Biochimica et Biophysica Acta, 614 (1980) 210—214

BBA Report

BBA 61374

EFFECT OF TRITON X-100 ON THE ELECTROPHORETIC MOBILITY OF SOLUBILIZED INTESTINAL BRUSH BORDER MEMBRANE DIPEPTIDYL PEPTIDASE IV

ROGER H. ERICKSON and YOUNG S. KIM

Gastrointestinal Research Laboratory, Veterans Administrations Hospital, San Francisco, CA 94121 and Department of Medicine, University of California, San Francisco, CA 94122 (U.S.A.)

(Received March 24th, 1980)

Key words: Dipeptidyl peptidase IV; Brush border membrane; Electrophoretic mobility; Triton X-100; (Intestine)

Summary

Dipeptidyl peptidase IV (dipeptidylpeptide hydrolase, EC 3.4.14.-) was solubilized from a rat intestinal mucosal brush border membrane preparation in varying concentrations of Triton X-100. Samples of a solubilized supernatant fraction were subjected to polyacrylamide gel electrophoresis, and the activities of several brush border enzymes were measured in gel slices following elution of the enzymes from the gel. At low concentrations of Triton X-100 (0.5%), two peaks of dipeptidyl peptidase IV activity were observed suggesting the presence of two electrophoretically distinct enzymes. However with increasing Triton X-100 concentrations, the slower migrating species was converted to a faster migrating form and only one major band of activity was observed at 10% Triton. These results indicate that there is only one form of the enzyme and that care must be taken when interpreting the electrophoretic patterns of detergent solubilized membrane bound enzymes.

Polyacrylamide gel electrophoresis has been widely used as a means to characterize and isolate a variety of soluble proteins. Membrane bound enzyme proteins, however, present special problems in that they must first be liberated from the lipid milieu, usually with detergents. These procedures inevitably result in the formation of soluble protein-lipid-detergent complexes. To avoid this problem various membrane solubilization techniques involve the use of denaturing conditions such as sodium dodecyl sulfate [1,2], acetic acid-urea [3], or phenol [4] treatment to achieve electrophoretic separation of membrane bound proteins. Such harsh procedures will have deleterious effects on

the activities of many membrane bound enzymes. Consequently, several milder procedures for solubilizing and electrophoresing membrane bound proteins have been described using low concentrations of the detergents Triton X-100 [5–7] sodium dodecyl sulfate [7–10] and Tween 20 [11]. The present paper demonstrates that the electrophoretic mobility of intestinal brush border membrane bound dipeptidyl peptidase IV can be modified when solubilized with different concentrations of Triton X-100 and further suggests that care must be exercised when assigning relative mobilities to other membrane bound enzymes monitored in this manner.

The mucosa was scraped from the whole small intestine of male Wistar rats (200–220 g) and homogenized with 6–8 strokes of a Potter-Elvehjem homogenizer in 6 vol. of 2 mM Tris-HCl/50 mM mannitol, pH 7.4. The homogenate was stored at −20°C and used as needed. Solubilization of the membrane bound enzymes was carried out at 4°C using either purified brush border membranes [12] or a membrane pellet fraction prepared as follows: An aliquot of the thawed (6 vols.) homogenate was diluted to 100 vol. in 2 mM Tris-HCl/50 mM mannitol, pH 7.4 and centrifuged at 27 000 × *g* for 1 h. The resulting membrane pellet or the purified brush border membrane fraction was brought to the same volume (100 vols.) of Tris-mannitol buffer containing the detergent Triton X-100 (0.5, 2 or 10% v/v). After a 15 s period of sonication on ice, the solubilized preparation was centrifuged at 27 000 × *g* for 90 min. The supernatant was carefully removed and an aliquot containing 3 μg of protein was used in the electrophoretic analysis.

A 10 cm resolving gel (7.5% acrylamide, 0.67% bisacrylamide) and 10.5 cm stacking gel (3.5% acrylamide, 6.15% bisacrylamide) both buffered with 125 mM Tris-HCl, pH 8.5 were used. The upper and lower reservoir buffer of 66 mM Tris-HCl/80 mM glycine pH 8.9 did not contain added Triton X-100. Electrophoresis was performed for 1 h at 1 mA per tube, and then at 2.5 mA per tube until the end of the run. Gels were removed from the tubes and sliced into 3-mm pieces. Each piece was soaked overnight at 4°C in 0.5 ml of 50 mM Tris-HCl buffer, pH 8.4, in order to elute the enzymes from the gel. Appropriate aliquots were taken for enzyme assays.

Dipeptidyl peptidase IV activity was monitored by the release of β-naphthylamide from the substrate L-glycine-L-proline-β-naphthylamide [13]. Aminopeptidase activity was measured in a similar fashion with L-leucyl-β-naphthylamide. The release of *p*-nitrophenol from *p*-nitrophenyl phosphate was used as a measure of alkaline phosphatase activity [14]. Protein was routinely measured by the method of Lowry et al. [15].

