Secretion of Biologically Active Recombinant Fibrinogen by Yeast*

(Received for publication, February 9, 1995, and in revised form, July 28, 1995)

Samar N. Roy, Bohdan Kudryk, and Colvin M. Redman†

From the Lindsley F. Kimball Research Institute of the New York Blood Center, New York, New York 10021

Fibrinogen (340 kDa) is a plasma protein that plays an important role in the final stages of blood clotting. Human fibrinogen is a dimer with each half-molecule composed of three different polypeptides (Aα, 67 kDa; Bβ, 57 kDa; γ, 47 kDa). To understand the mechanism of fibrinogen chain assembly and secretion and to obtain a system capable of producing substantial amounts of fibrinogen for structure-function studies, we developed a recombinant system capable of secreting fibrinogen. An expression vector (pYES2) was constructed with individual fibrinogen chain cDNAs under the control of a Gal-1 promoter fused with mating factor Fα1 prepro secretion signal (SS) cascade. In addition, other constructs were prepared with combinations of cDNAs encoding two chains or all three chains in tandem. Each chain was under the control of the Gal-1 promoter. These constructs were used to transform Saccharomyces cerevisiae (INVSc1; Mata his3-Δ1 leu2 trp1-289 ura3-52) in selective media. Single colonies from transformed yeast cells were grown in synthetic media with 4% raffinose to a density of 1 × 10⁶ cells/ml and induced with 2% galactose for 16 h. Yeast cells expressing individual chains contained fibrinogen precursors and fibrinogen and secreted about 30 μg/ml into the culture medium. The Bβ and γ chains were glycosylated. Glycosylation was inhibited by treatment of the yeast with tunicamycin. Intracellular and secreted recombinant fibrinogen, when treated with endoglycosidase H, CaCl₂, and galactosylase, was immunologically indistinct from the same fragments obtained from plasma fibrinogen. Fibrinogen was shown to be biologically active by forming a thrombin-induced clot, and factor XIIIa, could undergo γ-carboxylation of the Aα chain.

Human fibrinogen is a large plasma glycoprotein with diverse physiological functions. Its primary roles are in the final stages of blood coagulation, when it forms a fibrin clot and participates in platelet aggregation. Fibrinogen is a dimeric molecule with each half-molecule composed of three different polypeptides. The Aα chain has 610, the Bβ 461, and the γ 411 amino acid residues. The Bβ and γ chains are N-glycosylated. The six chains are connected by 29 disulfide bonds. The primary structure of fibrinogen is known, and structural studies indicate that fibrinogen is elongated and trinodal. The central E domain contains the NH₂ termini of the six polypeptide chains, and the two internal "D" nodes are formed by carboxyl-terminal globular domains of the Bβ and γ chains and a small (12-kDa) segment of the Aα chain. The COOH-terminal regions of the Aα and Bβ chains extend beyond the globular domains of the Bβ and γ chains and may fold back and contribute to the structure of the central nodal region. Central and terminal domains of the three chains are coiled together in a α-helical, rope-like manner. The Aα chain is glycosylated, and a "stem-loop" region and is essential for biological activity. To date, high resolution structural analysis has not been reached since fibrinogen is denatured in solution. However, a combination of biochemical and electron microscopy studies have provided a detailed model of fibrinogen structure that has been used in the design of recombinant fibrinogen for structural studies.

EXPERIMENTAL PROCEDURES

Materials—The expression vector pYES2 and the yeast strain INVSc1 (Mata his3-Δ1 leu2 trp1-289 ura3-52) were obtained from Invitrogen, Inc. (San Diego, CA). Medium to grow the yeast in selective conditions was purchased from Bio101, Inc (La Jolla, CA). Galactose, raffinose, and tunicamycin were purchased from Sigma. Restriction enzymes, Klenow fragment, and calf intestinal phosphatase were purchased from Boehringer Mannheim. Endoglycosidase H was obtained from Genzyme Corp. (Cambridge, MA), T4 DNA ligase from New England Biolab (Beverly, MA), L-[ß-35S]methionine (1100 Ci/mmol) from DuPont NEN, and agarose AminoLink® coupling gel was purchased from Pierce. Human plasma fibrinogen was prepared by IMCO and purchased from American Diagnostics Inc. (Greenwich, CT). Other reagents used have been described previously (8, 19–21).

