Characterization of CHAPS-solubilized prolactin receptors induced by estradiol in liver of male rats

Unmasking of cryptic sites by CHAPS

M. Gavish, T. Amit, R.J. Barkey and M.B.H. Youdim

Rappaport Medical Research Center, Department of Pharmacology, Faculty of Medicine, Technion–Israel Institute of Technology, PO Box 9649, Haifa 31096, Israel

Received 14 February 1983

Abstract not received

Prolactin receptor *CHAPS* *Solubilized prolactin* *Estradiol* *Cryptic site unmasking*

1. INTRODUCTION

Treatment of young and adult rats with estrone, estradiol (E_2) or ovine prolactin (oPRL) [1,2] significantly induces the appearance of specific PRL binding sites in liver and lung membranes. To characterize completely and purify such a receptor it needs to be solubilized from the membrane. There are few reports on the solubilization, purification and characterization of PRL receptors under conditions allowing the specific study of their binding properties [3–6]. The commonly used detergent Triton X-100 has been shown to cause aggregation of ^{125}I-oPRL during binding studies [3] and thus necessitated the use of ^{125}I-hGH. Recently, the zwitterionic detergent 3-[(3-cholamidopropyl) dimethylammonia]-1-propane-sulfonate·2 H_2O (CHAPS) has been used successfully to solubilize muscarinic cholinergic receptors from rat brain cortex [7] and was therefore used for this study of solubilization of the PRL receptor from E_2-induced male rat liver.

Binding of ^{125}I-oPRL to PRL receptor solubilized with 0.5% CHAPS was dependent upon the protein concentration. This receptor demonstrated the hormonal specificity typical of the membrane-bound PRL receptor and was bound to an anti-PRL receptor. Binding to the CHAPS-solubilized membranes was much greater than to the particulate preparation, suggesting an unmasking of cryptic sites. Gel filtration of the ^{125}I-oPRL–receptor complex on Sepharose 6B revealed an estimated $M_r \sim 340\,000$. These results show that CHAPS is particularly suitable for solubilization of PRL receptors since the properties of the receptor are maintained and since CHAPS does not interfere with the binding of ^{125}I-oPRL.

2. MATERIALS AND METHODS

oPRL (NIH-P-S15; 30.5 IU/mg), human GH (hGH; NIAMDD-hGH-RP-1), human PRL (hPRL; NIAMDD-hPRL-RP-1) rat PRL (rPRL-B-3) rat GH (rGH; GH-B-6) human FSH (hFSH; HS-1) were kindly supplied by the National Pituitary Agency of the NIAMDD. All the other reagents were purchased from commercial sources detailed in [14, 15]. Anti-PRL-receptor anti-serum (no. 151), raised in sheep against a partially purified rabbit mammary gland PRL receptor, was kindly provided by Dr P.A. Kelly (Molecular Endocrinology Laboratory, CHUL, Quebec). Sprague-Dawley female and male rats (200 g body wt) were used. For the induction of PRL binding sites, the male rats received daily s.c. injections of E_2 (0.4 mg) mixed with 10% PVP (w/v) for 7 days.

Published by Elsevier Science Publishers
00145793/83/0000–0000/$3.00

2.1. *Preparation of membrane fractions*

The crude membrane fractions containing PRL-receptors were prepared from livers of E_2-treated male rats as in [8]. The resultant 100 000 × *g* pellet was resuspended in 5 vol. (per gram of original tissue weight) ice-cold buffer containing 0.01 M Tris–HCl, 0.002 M KCl, 0.01 M $MgCl_2$ and 0.1% sodium azide (pH 7.6). The protein concentration as determined by a modification [9] of the method in [10] was ~ 200 μg/0.1 ml suspension.

2.2. *Iodination of oPRL*

oPRL was iodinated by the lactoperoxidase method in [1] and purified by chromatography on Sephadex G-100 as in [12]. The specific activity was ~ 80 Ci/g.

2.3. *Binding assay*

^{125}I-oPRL (1 ng/0.1 ml) was incubated with 0.1 ml particulate or solubilized membranes in the absence or presence of excess unlabelled oPRL (1 μg/0.1 ml) to determine non-specific binding as in [13]. Incubations were carried out in 0.3 ml final vol. at 20°C for 44 h. After incubation, the bound hormone was separated from the free by precipitation and filtration as in [14]. Filters were placed in plastic tubes and radioactivity was measured in an automatic γ-counter.

2.4. *Solubilization*

Solubilization was performed by adding 0.5% (w/v) CHAPS in 0.01 M Tris–HCl (pH 7.6) to the 100 000 × *g* resuspended membrane pellet, follow-

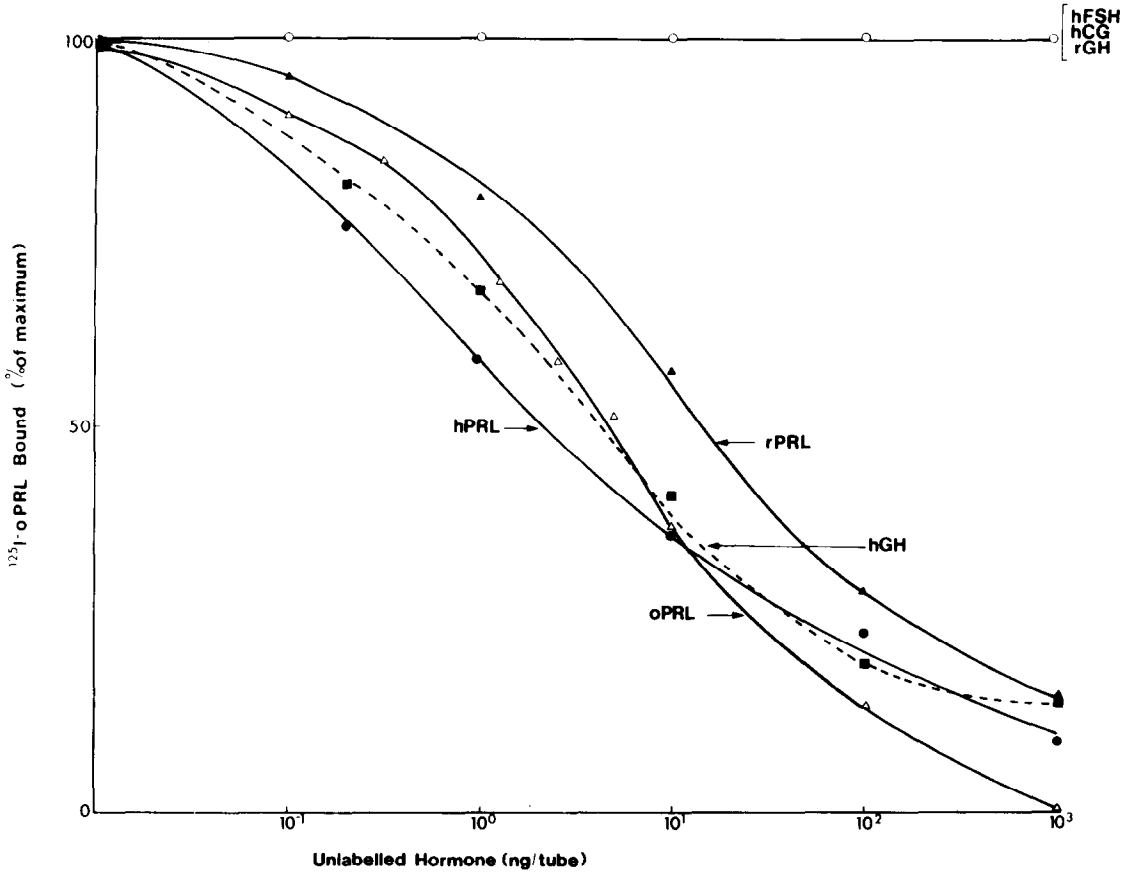

Fig. 1. Hormone specificity of ^{125}I-oPRL binding to CHAPS-solubilized liver membrane: ^{125}I-oPRL (1 ng/0.1 ml) was incubated with solubilized receptors (as in section 2) with increasing concentrations of unlabelled competing hormones. The specific binding of ^{125}I-oPRL was expressed as a percentage of that obtained in the absence of unlabelled hormone.

ed by homogenization and incubation for 60 min at 0°C. The reagent mixture was then centrifuged at 100 000 × *g* for 90 min at 4°C and the resultant supernatant used as the solubilized receptor preparation in binding studies.

2.5. *Gel filtration*

The hormone–receptor complex was chromatographed on a Sepharose 6B column (58 × 2.5 cm) at 5°C and eluted with 0.1 M Tris–HCl buffer (pH 7.6) containing 0.01% CHAPS. The column was calibrated with several marker proteins. The void volume (V_o) was determined by using blue dextran 2000 and the total volume (V_t with $Na^{125}I$. The chromatographic characteristic was estimated by calculation of the elution constant (K_{av}) based on the elution volume (V_e and the formula:

$$K_{av} = (V_e - V_o)/(V_t - V_o)$$

3. RESULTS

In preliminary studies, the ability of a variety of detergents to solubilize PRL receptors from the female rat liver was evaluated and only CHAPS (0.5%) was found to be both efficient in solubilizing the receptor and did not interfere in the binding assay. The use of $\geqslant$0.75% CHAPS resulted in inactivation of ~50% of the binding activity of the solubilized receptor.

In view of the high concentration of PRL receptors in livers of E_2-treated male rats [1,15], these receptors were solubilized with 0.5% CHAPS for the purpose of characterization. CHAPS-solubilized membranes demonstrated 75% greater binding activity than observed with the 100 000 × *g* pellet. Under these conditions 74% of the total microsomal membrane proteins were solubilized. ^{125}I-oPRL specific binding increased as a function of CHAPS-solubilized membrane protein concentration, reaching 55% of total cpm with 350 μg protein/tube. Non-specific binding was not significantly affected. In hormone specificity studies only unlabelled hormones with lactogenic properties effectively competed for the binding of ^{125}I-oPRL (fig. 1).

Scatchard analysis [16] of the competition data with oPRL revealed two populations of binding sites for the solubilized membranes, with K_a values of 2.4 × 10^{10} and 1.3 × 10^9 1/mol and respective binding capacities of 141.5 and 619.5 fmol/mg protein (fig. 2).

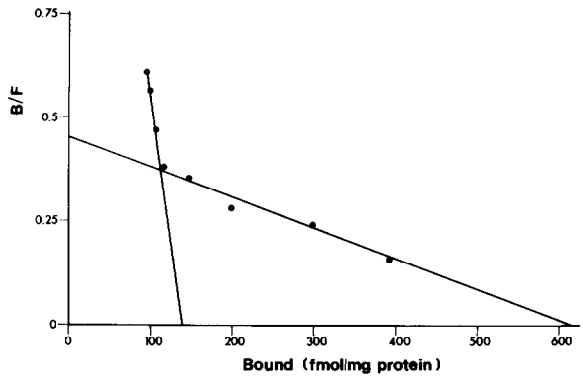

Fig. 2. Scatchard analysis of binding of ^{125}I-pPRL to CHAPS solubilized liver membranes from E_2-treated male rats: The competition data of fig. 1 were analyzed by Scatchard analysis [16]. The ordinate represents the ratio of bound/free and the abscissa the amount (fmol/mg) of ^{125}I-oPRL bound to CHAPS-solubilized receptors. The negative slope of the plot yields the affinity constant (K_a) and the intercept on the abscissa yields the binding capacity. The results presented are from a representative experiment which was repeated 3 times.

The addition of increasing concentrations of a sheep anti-PRL receptor antiserum to either particulate or CHAPS-solubilized membranes resulted in similar, concentration-related inhibition of ^{125}I-oPRL binding. The addition of control serum only did not significantly affect the binding, in the concentration range used (fig. 3).

Fig. 4 shows the elution pattern from Sepharose 6B of CHAPS-solubilized liver membrane from E_2-treated rats, incubated with ^{125}I-oPRL in the absence or presence of excesss unlabelled oPRL. The radioactive peak of fraction 32 (K_{av} = 0.15) represents the hormone–receptor complex since excess unlabelled oPRL resulted in its disappearance.

