The human glucagon receptor was expressed at high density in Drosophila Schneider 2 (S2) cells. Following selection with G418 and induction with CuSO₄, the cells expressed the receptor at a level of 250 pmol/mg of membrane protein. The glucagon receptor was functionally coupled to increases in cyclic AMP in S2 cells. Protein immunoblotting with anti-peptide antibodies revealed the expressed receptor to have an apparent molecular mass of 48 kDa, consistent with low levels of glycosylation in this insect cell system.

Binding of [fluorescein-Trp²⁵]glucagon to S2 cells expressing the glucagon receptor was monitored as an increase in fluorescence anisotropy along with an increase in fluorescence intensity. Anisotropy data suggest that the mobility of the fluorescein is restricted when the ligand is bound to the receptor. Kinetic analysis indicates that the binding of glucagon to its receptor proceeds via a bimolecular interaction, with a forward rate constant that is several orders of magnitude slower than diffusion-controlled. These data would be consistent with a conformational change upon the binding of agonist to the receptor. The combination of [fluorescein-Trp²⁵]glucagon with the S2 cell expression system should be useful for analyzing glucagon receptor structure and function.

Glucagon is a 29-amino acid peptide produced by proteolytic cleavage of the proglucagon gene product in the A cells of the pancreas. The peptide acts at the liver to increase the rate of gluconeogenesis and glycogenolysis, in this regard serving as the major counterregulatory hormone of insulin (for review, see Ref. 1). Glucagon binds to specific receptors on the surface of hepatocytes to stimulate increases in cyclic AMP, inositol phosphate, and intracellular calcium. The rat and human glucagon receptors have been cloned (2-4) and shown to contain seven putative transmembrane domains characteristic of the G protein-coupled family of receptors (5). The glucagon receptor shares significant sequence homology with the subfamily of G protein-coupled receptors that includes receptors for glucagon-like peptide-1 and parathyroid hormone (for review, see Ref. 6). This subclass of G protein-coupled receptors bears little sequence homology to the well characterized rhodopsin subfamily, and relatively little is known about the molecular interactions of ligands with receptors in this class.

Glucagon itself has been the subject of a wide variety of physical studies such as x-ray crystallography (7), NMR (8), and circular dichroism (9). However, these studies have been performed in various lipid or detergent solutions, which affect the conformation of the peptide. In addition, it has been observed that the secondary structure of glucagon is dependent on the concentration of peptide used in the experiment (9). For these reasons, the structure determined by these techniques may not reflect the physiological state of glucagon as it interacts with its receptor.

As is the case for most G protein-coupled receptors, biochemical and structural characterization of the glucagon receptor has been hampered by an inability to produce sufficient quantities of receptor protein. In the present study, we have expressed the glucagon receptor at high density using the Drosophila Schneider 2 (S2) cell system (10). [fluorescein-Trp²⁵]Glucagon (11) has been used as a tool for obtaining kinetic and structural information on the human glucagon receptor. The high levels of expression of human glucagon receptor obtained in S2 cells have allowed us to directly monitor changes in the fluorescence properties of this ligand as it binds to the receptor. This system has proven useful in understanding the environment of the ligand binding site of the human glucagon receptor and in monitoring conformational changes in both the receptor and the ligand during the binding interaction.

**Experimental Procedures**

Materials—Synthetic human glucagon was purchased from Sigma or Peninsula Laboratories or synthesized on an Applied Biosystems 432A peptide synthesizer. [2-thio-Trp²⁵]Glucagon was prepared from bovine porcine glucagon (Sigma) as described previously (12) and then reacted with 5-iodoacetic acid (Molecular Probes, Eugene, Oregon) to prepare [fluorescein-Trp²⁵]glucagon, as described in Ref. 11. An extinction coefficient of 26,000 M⁻¹ cm⁻¹ at 496 nm (11) was used to determine the concentration of [fluorescein-Trp²⁵]glucagon. Schröder’s Drosophila media (11720-018) was purchased from Life Technologies, Inc. [¹²⁵I]Glucagon was purchased from Dupont; Gpp(NH)p triethylium salt was from Boehringer Mannheim, and anti-fluorescein antibody was from Molecular Probes. The concentration of antibody was adjusted so that 10 μl quenched 90% of the fluorescence of 1 ml of a 5 nm fluorescein solution at pH 8.0.

Expression of the Human Glucagon Receptor in S2 Cells—Recombinant plasmid pVE2702 (4) was incubated with restriction endonuclease NheI, and the 5' ends were filled in by incubation with the Klenow fragment of DNA polymerase I and deoxynucleotide triphosphates (dNTPs). A 1.4-kilobase pair fragment encoding the human glucagon receptor was isolated by further cleavage of pVE2702 with XbaI. The 1.4-kilobase pair fragment was subcloned into Bluescript SK⁺ via the SmaI and XbaI sites (blue-hGlu). blue-hGlu was restricted with NotI, and the ends were filled in by incubation with Klenow polymerase and dNTPs. The 1.4-kilobase pair fragment containing the glucagon receptor cDNA was isolated by restriction with EcoRI and was subcloned into the expression plasmid pRmH43 (Ref. 10, kindly provided by Dr. L. S. B. Goldstein, University of Arizona) via the SmaI and EcoRI sites.

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Dept. of Molecular Pharmacology and Biochemistry, Merck Research Laboratories, Rahway, New Jersey 07065.

‡The abbreviations used are: Gpp(NH)p, guanyl-5'-yl imidodiphosphate; FACS, fluorescence-activated cell sorting.
This plasmid construct is subsequently referred to as pRm-hGlu. S2 cells were maintained and induced to express recombinant protein essentially as described by Bunch et al. (10). S2 cells (provided by Dr. L. S. B. Goldstein, University of Arizona) were maintained at 27°C in Schneider media supplemented with 10% heat-inactivated fetal calf serum, two mM glutamine, and 50 µg/ml gentamycin. S2 cells (10 × 10^6 cells) were cotransfected with 1 µg of pUCN80neo (gift of Dr. Hanno Steller) (13) and 20 µg of pRm-hGlu using the CaPO4 precipitation method. pUCN80neo encodes a gene for G418 resistance whose expression is driven by the Drosophila heat shock promoter. Twenty-four hours after transfection, G418 was added to a final concentration of 300 µg/ml. Cells were split approximately every 5–7 days. A stable population of G418-resistant cells was obtained in 3–4 weeks. S2 cells (1–2 × 10^6 cells/ml) were induced to express receptor by the addition of 1 mM CuSO4.

Fluorescence Flow Cytometry and Cell Sorting—Cells were analyzed for single cell fluorescence on a FACSScan/Vax flow cytometer (Benton Dickinon Immunocytometry Systems, San Jose, CA). The cells were sorted based on fluorescein intensity using a FACStar Dickinson Immunocytometry Systems, San Jose, CA). The cells were for single cell fluorescence on a FACScan/Vaxflow cytometer (Benton Dickinon Immunocytometry Systems, San Jose, CA). The cells were sorted based on fluorescein intensity using a FACStar Dickinson Immunocytometry Systems, San Jose, CA).

Determination of Glucagon Binding and Whole Cell cAMP Levels in S2 Cells—S2 membranes were prepared by hypotonic lysis, frozen in liquid N2, and stored at -80°C as described previously (14). [fluorescein-Trp25]Glucagon binding was performed in Buffer A (20 mM Tris-Cl, pH 7.4, 2.5 mM MgCl2, 1 mM dithiothreitol, 25 µM phenylmethylsulfonyl fluoride, 0.2 mM ethylenediaminetetraacetic acid (EDTA), 0.2 M phenylmethylsulfonyl fluoride, 3 mM o-phenanthroline). [fluorescein-Trp25]Glucagon binding assays were performed in Buffer A plus 0.05% bovine serum albumin. Membranes and [fluorescein-Trp25]Glucagon (100 pM) were incubated for 1 h at 22°C. Membranes were harvested by filtration over GF/C filters (Whatman) that had been presoaked in 0.5% polyethylenimine. Due to high levels of nonspecific binding, a direct determination of the Kd for [fluorescein-Trp25]Glucagon by saturation binding was not possible. At the low concentrations of [125I]glucagon employed in the competition binding experiments, the IC50 values observed for each state should approximate the mean Kd value.

RESULTS

Expression of the Human Glucagon Receptor in S2 Cells—The human glucagon receptor cDNA was subcloned into vector pRHmA3 and cotransfected with pUCN80neo into S2 cells. pRHmA3 contains a Drosophila metallothionein promoter that is tightly regulated in S2 cells (10). After 3 weeks of growth in 0.3 µg/ml G418, cells were induced to express glucagon receptor by incubation with 1 mM CuSO4. After 3 days of induction, the cells bound [125I]glucagon with a Bmax of 110 pmol/mg of membrane protein, whereas mock-transfected S2 cells displayed no specific [125I]glucagon binding.

In an attempt to select a subpopulation of cells with higher expression, cells induced to express the glucagon receptor were incubated with [fluorescein-Trp25]Glucagon and sorted by FACS. The % of the cells displaying the highest levels of binding were collected and expanded. Upon induction with 1 mM CuSO4, cells displayed a time-dependent increase in [125I]glucagon binding with maximal levels of 250 pmol/mg of protein achieved at 3–4 days after induction (data not shown). This represents about a 2-fold increase in receptor expression levels following FACS. After 4 months of continuous culture in the absence of CuSO4, no significant loss of receptor expression has been observed.

Immunoblotting of the Human Glucagon Receptor Expressed in S2 Cells—Antisera from rabbits injected with either peptide LX1 or LX2 recognized a protein with an apparent molecular mass of 48 kDa in transfected S2 cells induced with CuSO4 (Fig. 1, lanes C and F). This protein was absent in nontransfected or transfected but uninduced S2 cells (Fig. 1). The Mw = 48 kDa observed in this system differs from that measured by affinity labeling of the glucagon receptor in rat liver membranes (Mw = 63 kDa, Ref. 20), most likely reflecting differences in glycosylation between the receptor expressed in Drosophila versus mammalian cells.

Binding of [fluorescein-Trp25]Glucagon to the Receptor—The

\[ r(t) = A_1 + e^{-(t - t_1)} + A_2 + e^{-(t - t_2)} \]  

where A1, A2, t1, t2 are the amplitudes and relaxation times for two kinetic phases. The same equation was used for fitting intensity data (l(t)).

For radioligand and functional assays, IC50 (the concentration of ligand displacing 50% of the labeled ligand from the binding site) and EC50 (the half-maximal effective concentration of ligand in a functional assay) values were calculated using the Prism program (Graphpad Software). Bmax values (the concentration of ligand bound at saturation) were determined from the fitted competition curves as described previously (19).
displacement of $[^{125}]$Iglucagon by glucagon or [fluorescein-Trp$^{25}$]glucagon demonstrated that the affinity of the fluorescein-labeled glucagon was similar to that of unmodified glucagon (Fig. 2), as previously reported (11). Nonspecific interactions were examined by adding excess receptor was not practical under these experimental conditions. However, the signal from free ligand could rapidly be removed by adding anti-fluorescein antibody, which quenched up to 95% of the fluorescein fluorescence upon binding (21, 22). As shown in Fig. 4A, the addition of anti-fluorescein antibody to the [fluorescein-Trp$^{25}$]glucagon–receptor complex resulted in a rapid quenching of the fluorescein of the free ligand, while that of the bound ligand was protected from the antibody. As the ligand dissociated from the receptor, its fluorescence was quenched by the anti-fluorescein antibody, as observed by a slow decrease in intensity after the rapid quenching phase. While the intensity was decreasing during this period, the anisotropy remained stable (Fig. 4B). This anisotropy signal originated from the bound ligand and was determined to be 0.281 ± 0.001 (n = 2). Once the anisotropy of the bound ligand was determined, Equation 3 could be used to estimate the fraction of ligand bound to the receptor. Applying Equation 3 to the data in Fig. 4 (r$_{obs}$ = 0.176, r$_{free}$ = 0.096 [data not shown], r$_{bound}$ = 0.281) indicated that 43% of the ligand is bound under these conditions, consistent with the amount of fluorescein protected from quenching by the antibody (Fig. 4A).

Dissociation Kinetics of [fluorescein-Trp$^{25}$]Glucagon from Its
Receptor—The dissociation time course of [fluorescein-\text{Trp}^{25}]glucagon was measured in the absence of Gpp(NH)p using low concentrations (2–10 nM) of the agonist. Under these conditions, two distinct relaxation phases could be observed (Fig. 5, Table I), suggestive of agonist binding to two affinity states of the receptor. The slowly dissociating phase corresponds to the high affinity guanine nucleotide sensitive site noted in the competition binding experiments shown in Fig. 2, whereas the fast phase corresponds to the low affinity state of the receptor. These experiments, in which approximately 10 nM ligand and receptor were used, indicate that most of the glucagon receptor in the S2 cells is in the low affinity uncoupled state, as would be expected for a system in which the receptor is significantly overexpressed and G protein may be limiting. In contrast, at the submaximal concentration of [\text{125I}]glucagon used in the binding assays, most of the radioligand would bind to the high affinity state of the receptor, so that the high affinity state would be significantly overrepresented in the competition binding experiments (Fig. 2).

For more detailed kinetic analysis, Gpp(NH)p was included in the incubation to simplify the kinetics by converting all of the receptor to the low affinity state and to reduce the time needed for the reaction to come to completion. The addition of excess glucagon caused a decrease in anisotropy and intensity as the [fluorescein-\text{Trp}^{25}]glucagon dissociated from the receptor. The intensity decrease was about 9% (4.02 to 3.65) and was
determined by global analysis (6.6 s⁻¹, using Equation 3). The fitted value for the dissociation rate constant indicated that 23.3% of the ligand was bound under these conditions, compared with 30% bound ligand calculated using Equation 3. The fitted value for the dissociation rate determined by global analysis (6.6 × 10⁻³ s⁻¹) was similar to that determined by a local fit of the anisotropy data (5.8 × 10⁻³ s⁻¹). Because the magnitude of the effect of the intensity change on the anisotropy measurement was small and the intensity data were less precise than the anisotropy data, subsequent analysis used only the local fit of the anisotropy data for determination of rate constants.

Association Kinetics of [flucrescin-Trp²⁵]Glucagon with its Receptor—Association rates for [flucrescin-Trp²⁵]Glucagon were measured in a range of 5–150 nM ligand in the presence of 100 μM Gpp(NH)p (Fig. 7). Gpp(NH)p had minimal effects on the association rate of 10 nM [flucrescin-Trp²⁵]Glucagon with the receptor, lowering the total binding by 18% and increasing the rate of binding by about 20% (data not shown). The association rate data were adequately fit by a single relaxation time using Equation 5 and could be evaluated by a reversible one-step binding mechanism

\[ k_1 \]

\[ R + L \rightleftharpoons RL \] (Eq. 9)

where \( k_1 \) and \( k_-1 \) are the forward and reverse rate constants, respectively. The inverse relaxation time of ligand association (\( r^{-1} \)) would be defined as

\[ r^{-1} = k[L] + k_-1 \] (Eq. 10)

A plot of \( r^{-1} \) (also defined as the observed rate, \( k_{obs} \)) versus \( L \) should yield a straight line with the slope equivalent to \( k_1 \) and the intercept equivalent to \( k_-1 \). As shown in Fig. 7, inset, the data fit well to this model, with \( k_1 = 7.9 ± 1.1 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \) and \( k_-1 = 5.5 ± 0.01 \times 10^3 \text{ s}^{-1} \) (n = 2). The dissociation rate in the presence of Gpp(NH)p measured directly (5.9 ± 0.5 × 10⁻³ s⁻¹ n = 4; Fig. 6; Table I) was similar to the \( k_-1 \) value determined in this experiment, thus supporting the above model. The model was further supported by the agreement of the \( K_d \) derived from this association experiment (\( k_-1/k_1 = 69 ± 10 \text{ nM, n = 2} \)) with the thermodynamic \( K_d \) derived from the
amplitudes of the association kinetics fit to Equation 5. The fitted amplitudes and the quantum yield change upon binding were used in Equation 4 to calculate the fraction of bound and free [fluorescein-Trp25]glucagon. The data were then analyzed by a Scatchard plot to determine the thermodynamic Kd of 36 ± 9 nM (n = 2, data not shown). Both of these values are in reasonable agreement with the Kd value for the low affinity site derived from thermodynamic analysis of the displacement of [125I]glucagon (111 nM, Fig. 2).

**DISCUSSION**

Biophysical and structural characterization of the glucagon receptor, like that of other G protein-coupled receptors, has been hampered by the lack of availability of sufficient quantities of active receptor protein. Using a Drosophila S2 system, we have isolated a polydendostal cell population that expresses the human glucagon receptor to a level of 250 pmol/mg as measured by [125I]glucagon binding. Using this system, the interaction of the agonist [fluorescein-Trp25]glucagon with its receptor could be monitored via an increase in fluorescence anisotropy and intensity during the binding reaction. The anisotropy was more stable and less sensitive to nonspecific binding than the quantum yield increase and was therefore used as the primary means to monitor the binding of [fluorescein-Trp25]glucagon to the receptor.

The anisotropy of the ligand bound to the receptor was examined by using anti-fluorescein antibody to quench the free ligand in solution. The bound anisotropy was determined to be 0.281 ± 0.001, indicating that the fluorescein moiety is relatively immobile when anchored in the binding pocket of the glucagon receptor. For comparison, the anisotropy of fluorescein in [fluorescein-Lys3] substance P bound to the NK1 neurokinin receptor was only 0.17 (22) and that for fluorescein labeled epidermal growth factor bound to the epidermal growth factor receptor was determined to be 0.18 (26).

It is apparent from the equilibrium binding titrations (Fig. 2) and the dissociation rate studies (Fig. 5; Table I) that [fluorescein-Trp25]glucagon binds with two classes of receptor binding sites and that the conversion from high to low affinity is stimulated by Gpp(NH)p. These results indicate that the expressed glucagon receptor is coupled to G protein(s) in the S2 cells, consistent with the ability of glucagon to stimulate cAMP accumulation in these cells. In order to simplify the analysis of association rates, the receptor was converted to a single class of binding sites by preincubation with Gpp(NH)p. Gpp(NH)p had only a minimal effect on the observed association rate for [fluorescein-Trp25]glucagon. Monophasic association kinetics were observed in both the presence and absence of the guanine nucleotide, with only a small (~20%) increase in the association rate in the presence of Gpp(NH)p. Monophasic association kinetics were also observed in radioligand binding studies by Horwitz et al. (27) where association of [125I-Tyr10]glucagon was described by a single relaxation time.

A kinetic analysis of [fluorescein-Trp25]glucagon binding was performed in the presence of 100 μM Gpp(NH)p. The data were consistent with the model described in Equations 9 and 10, implying a simple bimolecular reaction between the ligand and the receptor. However, the association rate constant k1 (7.9 × 10^4 M^-1 s^-1) was much slower than that expected for a diffusion-controlled reaction, suggesting a more complex mechanism involving a slow conformational change in either the receptor or the ligand. Further biophysical studies will be required to address this possibility.

The increase in fluorescence intensity observed upon binding [fluorescein-Trp25]glucagon to the receptor is atypical, as the association of fluorescein with a protein is often accompanied by a decrease in intensity. These data suggest that the fluorescence of [fluorescein-Trp25]glucagon is quenched in solution by some internal quenching mechanism and that quenching is relieved upon receptor binding, perhaps reflecting the conformational change implied by the slow association kinetics. Thus, the receptor may actively "hold" the fluorescein away from the rest of the ligand, which would also be consistent with the high anisotropy determined for the bound ligand.

Previous structural studies of glucagon in solution have been performed at high (nonphysiological) concentrations of glucagon, or have used detergents or lipids to model receptor binding. The fluorescence analysis in the present study was performed at much lower ligand concentrations and gives direct information about the conformation of glucagon bound to its receptor. The combination of the S2 expression system with [fluorescein-Trp25]glucagon should allow further biophysical analysis of this receptor ligand interaction.

**Acknowledgments**—We thank Dr. Dennis Zaller for invaluable advice and comments regarding the S2 expression system, Hollis Williams for performing amino acid analysis, and Dr. Maria Bednarek for synthesizing glucagon. We also thank Dr. Margaret Cascieri, Dr. Jeff Toney, and Nancy Thornberry for helpful discussions and Dr. Robert Boltz for assistance with sorting the S2 cells.

**REFERENCES**

1. Defronzo, R. A., Bonadonna, R. C., and Ferrannini, E. (1992) Diabetes Care 15, 318–368
2. Jelinek, L. J., Lok, S., Rosenberg, G. B., Smith, R. A., Grant, F. J., Biggs, S., Bensch, P. A., Kuijper, J. L., Sheppard, P. O., Sprecher, C. A., O’Hara, P. J., Foster, D., Walker, K. M., Chen, L. H. J., McKean, P. A., and Kindsvogel, W. (1993) Science 259, 1614–1616
3. Lok, S., Kuijper, J. L., Jelinek, L. J., Kramer, J. M., Whitmore, T. E., Sprecher, C. A., Mathews, S., Grant, F. J., Biggs, S. H., Rosenberg, G. B., Sheppard, P. O., O’Hara, P. J., Foster, D. C., and Kindsvogel, W. (1994) Gene (Amst.) 140, 203–209
4. MacNeil, D. J., Occl, J. L., Hey, P. J., Strader, C. D., and Graziano, M. P. (1994) Biochem. Biophys. Res. Commun. 198, 328–334
5. Strader, C. D., Fong, T. M., Tota, M. R., and Underwood, D. (1994) Annu. Rev. Biochem. 63, 101–132
6. Segre, G. V., and Goldring, S. R. (1993) Trends Endocrinol. Metab. 4, 309–314
7. Sasaki, K., Dogi, S., Amadii, D. A., Tickle, J. I., and Blundell, T. (1975) Nature 254, 751–757
8. Braun, W., Wider, G., Lee, K. H., and Wurtzich, K. (1983) J. Mol. Biol. 169, 921–948
9. Moran, E. C., Chou, P. Y., and Fasman, G. D. (1977) Biochem. Biophys. Res. Commun. 77, 1300–1306
10. Bunch, T. A., Grinblat, Y., and Goldstein, L. S. B. (1988) Nucleic Acids Res. 16,
11. Heithier, H., Ward, L. D., Cantrill, R. C., Klein, H. W., Im, M., Pollak, G., Freeman, B., Schiltz, E., Peters, R., and Helmreich, E. J. M. (1988) Biochim. Biophys. Acta 971, 298–306
12. Wright, D. E., and Rodbell, M. (1980) J. Biol. Chem. 255, 10884–10887
13. Steller, H., and Pirrotta, V. (1985) EMBO J. 4, 167–171
14. Strader, C. D., Sigal, I. S., Register, R. B., Candelore, M. R., Randis, E., and Dixon, R. A. F. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4384–4388
15. Graziano, M. P., Hey P. J., Borkowski, D., Chichici, G. G., and Strader, C. D. (1993) Biochem. Biophys. Res. Commun. 196, 141–146
16. Posnett, D. N., McGrath, H., and Tam, J. P. (1988) J. Biol. Chem. 263, 1719–1725
17. Lakowicz, J. R. (1983) Principle of Fluorescence Spectroscopy, pp. 145–147, Plenum Publishing Corp., New York
18. Press, W. H., Flannery, B. P., Teukolsky, S. A., and Vetterling, W. T. (1988) Numerical Recipes in C, Cambridge University Press, Cambridge, United Kingdom
19. Fong, T. M., Yu, H., Huang, R.-R. C., and Strader, C. D. (1992) Biochemistry 31, 11806–11811
20. Iwanji, V., and Hur, K. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 325–329
21. Sklar, L. A., Fay, S. P., Seligmann, B. E., Freer, R. J., Muthukumaraswamy, N., and Mueller, H. (1990) Biochemistry 29, 313–316
22. Tota, M. R., Daniel, S., Sirotina, A., Mazina, K. E., Fong, T. M., Longmore, J., and Strader, C. D. (1994) Biochemistry 33, 13079–13086
23. Otto, M. R., Lillo, M. P., and Beechem, J. M. (1994) Biophys. J. 67, 2511–2521
24. Beechem, J. M. (1992) Methods Enzymol. 210, 37–53
25. Limbird, L. L. (1986) Cell Surface Receptors: A Short Course on Theory and Methods, pp. 88–91, Martinus Nijhoff Publishing, Boston, MA
26. Carraway, K. L., III, and Cerione, R. A. (1993) Biochemistry 32, 12039–12045
27. Horwitz, E. M., Jenkins, W. T., Hoosein, N. M., and Gurd, R. S. (1985) J. Biol. Chem. 16, 9307–9315