Construction of Expression Vectors—Expression vectors containing fibrinogen cDNAs for single chains, for combinations of two chains, and for all three chains were inserted into multiple cloning sites at the 23761
Expression vectors containing fibrinogen chain cDNAs. The full-length cDNAs for individual fibrinogen chains were inserted into multiple cloning sites at the 3′-end of Gal-1-SS promoter (pYES2Aα, pYES2Bβ, and pYES2γ). In the other constructs, combinations of two chains (pYES2AαBβ, pYES2Aαγ, and pYES2Bβγ) and all three chains (pYES2AαBβγ) were inserted in tandem. Each arrow indicates the cleavage site of the secretion signal.

Fig. 1. Expression vectors containing fibrinogen chain cDNAs. The full-length cDNAs for individual fibrinogen chains were inserted into multiple cloning sites at the 3′-end of Gal-1-SS promoter (pYES2Aα, pYES2Bβ, and pYES2γ). In the other constructs, combinations of two chains (pYES2AαBβ, pYES2Aαγ, and pYES2Bβγ) and all three chains (pYES2AαBβγ) were inserted in tandem. Each arrow indicates the cleavage site of the secretion signal.

The abbreviations used are: MF, mating factor; α1, preprosecretion signal; IP buffer, immunoprecipitation buffer; PAGE, polyacrylamide gel electrophoresis.
Fig. 2. N-Glycosylation of Bβ and γ 32 chains. Transformed yeast cells, expressing individual chains and all three fibrinogen chains, were metabolically labeled with L-[35S]methionine in the presence or absence of tunicamycin. Fibrinogen chains were isolated from the cell lysate by immunoprecipitation and separated by 7.5% SDS-PAGE. An autoradiogram is shown. Panel A, expression of individual fibrinogen chains (reduced samples). Panel B, expression of all three fibrinogen chains (nonreduced samples). The relative mobilities of molecular size markers are shown on the left of each autoradiogram. The locations of fibrinogen (Fbg), its intermediates, and free chains are shown on the right.

Fig. 3. Endoglycosidase H treatment of intracellular and secreted fibrinogen. Fibrinogen and its precursors were isolated from the cell lysate by immunoprecipitation, separated by SDS-PAGE, and detected by staining with Coomassie Blue and by Western immunoblotting with antibodies against the component chains on the left and, those treated with Endo H and by Glyko's monosaccharide composition kit. Recombinant fibrinogen, purified from the culture medium, was lyophilized and resuspended in 170 μl of water to a concentration of 2.35 mg/ml. The protein was denatured with 0.5% SDS, reduced with 1% β-mercaptoethanol, and separated by electrophoresis on a 7.5% polyacrylamide slab gel according to the manufacturer's protocol. Sialic acid content was measured by the method of H union and By Glyko's monosaccharide composition kit. Recombinant fibrinogen, purified from the culture medium was pretreated with digestion buffer (37 mM Tris-HCl, 10 mM CaCl2, 0.1% Na3VO4, and 0.5% Triton X-100) to a final concentration of 50 μg/ml (500,000 IU) Trasylol (Miles Laboratories, Inc., Elkhart, IN). The above digest was mixed with an equal volume of buffer A (40 mM Tris-HCl, 110 mM NaCl, 0.1% Na3VO4, pH 7.5) and applied to a 2-ml column containing approximately 40 μg of agarose-conjugated anti-fibrinogen fragment E monoclonal antibody (F4-7B3). Flow was stopped for 2 h to allow maximum binding. Nonadsorbed protein was removed by extensive washing with buffer A. Adsorbed protein was eluted with 4 ml of 3 M NaSCN in buffer A.

The above nonadsorbed fraction was applied to a 2-ml column containing approximately 40 μg of agarose-conjugated anti-fibrinogen fragment E monoclonal antibody (2N3H10). The sample was recycled several times over this column to allow maximum binding. Nonadsorbed protein was removed by extensive washing with buffer A. Adsorbed protein was eluted with 4 ml of 3 M NaSCN in buffer A.

Factor XIIa Cross-linking—Secreted recombinant fibrinogen was treated with thrombin (6.8 NIH units/ml) with or without factor XIIa (1.0 units/ml) to determine its ability to form a thrombin-induced clot and to cross-link. The fibrin complexes were separated by SDS-PAGE and detected by staining with Coomassie Blue and by Western immunoblotting with several chain-specific antibodies: IC2-2 (anti-fibrinogen αa/fibrin α) (28), Ea3 (anti-fibrinogen Bβ/fibrin β) (26), T2G1 (anti-fibrin β) (29), and 4-2 (anti-fibrinogen γ/fibrin γ-dimer) (29).
indicating lack of sialic acid, and treatment with endoglycosidase H; lane 4, partial wheat starch digest; lane 2, peptide-N-glycosidase F released oligosaccharide (starting material) treated with neuraminidase III; lane 3, starting material digested with endoglycosidase H; lane 4, partial digestion with \( \beta \)-galactosidase; lane 5, starting material obtained by peptide-N-glycosidase F treatment; lane 6, maltotetraose standard. Panel C, lane 1, partial wheat starch digest; lane 2, complete digestion of starting material with \( \beta \)-galactosidase; lane 3, digestion with combination of \( \beta \)-galactosidase and hexosaminidase III; lane 4, starting material obtained with peptide-N-glycosidase F treatment; lane 5, partial wheat starch digest.

Endoglycosidase H Treatment of Intracellular and Secreted Fibrinogen—Nascent glycoproteins present in the endoplasmic reticulum contain mannose-rich carbohydrate side chains, which are later trimmed and further processed in the trans Golgi compartment before secretion occurs. The mannose-rich oligosaccharides, but not the fully processed side chains, are cleaved from glycoproteins by endoglycosidase H. To determine the carbohydrate nature of intracellular and secreted fibrinogen, INVSCIA, INVSCIB, and INVSCI \( \gamma \) cells were metabolically labeled with \( \text{L-[35S]} \) methionine, and intracellular and secreted fibrinogen were treated with endoglycosidase H. Analysis on reduced SDS-PAGE showed that intracellular A chains, but not A\( \alpha \), were cleaved by endoglycosidase H, in contrast the secreted fibrinogen chains were cleaved by endoglycosidase H, which is to be expected since carbohydrates were fully processed.

Carbohydrate Structure and Composition—The peptide-N-glycosidase F released oligosaccharides (Fig. 4, panel A, lane 1) and \( \beta \)-galactosidase (Fig. 4, panel A, lane 1) released oligosaccharides (Fig. 4, panel B, lane 2) were purified from the gel where N-linked oligosaccharides migrated similarly to bands 1 and 2 (Fig. 4, panel A, lane 1). However, no other bands were noted in the blank sample (panel A, lane 4) or some other digests (panel B, lane 2). A third band that migrated in the position of N-linked oligosaccharides (panel C, lane 5 and panel C, lane 4). All digestions were performed with \( 100 \) pmol of fibrinogen, but sialic acid was not detected in the dialysis membranes, to accurately determine the molar concentration of the sugars (data not shown). Based on the relative migration of standard oligosaccharides, as compared with the bands obtained from partial digestion of wheat starch (data not shown), the main N-linked oligosaccharide (marked by an asterisk) obtained by peptide-N-glycosidase F digestion is consistent with it being an asialo-galactosylated biantennary oligosaccharide. Subsequent experiments confirmed the structure. Digestion with neuraminidase III (panel B, lane 2) had no effect on the major band, indicating lack of sialic acid, and treatment with endoglycosidase H (panel B, lane 3) also did not affect the mobility of the N-linked oligosaccharide, indicating, as was shown in a previous experiment (Fig. 3), that secreted recombinant fibrinogen glycoprotein is not of the high mannose type. Partial digestion with \( \beta \)-galactosidase (panel B, lane 4) showed the starting material and the appearance of two lower bands. This suggests that at least two galactose monomers were cleaved.

Further confirmation of the oligosaccharide structure was obtained by complete digestion with \( \beta \)-galactosidase (panel C, lane 2), which demonstrates removal of approximately two galactose units. Treatment of the starting material with a combination of \( \beta \)-galactosidase and a hexosaminidase (hexosaminidase III) (panel C, lane 3) indicated complete removal of galactose and GlcNAc from the nonreducing end of the starting oligosaccharide. