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Biochemistry

Specific gonadotropin binding to *Pseudomonas maltophilia*

(125I-labeled human chorionic gonadotropin/hormone binding sites in procaryotes/luteinized rat ovaries/porcine follicular fluid)

NANCY D. RICHERT AND ROBERT J. RYAN

Department of Molecular Medicine, Mayo Medical School and Foundation, Rochester, Minnesota 55901

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ABSTRACT **Binding of ^{125}I-labeled human chorionic gonadotropin to *Pseudomonas maltophilia* is dependent on time, temperature, and pH and the binding to this procaryotic species is hormone-specific and saturable. The equilibrium dissociation constant is 2.3×10^{-9} M. There are no cooperative interactions between binding sites (Hill coefficient, 1.05). The number of sites is estimated as 240 fmol/100 μg of protein. NaCl and KCl, at concentrations from 1 to 10 mM, have no effect on binding. Divalent cations (Mg^{2+} and Ca^{2+}) and 1 mM EDTA inhibit hormone binding. Binding is destroyed by heat or by treatment with Pronase or α-chymotrypsin and is increased by phospholipase C. Binding of the labeled gonadotropin is not observed with other gram-negative organisms—e.g., *Escherichia coli, Pseudomonas testosteroni, Pseudomonas aeruginosa, Enterobacter aerogenes,* or *Enterobacter cloacae.***

Two gram-negative, motile, bacteria were isolated from porcine ovarian follicular fluid and identified as *Pseudomonas maltophilia* and *Enterobacter cloacae* on the basis of physiological, nutritional, and biochemical characteristics. The properties of *P. maltophilia* have been described by Hugh and Ryschenkow (1) and Stanier *et al.* (2).

Subsequent outgrowth of the isolated strains in liquid media demonstrated that *P. maltophilia* was capable of binding ^{125}I-labeled human chorionic gonadotropin (^{125}I-hCG) with high affinity, whereas *E. cloacae* showed no gonadotropin binding. Because of the reported presence of a steroid-binding protein in *P. testosteroni* (3), the recent demonstration that steroid hormones influence the growth, infectivity and viability of *Neisserria gonorrhoeae* (4), and the presence of mammalian antigens on bacterial surfaces (5), we considered it of interest to further characterize the binding of gonadotropin to *P. maltophilia*. Except where noted, the follicular fluid isolate and subcultures of this strain were used in all experiments.

MATERIALS AND METHODS

Highly purified hCG CR 121 and CR 117α and β subunits were gifts from the National Institute of Child Health and Human Development and Dr. Robert Canfield, Columbia University. Purified human lutropin (hLH) was prepared in this laboratory. oLH-S18, ovine follitropin (FSH)-S7, and ovine prolactin (oPRL)-S6 were obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases. A commercial preparation of hCG (Ayerst) was used for injection of rats and for determinations of nonspecific binding. Pig ovaries were purchased from the Hormel Co. (Austin, Minnesota).

Reagents and enzymes were obtained from the following sources: carrier-free Na^{125}I, Amersham/Searle; trypticase soy broth and nutrient agar, BBL Div., Becton-Dickinson and Co.; eosin-methylene blue agar (Levine) plates, Gibco Diagnostics; protease, trypsin, phospholipase D, and hyaluronidase, Sigma; Pronase, Calbiochem; α-chymotrypsin, Schwarz/Mann; and phospholipase C, Worthington.

Bacterial Strains and Growth Conditions. *P. maltophilia* and *E. cloacae* were isolated from porcine ovarian follicular fluid of small (1–2 mm) follicles. *P. maltophilia* (ATCC 13637) and *P. testosteroni* (ATCC 11996) were obtained from the American Type Culture Collection, Rockville, Md. *P. aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Enterobacter aerogenes* are stock cultures maintained by the Mayo Clinic Diagnostic Microbiology Laboratory.

