but did not significantly alter the rate of dialysis at 5°C. In all studies, the concentration of BSA was measured after dialysis (data not shown), and the results clearly indicated 100% recovery.

Volumetric studies indicated similar results for both Spectrapor No. 2 and 3. The efficiency of dialysis was greatest for the smaller volumes (25 to 75 μl) dialyzed for 1 h (Fig. 4).

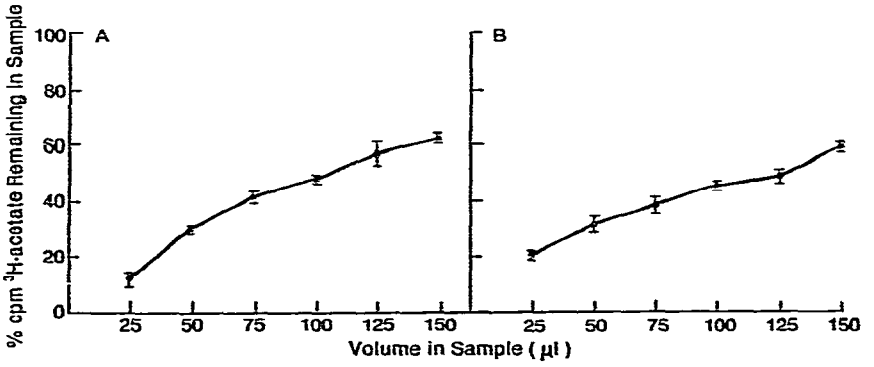

Fig. 4. Effect of sample volume on efficiency of dialysis at 25°C for 1 h. Dialysis was performed with [^{3}H]acetic acid and Spectrapor No. 2 membranes (A) and Spectrapor No. 3 membranes (B).

DISCUSSION

Studies using this microdialysis method have shown it to be a useful and predictable technique for small samples. The difference in rate of dialysis seen in the temperature change experiments was in keeping with the role that temperature plays in most equilibrium reactions. Colder temperatures tend to slow the progression to equilibrium, and this is illustrated by the experiments at 5 and 25°C. Pore size of the membrane also affected the rate of dialysis by limiting the flow of molecules through the dialysis chamber. Our experiments with Spectrapor No. 2 and 3 demonstrated this relationship of pore size to dialysis rate.

The experiments in which BSA was included presented interesting and unexpected results. The presence of protein in the dialysand should have caused an increase in osmotic pressure resulting in an increased dialysis rate. This increase occurred with the Spectrapor No. 2 membrane but not with the Spectrapor No. 3 membrane. We postulate that the pore size of the Spectrapor No. 3 compensated for the effect caused by the increased osmotic pressure. This suggestion is supported by the similarity of dialysis rates of the test and controls in the Spectrapor No. 3 experiments (Fig. 3).

Fig. 4 demonstrates the extent to which different volumes were dialyzed in 1 h. A direct relationship between sample size and extent of dialysis was expected. As the sample volume increased, the proportion of sample actually in contact with the membrane decreased, thus requiring longer dialysis times for large samples.

Certain precautions must be observed when using this microdialysis system. The seal made by the O-ring must be tight and should be examined before any sample is added to the chamber. One method of insuring a proper seal is to equilibrate the chambers in the dialyzing fluid before adding any sample. Leakage of fluid into the chamber will be evident before the sample is added. The use of double-thickness membranes greatly reduced leakage problems, while the use of single-thickness membranes led to more difficulty in obtaining a tight seal. Care also should be taken to avoid puncturing the membrane while introducing samples into the chamber.

The advantages of this microdialysis system include its simplicity, rapidity and reproducibility. The basic unit is easily constructed from readily available, inexpensive laboratory equipment. The microdialysis chambers, once placed in the dialyzing fluid, provide ready access to the samples during the dialysis process. A buffer change is not required during dialysis, and buffer temperature is easily regulated. Except for the dialysis membranes, the components of the system are reusable. An important advantage is the ability to recover samples. As was stated the recovery of BSA was virtually 100% in all cases, thus allowing confident dialysis of very small volumes. The microdialysis chamber (glass tube, membrane and O-ring) can be adapted to many situations. However, the O-ring applicator is essential for correctly positioning the membrane and O-ring. We used the electrophoresis cooling unit because it was available to our laboratory, but any other similar apparatus could be used. An ordinary test tube rack can be used to support the chambers in a refrigerator tray filled with desired buffer.

Application of this system is as varied as the techniques that require small volume samples. Our laboratory has used this system for dialysis of samples prior to electrophoretic and immunological assays. Testing enzyme activity during purifi-

cation studies is another indication for the use of this method. This microdialysis system should prove to be adaptable to suit many needs.

ACKNOWLEDGEMENTS

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