4. DISCUSSION

In this study the hepatic receptors for PRL from E^2-treated rats were solubilized with 0.5% CHAPS and partially characterized using ^{125}I-oPRL as a

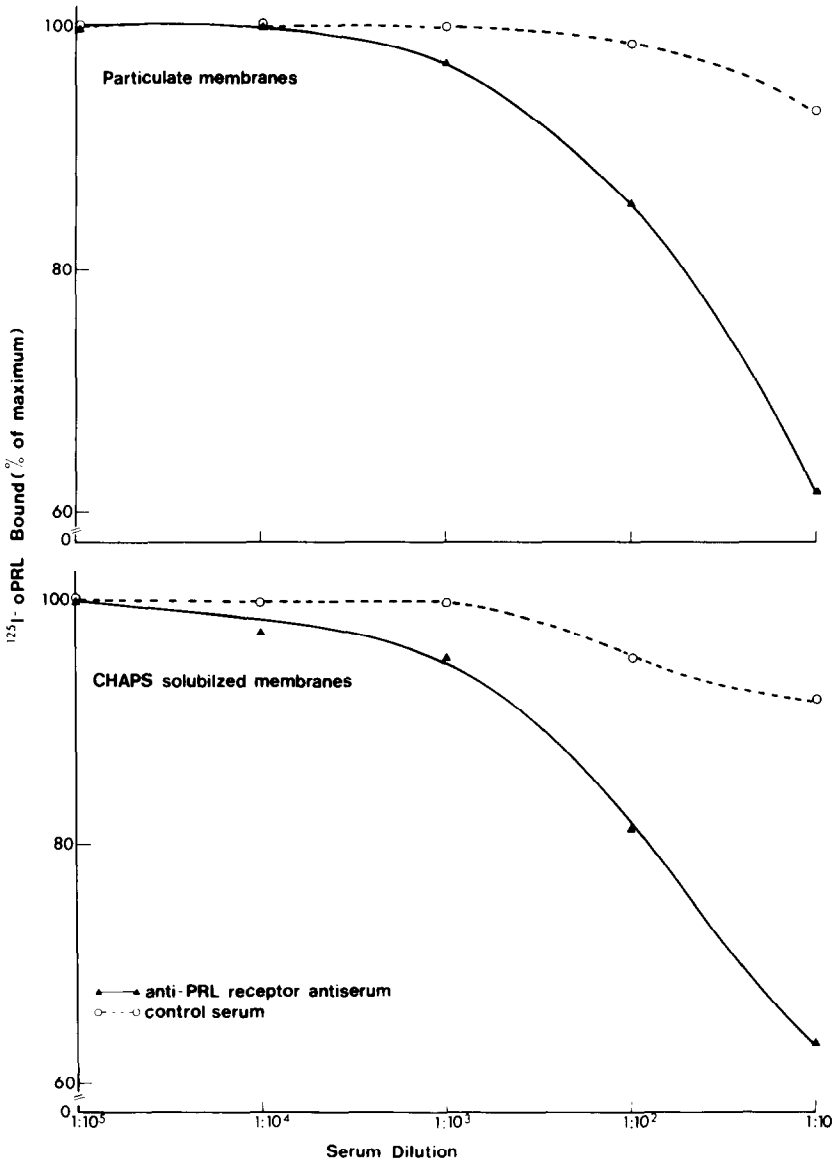

Fig. 3. Effect of anti-PRL receptor antiserum on the binding of ^{125}I-oPRL particulate or CHAPS-solubilized liver membranes, from E_2-treated rats: ^{125}I-oPRL (1 ng/0.1 ml) was incubated for 44 h at 20°C with particulate membranes (300 μg protein/reaction) or with CHAPS-solubilized membranes (80 μg protein/reaction), in the presence of increasing concentrations of either control serum or an antiserum from sheep, raised against a partially purified rabbit mammary PRL receptor. Binding was expressed as a percentage of binding in the absence of serum.

tracer in the binding studies. In contrast to Triton X-100, CHAPS did not alter the prolactin molecule as determined by its elution pattern on Sepharose 6B and did not interfere with its binding activity. Previously ^{125}I-hGH was used as a tracer in characterization of the Triton-solubilized receptors, since it has been shown not to be affected by the detergent, while ^{125}I-oPRL was shown to ag-

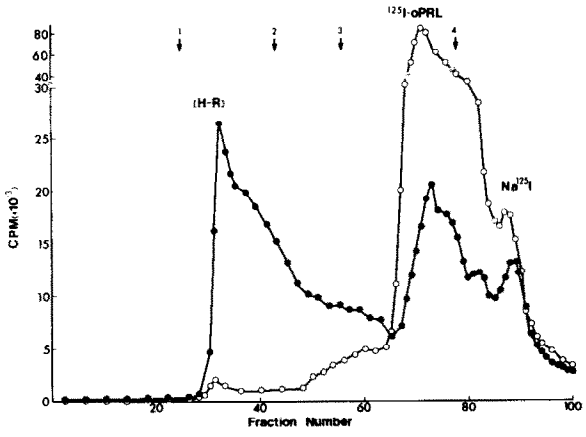

Fig. 4. Sepharose 6B chromatography of CHAPS-solubilized ^{125}I-oPRL–receptor complex. CHAPS-solubilized liver membranes of E_2-treated male rats were incubated with ^{125}I-oPRL in the absence (●) or presence (○) of excess unlabelled oPRL as in section 2. The incubation mixture was applied to Sepharose 6B column (58 × 2.5 cm) and eluted with 0.01% CHAPS in 0.01 M Tris–HCl buffer (pH 7.6). Fractions (3.8 ml) were collected, and the radioactivity was measured in an automatic γ-counter. The column was equilibrated for M_r determination using the following markers, whose position was monitored at 280 nm: (1) urease (480 000); (2) BGG (160 000); (3) BSA (69 000); (4) myoglobin (17 000).

gregate in Triton X-100 [3,17]. Even though hGH and oPRL bind to the same molecular lactogenic sites in the target tissue membranes and subsequently elicit similar biological responses, hGH has been shown to differ considerably from oPRL in a number of important points. While oPRL has a high affinity only for lactogenic sites, hGH is thought to recognize strongly at least 2 types of sites, namely lactogenic and somatogenic [18] and it has even been suggested that these two sites will not explain fully its binding properties to liver membranes [19]. oPRL and hGH have different primary structures [20] resulting in different three-dimensional geometry of the hormones. Thus it seems likely that the environmental requirements for each hormone's binding to its membrane receptors are different. Indeed, membrane modification differentially affects the binding of hGH and oPRL [21]. Thus it was important to find a detergent which did not interfere with ^{125}I-oPRL binding to characterize solubilized PRL receptors.

Out of a variety of detergents studied, CHAPS was able to solubilize as much as 74% of the particulate membrane protein and did not interfere in the binding activity of ^{125}I-oPRL. Further, ^{125}I-oPRl binding to this same solubilized preparation was 75% greater than to the original particulate membranes prepared from livers of E_2-treated male rats. This suggests the possibility that solubilization might have exposed masked or cryptic binding sites and is in keeping with findings that estradiol is capable of inducing cryptic PRL sites in livers of neonatally-treated female mice [22] and with the demonstration of cryptic cell-surface receptors in cultured rat mammary tumour cells [23]. This hypothesis is supported by the demonstration, by Scatchard analysis [16] of two types of PRL binding sites in the solubilized hepatic membranes.

The hormonal specificity of the solubilized PRL receptor was apparently not affected by the solubilization process, since all of the lactogenic hormones effectively competed for ^{125}I-oPRL binding, as shown for the membrane-bound receptors [8]. The lack of effect of CHAPS solubilization on the binding characteristics of the recognition site of the PRL receptor is further demonstrated by the inhibition of ^{125}I-oPRL by a specific PRL receptor antibody. The antiserum was equally effective on membrane-bound and on solubilized PRL receptors. This same antiserum has been shown to inhibit effectively the physiological action of PRL in both liver and mammary gland in vitro, in parallel to inhibition of PRL binding [24,25].

In gel chromatography studies on Sepharose 6B, the ^{125}I-oPRL–receptor complex was estimated to have an M_r ~340 000. Earlier studies of Triton X-100-solubilized rat liver or rabbit mammary gland membranes using hGH as their tracer obtained M_r-values for the lactogenic receptor from >150 000–330 000 [3–6,17]. In those studies, the particulate membrane solubilization was done at room temperature (20–25°C) for 30–60 min. While this study was in preparation two studies of solubilization of PRL receptors with zwitterionic detergents were reported [26,27]. Using zwittergent 3-12 PRL receptors were solubilized from rabbit mammary gland at 4°C for 1 h [26]. In that study, the M_r obtained was identical to the value presented here. In [27] the PRL receptor was

solubilized from lactating mouse liver membranes, also using CHAPS, but at room temperature (23°C) for 30 min with constant stirring and found the M_r to be 57 000–61 000 for the complex; they suggested that the peptide thus obtained might represent a binding subunit of the PRL receptor. It is possible that at 0°C (on ice), a milder solubilization is obtained such that a larger PRL receptor entity, rather than only a binding subunit, is separated from the membrane fraction. Nevertheless, one cannot rule out the possibility that we are looking at an aggregate of a smaller subunit not to mention the species and tissue differences. In support of the present findings, the M_r of a soluble PRL receptor studied in the 100 000 × g supernatant cytosolic fraction of liver from either female or E_2-treated male rats was identical to that of the CHAPS (0°C)-solubilized PRL receptor (submitted). Since no detergent was used for the study of the soluble cytosolic PRL receptor, it is implied that the use of CHAPS does not interfere with the M_r determination, and that we are in fact dealing with the PRL receptor molecule.

ACKNOWLEDGEMENTS

This study was supported by grants from the Wellcome Trust (UK) and the Ministry of Health (Israel) to M.B.H.Y. and R.J.B., and from the Batsheva de Rothchild Foundation to M.G.

REFERENCES

[1] Posner, B.I., Kelly, P.A. and Friesen, H.G. (1974) Proc. Natl. Acad. Sci. USA 71, 2407–2410.
[2] Amit, T., Ben Harari, R.R. and Youdim, M.B.H. (1981) Brit. J. Pharmacol. 74, 955.
[3] Shiu, R.P.C. and Friesen, H.G. (1974) J. Biol. Chem. 249, 7902–7911.
[4] Bonifacino, J.S., Sanchez, S.H. and Paladini, A.C. (1978) Biochem. Biophys. Res. Commun. 85, 62–69.
[5] Jaffe, R.C. (1982) Biochemistry 21, 2936–2939.
[6] Sasaki, M., Tanaka, Y., Imai, Y., Tsushima, T. and Matsuzaki, F. (1982) Biochem. J. 203, 653–662.
[7] Gavish, M. and Sokolovsky, M. (1983) Biochem. Biophys. Res. Commun. in press.
[8] Shiu, R.P.C., Kelly, P.A. and Friesen, H.G. (1973) Science 180, 968–971.
[9] Miller, G.L. (1959) Anal. Chem. 31, 964.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.N. (1951) J. Biol. Chem. 193, 265–275.
[11] Rogol, A.D. and Chrambach, A. (1975) Endocrinology 97, 406–417.
[12] Barkey, R.J., Shani, J., Amit, T. and Barzilai, D. (1977) J. Endocr. 74, 163–173.
[13] Barkey, R.J., Shani, J., Amit, T. and Barzilai, D. (1979) J. Endocr. 80, 181–189.
[14] Gavish, M., Goodman, R.R. and Snyder, S.H. (1982) Science 215, 1633–1635.
[15] Amit, T., Barkey, R.J. and Youdim, M.B.H. (1983) Mol. Cell. Endocr. in press.
[16] Scatchard, G. (1949) Ann. NY Acad. Sci. 51, 660–672.
[17] Carr, F.E. and Jaffe, R.C. (1981) Endocrinology 109, 943–949.
[18] Posner, B.I. (1976) Endocrinology 98, 645–654.
[19] Fix, J.A., Leppert, P. and Moore, M.V. (1981) Horm. Metab. Res. 13, 510–515.
[20] Bewley, T.A. and Li, C.H. (1970) Science 168, 1361–1362.
[21] Battacharya, A. and Vonderhaar, B.K. (1981) Proc. Natl. Acad. Sci. USA 78, 5704–5707.
[22] Alhadi, T. and Vonderhaar, B.K. (1982) Endocrinology 110, 254–259.
[23] Costlow, M.E. and Hample, A. (1982) J. Biol. Chem. 257, 6971–6977.
[24] Rosa, A.A.M., Djiane, J., Houdebine, L.M. and Kelly, P.A. (1982) Biochem. Biophys. Res. Commun. 106, 243–249.
[25] Djiane, J., Houdebine, L.M. and Kelly, P.A. (1981) Proc. Natl. Acad. Sci. USA 78, 7445–7448.
[26] Church, W.R. and Ebner, K.E. (1982) Experientia 38, 434–435.
[27] Liscia, D.S., Alhadi, T. and Vonderhaar, K. (1982) J. Biol. Chem. 257, 9401–9405.