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CARTA AL EDITOR

Solubilization of Membrane-Bound Transpeptidase from Streptomyces Strain K-11 with 2,3-Dimethylmaleic Anhydride *

The protein reagent 2,3-dimethylmaleic anhydride modifies amino groups substituting a negative charge for a positive one (8). When protein-containing structures are treated with this reagent, the drastic change in electrostatic properties brought about by modification can be accompanied by dissociation of individual proteins. At moderately acid pH, the reagent moieties are released with regeneration of the modified amino groups (2). This property should allow the separation of the dissociated proteins and the subsequent regeneration of their amino groups under fairly mild conditions. Dimethylmaleic anhydride has recently been used to specifically dissociate certain proteins from *Escherichia coli* ribosomes, with the production of protein-deficient ribosomal particles from which active ribosomes can be reconstituted by incubation with the corresponding split proteins (9). The bacterial wall peptidoglycan crosslinking enzyme system, which is the target of penicillin action (5), consists of a set of membrane-bound DD-transpeptidase and DD-carboxypeptidase enzymes. Up to now, attempts to solubilize membrane-bound transpeptidases in a functional state have failed, with the exception of that of *Streptomyces* sp (4). The *Streptomyces* transpeptidase can be studied by means of welldefined artificial peptide substrates (3, 6). It is the major penicillin-binding component found in the isolated membranes. By sodium dodecylsulfate gel electrophoresis, the (^{14}C)benzylpenicillin-enzyme complex has an apparent molecular weight of about 20,000-25,000 (J. Dusart & M. Leyh-Bouille, unpublished results). So far, this essential enzyme has been successfully solubilized only by the use of *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (4). The use of this detergent, however, is a serious drawback for the final purification of the enzyme and the study of its properties. The present paper reports the utilization of dimethylmaleic anhydride to dissociate and solubilize, in the absence of detergents, the transpeptidase activity of *Streptomyces* membranes. *Streptomyces* K-11 has been used as a model because of its high content in membrane-bound transpeptidase activity (M. Leyh-Bouille, unpublished results).

Treatment of membranes from *Streptomyces* K-11 with dimethylmaleic anhydride produces inactivation of transpeptidase, but reactivation takes place upon regeneration of the amino groups at pH 6.0 (table I). Membranes were treated at pH 8.2 and room temperature with 2.5 times their protein weight of solid dimethylmaleic anhydride. Since modification of amino groups and hydrolysis of the reagent are accompanied by release of hydrogen ions, the pH had to be maintained

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Table I. *Recovery of transpeptidase activity of membranes after modification with dimethylmaleic anhydride and regeneration of the amino groups.*

Preparation	Transpeptidase activity (Relative values)	
	Prior to incubation at pH 6.0	After incubation at pH 6.0 and dialysis
Control membranes	100	160
Modified membranes	25	200

Streptomyces K-11 membranes (30 mg protein), prepared as described previously (6), were treated in 4 ml of 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (K^+) (pH 8.2) with 75 mg of dimethylmaleic anhydride. After modification, the reagent-treated preparation as well as an untreated control were incubated at pH 6.0 to regenerate the amino groups and dialyzed, as described in the text. Transpeptidase activity was determined in aliquots of the modified membranes and the untreated control immediately after modification with dimethylmaleic anhydride, and after incubation at pH 6.0 and dialysis. Transpeptidase activity was assayed by measuring the radioactivity of D-(^{14}C)alanine incorporated into Ac_2-L-Lys-D-Ala-D-(^{14}C)Ala, according to the following catalyzed reaction: Ac_2-L-Lys-D-Ala-D-Ala + D-(^{14}C)Ala = D-Ala + Ac_2-L-Lys-D-Ala-D-(^{14}C)Ala. Protein was estimated according to LOWRY *et al.* (7), but in the presence of 1 % (final concentration) of sodium dodecylsulfate.

at 8.2 by addition of base. After 2 h the pH was stabilized, indicating that all dimethylmaleic anhydride had reacted. To regenerate the amino groups, the pH was brought to 6.0, and the preparation was incubated at 37° C for 2 h. The regenerated preparation was dialyzed to eliminate the hydrolyzed reagent and to raise the pH to 7.5. A control was subjected to the same treatments except that the membranes were not exposed to the reagent. Table I shows that regeneration of the amino groups is accompanied by reactivation of transpeptidase, the activity of which reaches a value twice as large as that of the original membrane preparation. Table I also shows that the various treatments applied in the absence of dimethylmaleic anhydride have a stimulating effect on the enzyme activity.

Membrane-bound transpeptidase was dissociated from the membranes and obtained in a soluble form by the following procedure. *Step 1.* Membranes from *Streptomyces* K-11 were isolated as described previously (6). Specific activity was 17 pmol transpeptidated product $\times$ min^{-1} $\times$ mg^{-1} protein. *Step 2.* Isolated membranes (180 mg protein) in 24 ml of 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (K^+) (pH 8.2) were treated with 2.5 times their protein weight of dimethylmaleic anhydride, with stirring. The pH was maintained at 8.2 by addition of 5 N KOH. The reaction was completed after 4 h of incubation at room temperature. The preparation was centrifuged (180,000 $\times$ g, 3 h, 4° C), and the released proteins (supernatant) were separated from the residual membranes (pellet). This treatment caused solubilization of about 60 % (108 mg) of the total membrane proteins. *Step 3.* The pH of the supernatant containing the released proteins was lowered to a value close to 6.0 by addition of 0.5 volume of 1 M cacodylate buffer (pH 6.0), and the preparation was incubated at 37° C for 2 h. During incubation a precipitate was formed that was separated by centrifugation, and dissolved in 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (K^+) (pH 8.2) containing 0.5 % of *N*-dodecyl-*N,N,N,*-trimethylammonium chloride. This latter fraction contained 37 mg protein and was devoid of transpeptidase activity. *Step 4.* The supernatant from the preceding step was concentrated to 3 ml by ultrafiltration and dialyzed against 25 mM Tris-HCl (pH 8.0) at 0-5° C for 18 h. The dialyzed preparation contained 18 mg protein with a specific activity of 210 pmol $\times$ min^{-1} $\times$ mg^{-1}. The residual membranes separated from the released proteins and then further subjected to regeneration of amino groups and dialysis

Fig. 1. *Dodecylsulfate slab gel electrophoresis of the solubilized transpeptidase preparation (after step 4).* Detection with Coomassie brilliant blue (track 1) and by fluorography after treatment with (^{14}C)benzylpenicillin (track 2). Conditions for electrophoresis and fluorography are those described in (1). Coelectrophoresis with standard proteins indicates that the major (^{14}C)bencylpenicillin-binding protein in track 2 has a molecular weight of about 20,000-25,000.

retained only 5 % of the original transpeptidase activity, indicating that practically all the enzyme had been released from the membranes.

The soluble transpeptidase preparation obtained by the described procedure was treated with (^{14}C)benzylpenicillin and subjected to polyacrylamide slab gel electrophoresis in sodium dodecylsulfate as described in (1). Only a few protein bands were observed. One of them corresponded to a major highly labeled penicillin-binding component with an apparent molecular weight of about 20,000-25,000 (figure 1). Two minor penicillin-binding proteins of higher molecular weights were also detected.

The solubilization of a membrane-bound transpeptidase in the absence of detergents opens new possibilities for the study of the structure and activity of this interesting group of enzymes, and for the elucidation of the mode of action of the β-lactam antibiotics. In addition, as indicated by the large amount (60 %) of membrane proteins released by dimethylmaleic anhydride, this reagent may be useful as a tool for isolating other membrane proteins.

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E. Palacián *

Service de Microbiologie,
Faculté de Médicine,
Institute de Botanique,
Université de Liège,
Sart Tilman, B-4000 Liège (Belgium)

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* Permanent address: Instituto de Bioquímica de Macromoléculas, Centro de Biologia Molecular, C.S.I.C. y Universidad Autónoma de Madrid, Canto Blanco, Madrid-34 (Spain).