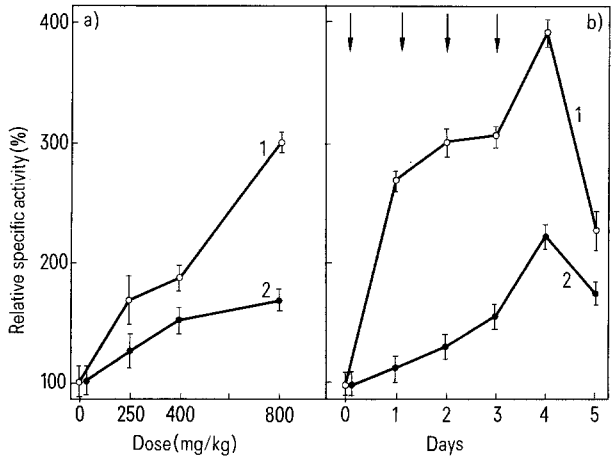
800 mg/kg for 4 days was more than 1.25-fold ($p \leq 0.01$) greater than in the controls. These results are in a good agreement with previous reports[2,14]. The change in peroxisomal enzyme content was accompanied at the same time by the increase of rat liver alkaline phosphatase and histidine-glyoxylate aminotransferase activities in the clofibrate treated animals (table). The total and the specific activities of acid phosphatase and glucose-6-phosphatase were unchanged. The effect of clofibrate on the alkaline phosphatase and histidine-glyoxylate aminotransferase activities was not observed after incubation (37 °C, 15 min) of rat liver homogenates with 1 mM drug (final concentration) or its pharmacologically active derivative chlorophenoxy isobutyric acid. More pronounced changes of enzyme activities were obtained after treatment with higher doses of clofibrate (fig. a). The time-course of enzyme induction by clofibrate showed (fig. b) that the both activities reached the maximum at 4 days and returned rapidly towards the normal values after the end of clofibrate injections. Cycloheximide (1.5 mg/kg) or puromycin (5 mg/kg) injected i.p. inhibited induction of enzyme activities in rats following a single clofibrate administration (800 mg/kg, animals were killed 28 h after the drug treatment). This result suggests that de novo protein synthesis is essential for the induction of hepatic alkaline phosphatase and histidine-glyoxylate aminotransferase by clofibrate. To clarify the possible effects of clofibrate on the subcellular distribution of both enzymes, plasma membranes, mitochondria, peroxisomes and soluble fraction were isolated from control and treated rat livers. All preparations of plasma membranes showed similar recoveries of the alkaline phosphatase activities (20–23% of the total homogenate activity) and purification ratios of the enzyme (25–28-fold). This indicates that alkaline phosphatase induced by clofibrate was located mainly in the plasma membranes, as observed in intact livers.

After fractionation of liver homogenates by differential centrifugation, the highest specific activity of the histidine-glyoxylate aminotransferase was observed in the 'light' mitochondrial fraction together with catalase, a peroxisomal marker. The soluble fractions from the livers of control and clofibrate-treated animals contained 26.8% and 46.1% of total enzyme activity, respectively. The difference may be a consequence of a greater fragility of peroxisomes in treated rats[14]. In order to confirm the distribution of histidine-glyoxylate aminotransferase in peroxisomes and mitochondria, the 'light' mitochondrial fraction was further fractionated by sucrose density gradient centrifugation. The enzyme was widely distributed in both mitochondria and peroxisomes and its specific activity in both organelles doubled after clofibrate treatment. Thus the clofibrate-induced increase of histidine-glyoxylate aminotransferase in rat liver may be the consequence of the enhancement of the activity in mitochondria and peroxisomes.



Effect of clofibrate on the activity of alkaline phosphatase (1) and histidine-glyoxylate aminotransferase (2) in rat liver homogenate. *a* Effect of various clofibrate doses. Clofibrate (250, 400 and 800 mg/kg) was given once a day for 3 days. *b* Time course of changes in enzyme activities. The arrow indicates the injections of clofibrate (800 mg/kg). Results are expressed as the means ± SD for 3–4 rats.

