THE EFFECT OF CYTOPLASMIC PROTEINS ON THE REAGGREGATION OF DISSOCIATED GHOST MEMBRANES OF *BACILLUS MEGATERIUM* KM

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SUMMARY

The effect of cytoplasmic proteins on the reassociation of membrame proteins and lipids which have been solubilized in sodium dodecyl sulfate and urea has been investigated. The cytoplasmic proteins have been found to inhibit the reassociation of the membrane proteins. Moreover, approximately 15% of the cytoplasmic proteins co-aggregate with the membrane components after removal of the sodium dodecyl sulfate and urea.

INTRODUCTION

As part of a general study of the structure and function of the membranes of *Bacilli* (1,2,3) we have been investigating the possibility that the components of the cytoplasmic membrane of *Bacillus megaterium* KM can be reaggregated after complete dissociation to individual molecular constituents. It is hoped that ultimately such studies will allow one to determine whether the reassembly process could result in formation of a membrane with functional and morphological characteristics similar to those of the original membrane.

We have previously reported that membranes from *Bacillus megaterium* KM can be almost completely dissociated by a mixture of sodium dodecyl sulfate and urea, and that the dissociated lipid and protein components of the membrane reassociate upon removal of the sodium dodecyl sulfate and urea (3). In this report we present experiments which were designed to investigate two

questions. First, is the reassociation of dissociated membrane lipids and proteins specific for membrane proteins alone, or will other proteins, such as those of the cytoplasm of Bacillus megaterium, also associate with the membrane proteins and lipids upon removal of the dissociating reagents? Secondly, does the presence of non-membrane proteins, e.g. cytoplasmic proteins, inhibit reassociation of membrane proteins?

MATERIALS AND METHODS

Bacillus megaterium KM ATCC 13632 cells were grown to mid log phase as previously described (3). Unlabeled and ^{14}C- or ^{3}H-leucine labeled ghosts were prepared as described (3) except that the final wash and resuspension was done in 50 mM Tris-HCl, pH 7.0. Unlabeled and ^{14}C or ^{3}H-leucine labeled cytoplasmic protein was obtained by hypotonic lysis of protoplasts following lysozyme treatment. After centrifugation of the lysed protoplast suspension at 10,000 xg for 15 min, the resulting supernate was treated with deoxyribonuclease and ribonuclease and then centrifuged at 100,000 xg for 3 hours. The pellet was discarded and the cytoplasmic proteins in the supernate were used as described below.

For dissociation and reassociation experiments 1 mg (5×10^4 cpm) of ^{3}H-ghost protein and 6 mg (4×10^5 cpm) of ^{14}C-cytoplasmic protein, were mixed in 50 mM Tris-HCl, pH 7.0. This proportion of cytoplasmic and membrane protein was chosen because it approximates that found in the intact cell. To this mixture were added in the order indicated: Tris-HCl, pH 7.0, to a final concentration of 50 mM; urea, to a final concentration of 7.5 M; and sodium dodecyl sulfate to a final concentration of 2 mg/ml. The protein concentration was 2 mg/ml. This mixture was stirred for 30 minutes at 0° and centrifuged at 100,000 xg at $2\text{-}3^{\circ}$ for 3 hours. The supernate, which constituted 98% of the input protein, was removed and dialyzed with stirring initially against 7.5 M urea, 50 mM Tris-HCl, pH 7.0, for 24 hours, and then against 50 mM Tris-HCl, pH 7.0, 20 mM $MgCl_2$ for 36 hours. The dialyzed sample was then centrifuged at 100,000 xg for 1 hour. The supernate was removed and

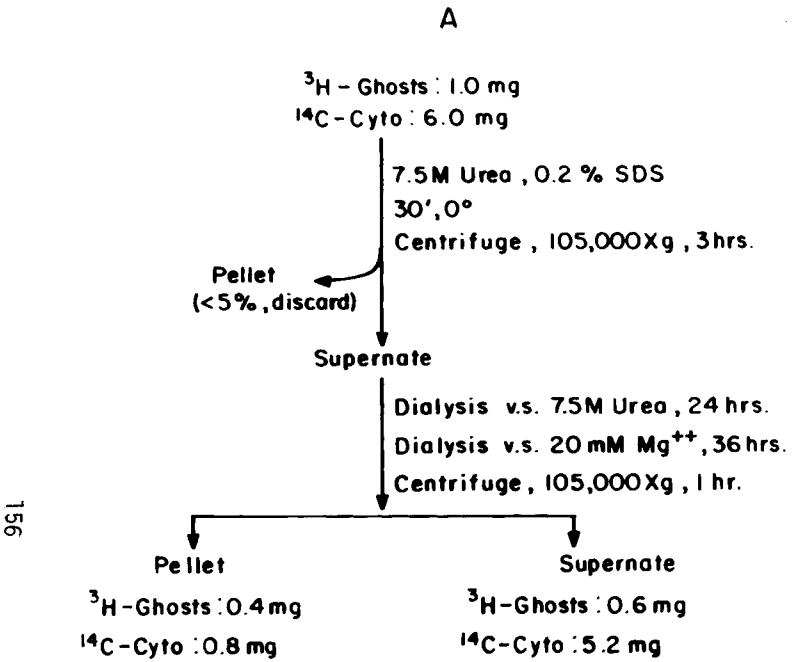
the pellet was washed by resuspension in 2.0 ml 50 mM Tris-HCl, 20 mM $MgCl_2$, followed by centrifugation at 100,000 xg for 3 hours. The final pellet was resuspended in 1.0 ml 50 mM Tris-HCl, pH 7.0. Another mixture of cytoplasmic protein and ghosts was carried through the procedure except that the label was reversed and ^{14}C-labeled ghosts and ^{3}H-labeled cytoplasmic protein were used. In Figure 1A an outline of the dissociation-reassociation procedure is shown.

The resuspended pellet (0.9 ml) was placed on top of a continuous sucrose gradient (5 ml) of 30 to 60% sucrose (w:v). After centrifugation for 16 hours at 25,000 rpm in a Spinco SW 25 rotor fractions of twenty drops were collected by means of a Buchler DensiFlow gradient sample collector and counted in a scintillation counter.

The, isotopically labeled amino acids L-leucine - C^{14}(U) and L-leucine - 4, 5 - H^3 were purchased from New England Nuclear Corp., Boston, Mass. Liquid scintillation counting was done on a Packard Model 3310 scintillation spectrophotometer. Corrections were made for quenching and for C^{14} spillover into the H^3 channel. Protein determinations were done by the method of Lowry et.al. (4).

RESULTS AND DISCUSSION

Previous work in this laboratory has shown that when purified ghosts of Bacillus megaterium KM are subjected to dissociation by urea-SDS treatment, followed by sequential removal of the SDS and then the urea in the presence of 20 mM $MgCl_2$, over 85% of the membrane protein and 95% of the membrane lipid reaggregate into a readily sedimentable form (3). Current investigations are concerned with the morphology of this reaggregated material and its functional activity. Preliminary experiments indicate that under certain conditions a high percentage of the original activity of several of the enzymes involved in lipid biosynthesis are recovered in the reaggregate (Devor, Makula and Lennarz, unpublished studies).