Fig. 1 (A, B and C) illustrates the electrophoretic profiles obtained for dipeptidyl peptidase IV solubilized from the intestinal mucosa brush border membranes with increasing concentrations of Triton X-100. The presence of two peaks of peptidase activity (Fig. 1A) suggested that there were two distinct electrophoretic forms of the enzyme when the membranes were solubilized with 0.5% Triton. However, with increasing concentrations of detergent, the enzyme profile changes, with a progressive increase in the fast migrating enzyme concomitant with a reduction in the slower migrating form (Fig. 1B and 1C). The electrophoretic profile shown in Fig. 1D demonstrates that the slow migrating enzyme can be converted to the fast migrating form. In this experi-

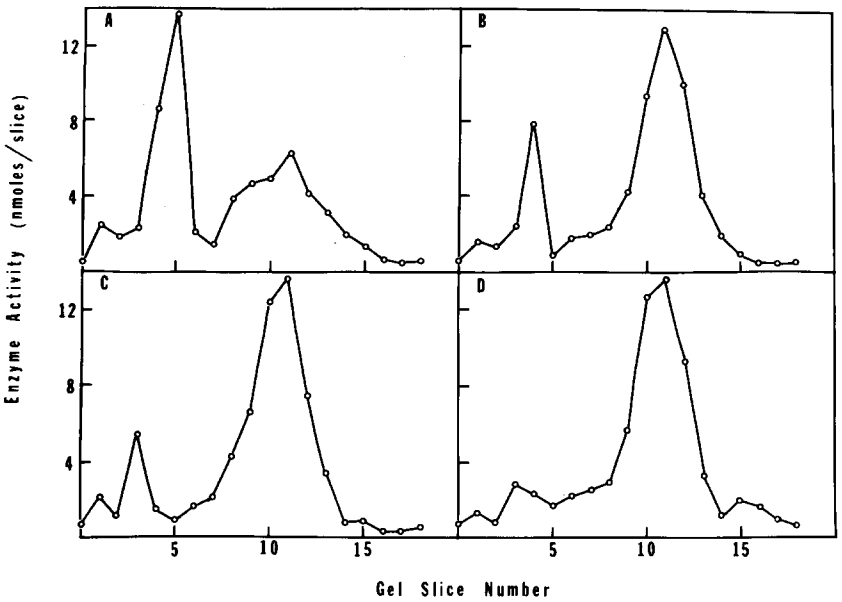

Fig. 1. Effect of Triton X-100 concentration on solubilized dipeptidyl peptidase IV electrophoretic patterns. The enzyme was solubilized from rat intestinal mucosa and electrophoresed as described. The following concentration of Triton X-100 was used: A, 0.5%; B, 2%; C, 10%, D, an aliquot of the 0.5% supernatant was taken and the detergent concentration increased to 10%. The zero position on the gel slice axis represents the stacking gel.

ment, the detergent concentration of the solubilized supernatant from Fig. 1A (0.5% Triton X-100) was increased to 10%. The electrophoretic mobility of the slow migrating enzyme observed in Fig. 1A was shifted to the faster form suggesting that the two enzymes are the same, with electrophoretic mobility dependent upon the detergent concentration. The results were the same when either a partially purified or highly purified brush border membrane preparation was used.

In order to determine whether this phenomenon could be observed with other intestinal brush border membrane enzymes, the electrophoretic behavior of two additional detergent solubilized membrane bound enzymes was monitored under similar conditions. Fig. 2A shows that in the 0.5% Triton extract, a single major peak of enzyme activity consisting of aminopeptidase and alkaline phosphatase overlapped precisely at the slow migrating peak of dipeptidyl peptidase IV. Extraction with 10% Triton shifted the dipeptidyl peptidase IV peak to the expected fast migrating position but had virtually no effect on the position of alkaline phosphatase (Fig. 2B). No aminopeptidase activity could be detected in the 10% Triton X-100 extract indicating that the enzyme was inactivated at this concentration of detergent.

In view of the above observations, it is reasonable to conclude that the slow moving electrophoretic peak represents an aggregate of various proteins, lipids and detergents with dispersal of this aggregate requiring a much higher detergent concentration. This is surprising in view of the fact that the detergent/protein ratio (mg/mg) was 140 during solubilization in 0.5% Triton. Thus, there should have been more than enough detergent to accomplish complete

disaggregation of the membrane components. It is interesting that when the detergent concentration was increased to 10% there was no significant effect on the mobility of alkaline phosphatase.