1 J. Reddy and D. Svoboda, Am. J. Path. *63*, 99 (1971).
2 M. Kahonen, Biochim. biophys. Acta *428*, 690 (1976).
3 P.B. Lazarow and C. de Duve, Proc. natl Acad. Sci. USA *73*, 2043 (1976).
4 A.R. Gear, A.D. Albert and J.M. Bednarek, J. biol. Chem. *249*, 6495 (1974).
5 L.F. Panchenko, V.D. Antonenkov and A.M. Gerasimov, Int. J. Biochem. *7*, 409 (1976).
6 V.D. Antonenkov and L.F. Panchenko, FEBS Lett. *88*, 151 (1978).
7 G. Toda, H. Oka, T. Oda and H.N. Muhro, Biochim. biophys. Acta *55*, 561 (1962).
8 H. Hayashi, T. Suga and Sh. Niinobe, Biochim. biophys. Acta *252*, 58 (1971).
9 V. Patel and A. Tappel, Biochim. biophys. Acta *191*, 86 (1969).
10 F. Leighton, B. Poole, H. Beaufay, P. Baudhuin, J.W. Coffey, St. Fowler and C. de Duve, J. Cell Biol. *37*, 482 (1968).
11 T. Noguchi, E. Okuno, Y. Minatogawa and R. Kido, Biochem. J. *155*, 107 (1976).
12 R.J. Cenedella, J.J. Jarrell and L.H. Saxe, J. Atheroscler. Res. *8*, 903 (1968).
13 O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
14 F. Leighton, L. Colona and C. Koenig, J. Cell Biol. *67*, 281 (1975).

Solubilization of prolactin receptor by a Zwitterionic detergent[1]

W.R. Church and K.E. Ebner

Department of Biochemistry, University of Kansas Medical Center, Kansas City (Kansas 66103, USA), 7 July 1981

Summary. About 60% of the prolactin receptors were solubilized from rabbit mammary gland membranes by Zwittergent 3-12. The use of Zwittergent 3-12 resulted in increased sensitivity of the receptor assay and permitted use of ovine prolactin instead of human growth hormone in the receptor assay.

Purification of protein hormone receptors requires the use of a suitable detergent to remove the receptors from the membrane. The nonionic detergent Triton X-100 has been used to solubilize prolactin receptors[2,3] but has several undesirable properties, including absorbance of UV-light, interference with common protein determination methods,

affecting the physical properties of ^{125}I-prolactin so that it cannot be used in assays, and at concentrations above 0.1% (v/v) interferes with the selective precipitation of the ^{125}I-prolactin receptor complex from free ^{125}I-prolactin by polyethylene glycol. In contrast, Zwittergent 3-12 does not have these undesirable properties and is very useful for purifying prolactin receptors.

Materials and methods. Ovine prolactin (NIH-P-S-12) was obtained from the National Institutes of Health, Bethesda, Maryland. 3-(dodecyldimethylammonio)-1-porpanesulfonate, Zwittergent 3-12, was from CalBiochem. La Jolla, California. Prolactin was iodinated with lactoperoxidase and rabbit mammary membranes were prepared as previously reported[4] and had a specific radioactivity between 30 and 60 μCi/μg. Prolactin receptor was solubilized by stirring the membrane suspension in 0.3 M sucrose or 50 mM Tris, pH 7.4 with 1% detergent for 1 h at 4 °C followed by centrifugation at 100,000 × g for 2 h at 4 °C. The preparation represents a starting point for further purification and at this stage, detergent must be present at above the critical micelle concentration of 0.2% to prevent aggregation that slowly occurs.

Binding was measured by incubation 1.0×10^5 cpm ^{125}I-prolactin with aliquots of particulate or solubilized membranes in 20 mM Tris, 10 mM $MgCl_2$, 0.5% BSA, pH 7.6 in a total volume of 0.5 ml for 5 h at room temperature or overnight at 4 °C. Following incubation, 0.5 ml of 0.1% (w/v) human γ-globulin in 0.1 M sodium phosphate, pH 7.4 and 0.7 ml of 25% polyethylene glycol (mol. wt 6000) in water were added to the solubilized samples and the tubes were placed in an ice bath for 10 min. Each sample was individually filtered through 0.5 μm Millipore filters and rapidly rinsed with 4 ml of ice-cold 8% polyethylene glycol in 0.1 M Tris, pH 7.4. The filters were carefully removed from the filter apparatus (Hoeffer Scientific, San Francisco, California), wrapped in small pieces of aluminium foil and counted in a Beckman Biogamma counter. Specific binding is defined as the difference in the cpm of ^{125}I-prolactin bound in the absence and presence of 10 μg unlabeled prolactin.

Results. The optimum concentration of detergent for solubilization was determined to be between 1 and 2% (w/v), and under these conditions 50% of the total membrane proteins and 60% of the prolactin receptors were solubilized from rabbit mammary membranes. Typical binding data as a function of the amount of membranes are presented in figure 1. The specific binding was constant up to a concentration of detergent of 0.15% (w/v). Higher concentration of detergent tends to inhibit the precipitation of the prolactin-receptor complex by polyethylene glycol. The molecular weight of the receptor-detergent micelle was estimated on a Sepharose Cl-6B to be 320,000 (fig. 2). In addition, Zwittergent 3-12 did not interfere with protein determination[5] as was the case with Triton X-100.

Discussion. As shown in figure 1, Zwittergent 3-12 readily solubilized prolactin receptor and it could be assayed with ovine prolactin rather than with human growth hormone which is necessary when using Triton X-100 as the solubilizing detergent[2], since it tends to aggregate the prolactins. The prolactin receptor complex was readily precipitated with 10% polyethylene glycol and this resulted in an increase in the sensitivity of the assay for receptor when compared to using Triton X-100 and human growth hormone. Since Zwittergent 3-12 does not absorb in the UV, columns are readily monitored as shown with the determination of the molecular weight of the receptor on Sepharose Cl-6B. The molecular weight is somewhat higher than that reported in the presence of Triton X-100[2]. Other Zwittergent detergents also were satisfactory, but were not examined in detail. The improvement in the polyethylene glycol assay for lactogenic receptors and the greater utility of Zwittergent detergents should assist studies on the purification and solubilization of membrane protein receptors.

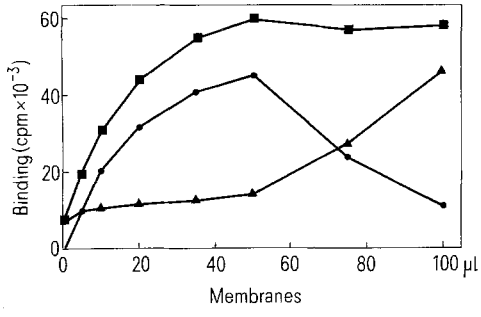


Figure 1. Total (■), specific (●), and nonspecific (▲) binding of ^{125}I-prolactin to Zwittergent 3-12 solubilized rabbit mammary gland membranes. Increasing amounts of 1% Zwittergent 3-12 solubilized membranes were incubated as described in 'methods' with 1.0×10^5 cpm ^{125}I-prolactin in the presence (▲) and absence (■) of 10 μg unlabeled prolactin. The protein concentration of the solubilized receptor preparation was 12.9 mg/ml. Each point represents an average of duplicate samples.

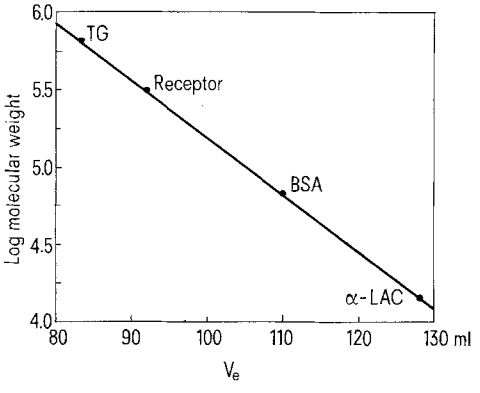


Figure 2. Molecular weight of solubilized prolactin receptor on Sepharose Cl-6B in the presence of Zweittergent 3-12. A 5 ml sample of 1% Zwittergent 3-12 solubilized receptor (40 mg protein) was chromatographed on a 80 × 1.5 cm column of Sepharose Cl-6B, equilibrated and eluted with 20 mM Tris, 0.1 M NaCl, 0.2% Zwittergent 3-12, pH 7.4 at 4 °C. Fractions of 2 ml were collected and 100 μl aliquots of each fraction were assayed for receptor activity using the prolactin binding assay described in 'methods'. The sample was dialyzed overnight at 4 °C against the column buffer prior to gel filtration. Protein was monitored at 280 nm. Thyroglobulin (TG), bovine serum albumin (BSA), and α-lactalbumin (α-LAC), 4 mg each in 2 ml of column buffer, were used as reference proteins. The estimated molecular weight of the solubilized receptor was 3.2×10^5.

1 Acknowledgments. This work was supported by grant AM 17476 from the National Institutes of Health.

2 R.P.C. Shiu and H.G. Friesen, J. biol. Chem. *249*, 7902 (1974).
3 J.S. Bonafacino, S.H. Sanchez and A.C. Paladini, Biochem. biophys. Res. Commun. *85*, 62 (1978).
4 T.T. Andersen, M.M. Zameriowski and K.E. Ebner, Archs Biochem. Biophys. *192*, 112 (1979).
5 M.M. Bradford, Analyt. Biochem. *72*, 248 (1976).