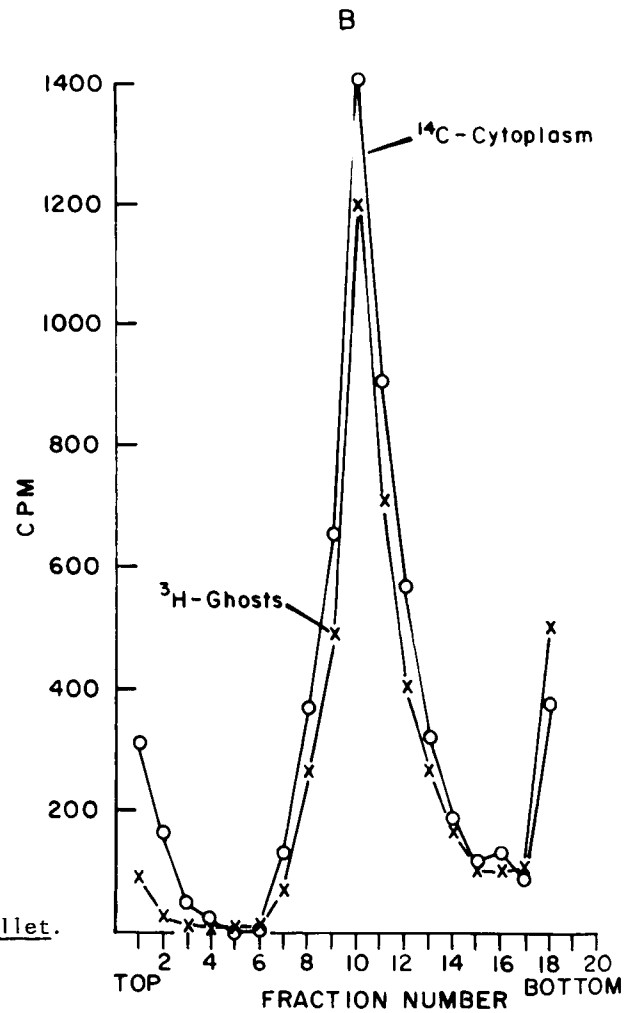


Fig. 1A. Dissociation and Reassociation Procedure for Cytoplasm - Ghost Mixture.

Fig. 1B. Sucrose Density Gradient Centrifugation of the Reassociated Pellet.

In connection with these studies it was of interest to investigate the specificity of this reaggregation process with regard to the protein component and the effect of the presence of non-membrane proteins on the reaggregation of the membrane proteins. When cytoplasmic protein is present during the dissociation and reaggregation process only 50% of the ghost protein reaggregates to a readily sedimentable form (Fig. 1A). Thus, cytoplasmic protein inhibits the reaggregation of membrane proteins. Also of interest is the finding that a small but significant proportion of the original cytoplasmic protein (ca. 15%) is found in the reaggregated membrane pellet. When the label was reversed and the experiment was repeated using ^{14}C-labeled ghosts and ^{3}H-labeled cytoplasm identical results were observed.

Because it was possible that the cytoplasmic proteins found in the ghost pellet were present simply because they existed as large aggregates of denatured protein rather than because they co-aggregated with the membrane protein-membrane lipid complex, the pellet containing ^{3}H-labeled ghost protein and ^{14}C-labeled cytoplasmic protein was subjected to isopycnic centrifugation in a sucrose gradient (Fig. 1B). Previous studies with reaggregated ghosts have shown that the ghost proteins and ghost lipid co-sediment and the aggregate has a density only slightly lower than that of the original membranes (3). The reaggregate obtained in the present study behaved similarly, however it is clear that the relatively small proportion of cytoplasmic protein found in the pellet does co-sediment with the reaggregated membrane, thus suggesting that it interacts in some manner with the membrane protein-membrane lipid complex.

In summary, these studies indicate that, at least under the conditions employed, the specificity and the degree of reaggregation of the membrane proteins are affected by the presence of 'non-membrane' proteins, such as those of the cytoplasm.

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BIBLIOGRAPHY

1. Patterson, P.H., and Lennarz, W.J. (1971) J. Biol. Chem., 246, 1062.
2. Devor, K.A., Makula, R.A., Patterson, P.H., Kennedy, M., Decker, G., Greenawalt, J.W., and Lennarz, W.J. (1972) J. Biol. Chem., 247, 1288.
3. Makula, R.A., Devor, K.A., and Lennarz, W.J. (1972) Biochem. J., in press.
4. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem., 193, 265.