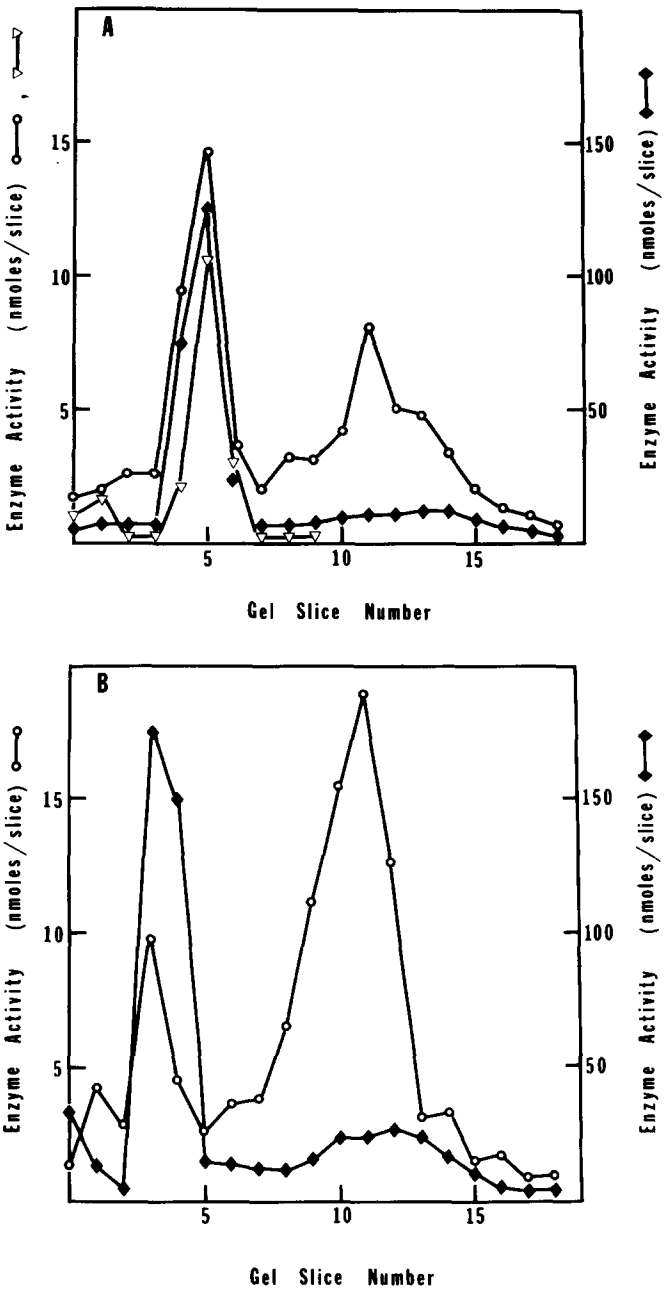

Fig. 2. Effect of Triton X-100 on the electrophoretic profiles of alkaline phosphatase and aminopeptidase. A, 0.5% Triton X-100; B, 10% Triton X-100. 0-0, dipeptidyl peptidase IV: △-△, aminopeptidase; ♦—♦, alkaline phosphatase. The zero position on the gel axis represents the stacking gel.

Intestinal brush border membrane dipeptidyl peptidase IV has recently been solubilized with Triton and purified to homogeneity in our laboratory (Bella, A. and Kim, Y.S., unpublished data). When the purified enzyme was electrophoresed in the above system, only one enzyme band was observed in the fast migrating position. Addition of Triton X-100 (0.5 or 10%) had no effect on its mobility (not shown). Therefore, the faster migrating band probably represents the electrophoretic mobility of dipeptidyl peptidase IV in the disaggregated state.

The solubilization of membrane proteins with detergents under non-denaturing conditions is a widely used technique in the field of membrane biology. This paper demonstrates that the monitoring of enzyme electrophoretic profiles in detergent solubilized preparations can be subject to error unless adequate measures are taken to ensure complete dispersal of the components of interest.

This work was supported by grant AM 17938 from the National Institutes of Health, and the Veterans Administration Medical Research Service.

References

1 Laemmli, U.K. (1970) Nature, 227, 680—685
2 Maestracci, D., Schmitz, J., Preiser, H. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 113—124
3 Lim, R. and Tadayton, E. (1970) Anal. Biochem. 34, 9—15
4 Cotman, C.W., Mahler, H.R. and Hugli, T.E. (1968) Arch. Biochem. Biophys. 126, 821—837
5 Tulsiani, D.R.P., Opheim, D.J. and Touster, O. (1977) J. Biol. Chem. 252, 3227—3233
6 Louvard, D., Maroux, S., Vannier, C. and Desnuelle, P. (1975) Biochim. Biophys. Acta 375, 236—248
7 Dulaney, J.T. and Touster, O. (1970) Biochim. Biophys. Acta 196, 29—34
8 Hauri, H.P., Kedinger, M., Haffen, K., Freiburghaus, A., Grenier, J.F. and Hadorn, B. (1977) Biochim. Biophys. Acta 467, 327—339
9 Simon, P.M., Kedinger, M., Raul, F., Grenier, J.F. and Haffen, K. (1979) Biochem. J. 178, 407—413
10 Green, J.R. and Hadorn, B. (1977) Biochim. Biophys. Acta 467, 86—90
11 Johansson, K.E., Blomqvist, I. and Hjerten, S. (1975) J. Biol. Chem. 250, 2463—2469
12 Schmitz, J., Preiser, H.,Maestracci, D., Ghosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98—112
13 Goldbarg, J.A. and Rutenburg, A.M. (1958) Cancer II, 283—291
14 Fujita, M., Hidehiko, O., Kawai, K., Matuse, H. and Nakao, M. (1972) Biochim. Biophys. Acta 274, 336—347
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275