Stock cultures were maintained on eosin-methylene blue agar plates and nutrient agar slants at room temperature. For binding studies, several small (1–2 mm) granular colonies were inoculated into a sterile tube containing 5 ml of trypticase soy broth, pH 7.4. After overnight incubation at 30° in a shaking water bath, the culture was transferred to a 2-liter sterile flask containing 500 ml of trypticase soy broth and grown to stationary phase at 30° in a shaking water bath (100 oscillations/min).* Stationary phase cultures had an optical density (turbidity) at 625 nm of 3.5–9.0, depending on the bacterial strain. Although binding activity was tested twice a day during all phases of growth, maximal binding in *P. maltophilia* generally occurred within 48 hr after the culture reached stationary phase.

Preparation of Bacterial Pellets for Binding Assays. Bacteria were harvested by centrifugation at 20,000 × *g* for 20 min at 4° and washed twice with an equivalent volume of 40 mM Tris·HCl, pH 7.4. The pellets were resuspended at a 10-fold concentration in 40 mM Tris·HCl, pH 7.4, containing 10% sucrose. These pellets could be stored at −70° for 6 months without appreciable loss of binding activity. For the binding assay, bacterial pellets were used undiluted or were diluted with 40 mM Tris·HCl to a protein concentration of 1 mg/ml. Protein determinations were performed by the method of Lowry *et al.* (6) with bovine serum albumin as standard.

Preparation of 2000 × *g* Ovarian Pellets. Pseudopregnant rat ovaries 7–10 days after priming with pregnant mare's serum gonadotropin and hCG were used for all experiments. Preparation of ovarian homogenates (7) and the 2000 × *g* pellet fraction thereof (8) have been described.

Binding Assays. The preparation of ^{125}I-hCG, having a specific activity of 30–40 μCi/μg, has been described previously (8). In a standard binding assay, 100 μg of bacterial or ovarian pellet protein was incubated with 4 ng of ^{125}I-hCG in a final volume of 1 ml in 40 mM Tris·HCl buffer, pH 7.4, containing 0.1% bovine serum albumin. Nonspecific binding was measured in a duplicate set of tubes containing unlabeled hCG (200 international units/ml). After incubation at 24° for 20 hr, bound radioactivity was precipitated with Carbowax (9) or by cen-

Abbreviations: hCG, human chorionic gonadotropin; hLH, human lutropin (luteinizing hormone); oLH, ovine LH; FSH, follitropin (follicle-stimulating hormone); oPRL, ovine prolactin; K_d, equilibrium dissociation constant.

* These culture conditions are necessary for optimal and reproducible binding results. The suspension cultures must be adequately aerated. Subcultures from suspension cultures often show lower degrees of binding.

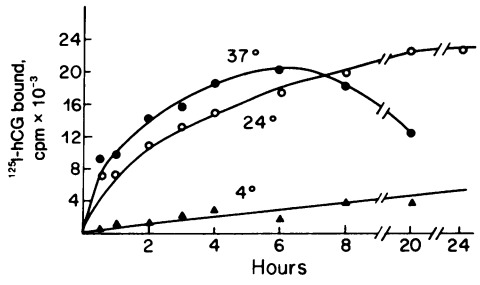

FIG. 1. Specific binding of ^{125}I-hCG as a function of time and temperature. Four nanograms of ^{125}I-hCG (about 200,000 cpm) was incubated with bacterial protein, 150 μg/ml, in 40 mM Tris buffer as described in *Materials and Methods*. Control incubations contained unlabeled hCG. Each point represents the mean of triplicate determinations.

trifugation at 2000 × *g* after the volume was adjusted to 5 ml with 40 mM Tris·HCl, pH 7.4. The 2000 × *g* pellets were washed a second time with 5 ml of the same buffer. Radioactivity was measured in an Autogamma spectrometer. Specific binding is expressed as the difference between total and nonspecific ^{125}I cpm.

RESULTS

Time- and Temperature-Dependence of Gonadotropin Binding. Binding of ^{125}I-hCG to *P. maltophilia* depends on both time and temperature (Fig. 1). At 37° specific binding increased during the first 6 hr and progressively declined thereafter. At 24°, the rate of binding was slower than at 37°, requiring 20–24 hr to reach maximum. The maximal binding at 24° was slightly higher than at 37° and this level of binding was maintained to 48 hr of incubation (data not shown). At 4° binding was significantly decreased and did not reach equilibrium during 24 hr of incubation.

Effect of Bacterial Concentration. Fig. 2 shows that binding of ^{125}I-hCG is proportional to the concentration of bacterial protein used. Specific binding was curvilinear but approached linearity when the protein concentration per assay tube ranged from 1 to 100 μg/ml. In contrast, nonspecific binding of ^{125}I-hCG was linear at all protein concentrations. Specific binding approached a maximum at 1200 μg of protein per ml, at which 20% of the added labeled hormone was bound.

pH Dependency of Gonadotropin Binding. Binding of ^{125}I-hCG to *P. maltophilia* was optimal at pH 7.0 (data not shown) with nearly maximal binding being observed in the pH range 6.8–7.5. Binding decreased abruptly on either side of these limits.

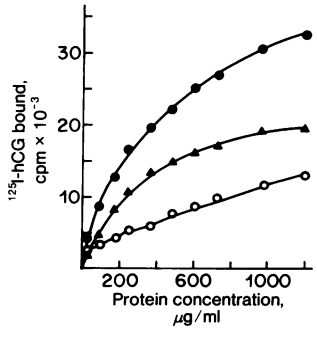

FIG. 2. Effect of bacterial protein concentration on binding of ^{125}I-hCG. Increasing concentrations of bacterial 20,000 × *g* pellet protein were incubated with 2 ng of ^{125}I-hCG for 20 hr at 24°. Nonspecific binding (○) measured in the presence of unlabeled hCG was subtracted from total binding (●) to determine specific binding (▲). Each point represents the mean of triplicate determinations.

Table 1. Effect of salts on ^{125}I-hCG binding to *P. maltophilia*

Metal ion or chelator	Specific ^{125}I cpm bound				
	0*	1 mM	10 mM	100 mM	150 mM
NaCl	37,699	34,604	32,929	20,197	15,247
KCl	—	38,854	32,944	18,535	14,013
$CaCl_2$	—	35,052	21,578	4,603	2,729
$MgSO_4$	—	33,983	20,414	4,819	5,253
EDTA	—	17,413			

* The control solution was 40 mM Tris·HCl, pH 7.4, with 0.1% bovine serum albumin. All other salts and EDTA were added to this buffer in the concentrations indicated. Assays were terminated by centrifugation at 2000 × *g*.

Effect of Metal Ions on Gonadotropin Binding. NaCl and KCl had no effect on specific binding of ^{125}I-hCG to *P. maltophilia* at concentrations ranging from 1 to 10 mM (Table 1). Higher concentrations of these ions, 100–150 mM, inhibited binding by approximately 50%. Binding was sensitive to the presence or absence of divalent cations in the assay medium. Addition of a metal ion chelating agent (1 mM EDTA) substantially decreased specific binding to 46% of control values. Calcium and magnesium ions, at concentrations from 10 to 150 mM, also inhibited binding although low concentrations (1 mM) had no effect.

Hormonal Specificity of Binding. The hormonal specificity of binding was determined by incubating *P. maltophilia* with ^{125}I-hCG in the presence of increasing amounts of unlabeled hormones. Binding in the absence of unlabeled hormones was regarded as 100%. Parallel incubations were performed with 2000 × *g* ovarian pellets (data not shown). The results (Fig. 3) show that hLH, hCG, and oLH competitively inhibited the binding in a similar manner. Maximal competition occurred when the concentration of unlabeled hormone was 100 μg/ml. Specific binding at this point was decreased to 20% of the control value.

oFSH was effective only at high concentrations consistent with the level of LH contamination in the preparation.† The α and β subunits of hCG, which have no *in vitro* binding activity to ovarian (10) or testicular gonadotropin receptors (11), did not compete with ^{125}I-hCG for binding. Similarly, oPRL did not compete.

Table 2 compares the relative potencies of gonadotropins as measured by radioreceptor assays using *P. maltophilia* and ovarian 2000 × *g* pellets. The two methods of assay show relatively good agreement.

To determine whether *P. maltophilia* binds the sugar moiety of the glycoprotein hormone, various sugars were added at a 50,000-fold excess of labeled hormone. The results (Fig. 3) indicate that galactose, glucose, mannose, maltose, D-glucosamine, and *N*-acetylneuraminic acid do not compete with ^{125}I-hCG for binding. Other glycoproteins—e.g., ovomucoid and ovalbumin—only competed at concentrations of 10 mg/ml. The competition probably was due to a nonspecific protein effect on the assay. Similar competition was observed with bovine gamma globulin and with these proteins in an ovarian

† Highly purified hFSH shows 3% cross-reactivity in the bacterial competition experiments. This is compatible with the level of LH contamination in this preparation as measured by radioimmunoassay.

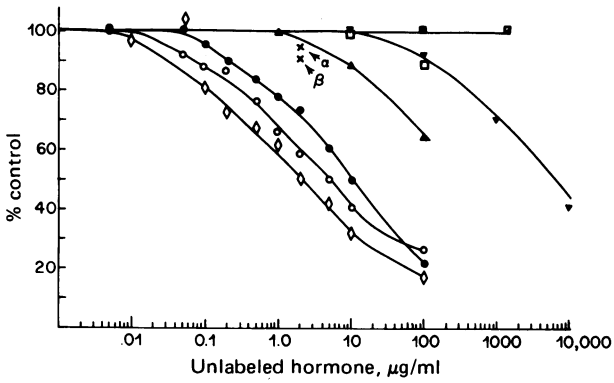

FIG. 3. Competition for binding of ^{125}I-hCG to *P. maltophilia* by various unlabeled hormones. Two ng of ^{125}I-hCG was incubated with 210 μg of bacterial protein in the presence of increasing concentrations of unlabeled hormones. Incubations were performed at 24° overnight and terminated with Carbowax. ◊, hLH; ○, hCG; ●, oLH; ×, hCGα and β subunits; △, oFSH; □, oPRL; ▼, ovalbumin, ovomucoid, bovine gamma globulin; ■, sugars (glucose, galactose, mannose, maltose, D-glucosamine, *N*-acetylneuraminic acid); □, KI.

receptor assay (data not shown).‡ Excess KI (10^{-5} M) did not inhibit the binding of labeled hormone, suggesting that binding is not due to selective uptake of ^{125}I.

Stability of Gonadotropin Binding. The binding of ^{125}I-hCG to *P. maltophilia* was saturable when increasing concentrations of labeled hormone were incubated with a fixed amount of bacterial protein (Fig. 4*A*). Because a relatively large amount of labeled hormone is required for saturation, the bacterial protein concentration in the assay must be kept low (about 50 μg/ml).

In contrast to the ovarian gonadotropin receptor–hormone interaction (8), there was no inactivation of unbound hormone during the incubation with *P. maltophilia*. Labeled hCG remaining in the supernatant after the incubation bound equally as well to fresh ovarian pellets as did hormone incubated in buffer alone.

A Scatchard plot (12) of the data is shown in Fig. 4*B*. The equilibrium dissociation constant (K_d), the reciprocal of the slope, was 2.3×10^{-9} M. The number of sites (n) can be estimated from the intercept at the abscissa as 240 fmol/100 μg of protein. From a Hill plot of the data, the Hill coefficient, n, was calculated to be 1.05, indicating no cooperativity between binding sites.

Growth of *P. maltophilia* and Bacterial Specificity of Gonadotropin Binding. A growth curve of *P. maltophilia* in trypticase soy broth suspension culture at 30° is shown in Fig. 5*A*. After a lag phase of 2 hr, the bacterial growth was exponential during the first 24 hr of incubation. Thereafter, the culture became stationary.

Hormone binding was measured by harvesting aliquots of the culture at various times and preparing 20,000 × *g* pellets as described in *Materials and Methods*. Maximal binding to *P. maltophilia* occurred 24 hr after the culture reached stationary phase. At maximal binding, 22.5% of the total labeled hormone was specifically bound. In contrast, a batch culture of *P. aeruginosa* grown under the same conditions bound less than 2% of the total hormone throughout all phases of growth (Fig. 5*B*).

Other pseudomonads and various other gram-negative organisms were similarly tested for binding after growth in trypticase soy broth suspension cultures. The results (Table 3) demonstrate that gonadotropin binding is not restricted to the *Pseudomonas* isolate from porcine follicular fluid, because the ATCC strain of *P. maltophilia* bound ^{125}I-hCG equally as well. *P. testosteroni*, *P. aeruginosa*, *E. coli*, *E. aerogenes*, and *E. cloacae* and one gram-positive bacillus (isolated from follicular fluid) failed to showed significant hormone binding.

Although this survey is limited, it illustrates that gonadotropin binding is not widespread among all gram-negative organisms, nor is it a common feature of all pseudomonads. It further suggests that the gonadotropin binding to *P. maltophilia* is not an artifact of the culturing conditions, because all bacteria were grown under identical conditions.

Enzyme Sensitivity of the Gonadotropin Binding Sites. To determine the nature of the gonadotropin binding sites, various enzyme treatments were used to digest *P. maltophilia* prior to the binding assay. Controls were incubated under the same conditions in buffer alone. The results (Table 4) show that the binding is not uniformly sensitive to proteolytic enzymes. Trypsin and protease had little or no effect, whereas α-chymotrypsin and Pronase caused substantial decreases in binding. Binding was destroyed by heating, which suggests that the binding specificity does not reside in the thermostabile polysaccharide portions of the gram-negative envelope. Phospholipase C caused a significant increase in binding (156% of control values) while phospholipase D, hyaluronidase, and

Table 2. Relative potency estimates of various hormone preparations by radioreceptor assay with ovarian receptors or *P. maltophilia*

Standard	Hormone	Relative potency (S1 units/mg) *P. maltophilia*	Ovarian	I.D.
oLH S18*	hLH	4.85	3.68†	1.31
	oFSH	0.032	0.024	1.33
	oPRL	0.00139	0.00078	1.78
hCG CR 121	CR117 hCGα	<0.04	0.009†	
	CR117 hCGβ	<0.04	0.006†	

I.D. = Index of discrimination—bacterial receptor value/ovarian receptor value.
* 1.03 NIH oLH S1 units/mg.
† Lee and Ryan (10).

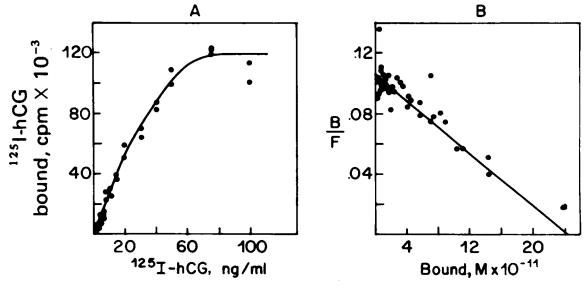

FIG. 4. (*A*). Binding as a function of the concentration of ^{125}I-hCG. Increasing concentrations of ^{125}I-hCG were incubated with bacterial protein, 50 μg/ml, at 24° for 20 hr. Nonspecific binding in the presence of hCG was subtracted from the total. (*B*). Scatchard plot of ^{125}I-hCG binding to *P. maltophilia*. Data from Fig. 4*A* and two other experiments were plotted according to Scatchard after normalizing the binding data to 100 μg of protein. The slope of the line and the intercept at the abscissa were determined by linear regression analysis.

‡ Subsequent experiments have shown that this inhibition is an artifact of the Carbowax separation system.

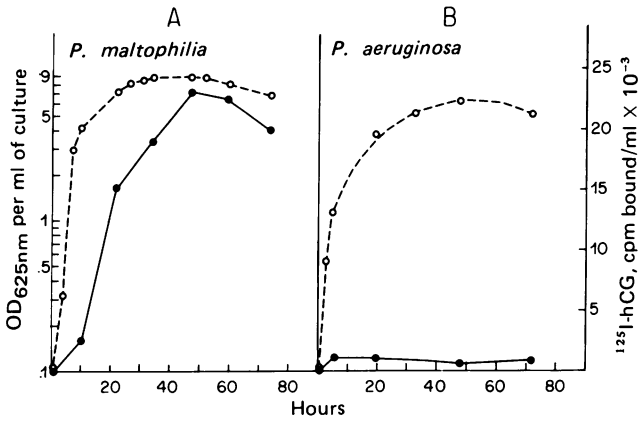

FIG. 5. Growth curve (○) and ^{125}I-hCG binding (●) in trypticase soy broth at 30° with *P. maltophilia* (*A*) and *P. aeruginosa* (*B*). Batch cultures (500 ml) were inoculated at time 0 as described in *Materials and Methods*. At various times, duplicate 10-ml aliquots were removed from the flask for measurement of optical density of the culture at 625 nm with trypticase soy broth as a blank. For binding of ^{125}I-hCG, the aliquots were centrifuged at 20,000 × *g*, washed, and resuspended in 1.0 ml of 40 mM Tris/10% sucrose buffer. Binding assays contained 2 ng of ^{125}I-hCG and 100 μl of the final bacterial pellet in a volume of 1 ml of 40 mM Tris. Nonspecific binding was measured in the presence of unlabeled hCG.

DNase had no effect. Although RNase decreased the binding by about 50%, a similar and as yet unexplained phenomenon occurs when ovarian homogenates are incubated with this enzyme (7).

DISCUSSION

Table 5 compares the properties of the hCG binding sites in *P. maltophilia* with gonadotropin receptors from luteinized rat ovaries. In both systems, binding is a temperature-dependent, saturable process. Although *P. maltophilia* receptors require a considerably longer time to reach equilibrium at 37° and 24° than do the ovarian receptors, the kinetics of bacterial binding are similar to those described for testicular gonadotropin receptors (13).

In both the ovary and the bacteria, gonadotropin binding is linear at protein concentrations ranging from 1 to 100 μg/ml. At the present time, it is not known why maximal specific binding of labeled hormone does not exceed 20% in the bacterial system. Perhaps it is due to steric hindrance when large numbers of bacterial cells are incubated in a relatively small volume of assay buffer. The fact that, under similar incubation conditions, the ovarian receptors can achieve a maximal binding approaching 80% suggests that the bioactivity of the labeled hormone preparation is not a limiting factor. Furthermore, there is no extensive degradation or inactivation of unbound labeled hormone by the bacteria, because unbound hormone exhibits normal binding to fresh ovarian pellets. The possibility of inactivation of bacterial binding sites during the assay has not yet been investigated.

The pH optima for binding in the ovary and the bacteria are similar. Unlike the ovary (7), *P. maltophilia* binding is sensitive to 1 mM EDTA and 10 mM calcium and magnesium. This could be related to the alteration of the structural integrity of the bacterial outer membrane by these agents (14–16).

The bacterial K_d (2.3×10^{-9} M) suggests that the bacterial binding site has a lower affinity for hCG than the ovarian receptor. Nevertheless, it is the same order of magnitude as in other mammalian receptors—e.g., oPRL receptors in the rat mammary gland (17) and FSH receptors in the rat testis (18).

Table 3. Binding of ^{125}I-hCG by various procaryotic organisms

Organism (source)	% ^{125}I-hCG bound: per ml culture	% ^{125}I-hCG bound: per mg protein
P. maltophilia (follicular fluid)	24.7	19.3
P. maltophilia (ATCC 13637)	15.7	13.5
P. testosteroni (ATCC 11996)	0.5	0.8
P. aeruginosa (ATCC 27853)	1.5	1.5
E. cloacae (follicular fluid)	1.8	N.D.
E. aerogenes (Mayo Clinic)	1.4	1.0
E. coli (ATCC 25922)	0.9	1.0
Bacillus sp. (follicular fluid)	1.2	3.5

Suspension cultures were sampled at various times for growth and binding determinations (see legend, Fig. 5). Binding data represent maximal binding in all cultures at stationary phase.

Table 4. Sensitivity of *P. maltophilia* binding sites to enzyme digestion and heat

Treatment	Concentration (μg/ml)	% of control binding
Trypsin	250	98.1
	500	81.2
	1000	72.9
α-Chymotrypsin	250	39.0
Pronase	250	9.5
Protease	250	89.6
Phospholipase C	250	156.0
Phospholipase D	250	90.2
Hyaluronidase	250	79.7
DNase	100	97.8
RNase	100	52.0
Heat (100°, 30 min)		7.8

A stationary phase culture of *P. maltophilia* was centrifuged at 20,000 × *g* for 20 min and washed twice in 40 mM Tris buffer. The culture was resuspended in Tris buffer to OD_{625} of 0.5 (approximately 100 μg of protein per ml) and divided into aliquots for enzyme treatment at the concentrations indicated. After incubation at 37° for 30 min, the enzymes were removed by centrifugation at 20,000 × *g* and two washes with 40 mM Tris buffer. The resulting bacterial pellets were resuspended in 40 mM Tris/10% sucrose buffer and assayed for binding of ^{125}I-hCG.

Table 5. Comparison of hCG binding sites of *P. maltophilia* and ovarian gonadotropin receptors

Properties	Receptors: *P. maltophilia*	Receptors: Ovary
Binding equilibrium time (hr)		
37°	6	0.5
24°	20	4
4°	>24	>24
pH optimum	7.0(6.8–7.5)	7.0(6.0–8.0)
Binding constants		
K_d at 24°	2.3×10^{-9} M	3.5×10^{-11} M
Sites		
(fmol/100 μg of protein)	242	84
Hill coefficient	1.05	1.1

The competition experiments with unlabeled hCG and other hormones suggest that binding of gonadotropin to *P. maltophilia* is not simply an ionic interaction between cationic ligands and the anionic lipopolysaccharide complex of the bacterial outer membrane (19). The hormones that show maximal binding, hCG and hLH, have isoelectric points of 3.0 (20) and 5.4 (21), respectively. These hormones would be negatively charged rather than positively charged at pH 7.4 in the assay. Also, the relative potencies of the hormones used in the competition assay bear no relationship to their respective isoelectric points. Finally, the hormone relative potency estimates for the bacterial binding are similar to those obtained with the ovarian receptor.

The gonadotropin binding site in *P. maltophilia* appears to be protein in nature although it is not as uniformly sensitive to proteolytic enzymes as in the ovary (7). This may suggest that some of the enzyme-sensitive residues are buried or masked. Alternatively, it may be related to unique structural properties of the binding protein itself. Outer membrane (envelope) proteins in *E. coli* show differential sensitivity to trypsin and Pronase (22). Similarly, flagellar filaments of *Salmonella typhimurium* are sensitive to α-chymotrypsin whereas purified flagellin is resistant to trypsin, pepsin, and papain digestion (23).

Although the gonadotropin binding site in *P. maltophilia* resembles the ovarian hCG receptor, further studies are needed regarding the nature of the site and its physiological significance before one can ascribe the term "receptor" to this procaryotic binding site. One would predict that the binding site is localized on the bacterial outer surface because of its accessibility to the relatively large hCG molecule (molecular weight, 40,000). Also, the sensitivity to phospholipase C, EDTA, and proteolytic enzymes suggests that the binding site is a protein or lipoprotein in the outer membrane. Other superficial structures—e.g., flagella—are insensitive to EDTA (23) and lack phospholipid (23, 24). Additional studies are required for a more definitive localization.

It is also of critical importance to demonstrate some altered physiological function subsequent to hCG binding. Preliminary experiments have failed to demonstrate any increased adenyl cyclase activity in response to hormone binding. However, the procaryotic enzyme is thought to be insensitive to mammalian hormones (25). It is possible that the binding of hCG induces some other more generalized response (e.g., cell growth, cell death) which is not regulated by adenosine 3′:5′-cyclic monophosphate.

If hCG binding can be correlated with some physiological function in bacteria, then the question arises as to whether there is an hCG-like molecule normally produced by *P. maltophilia* cultures. Preliminary data indicate that the bacterial culture medium contains a large molecule that crossreacts with antisera to hCG native and hCGβ and a smaller heat-labile molecule that stimulates rat ovarian adenylate cyclase (N. D. Richert, M. Ramos-Uribe, and R. J. Ryan, unpublished data). Recent, independent studies in other laboratories (26, 27) also suggest bacterial synthesis of hCG-like molecules that compete in a radioimmunoassay and radioreceptor assay in a manner identical to that of highly purified hCG.

If no functional significance can be attributed to the binding of hCG, it is possible that the binding sites are structurally related to ovarian receptors but function in a more primitive manner as receptors for bacteriophages or colicins. Structural similarity between microbial surface antigens and eucaryotic cell membrane proteins is exemplified by the cross-reactivity of human blood group antigens and the O-antigenic side chains of many gram-negative bacteria and constitutes the subject of a recent review (5).

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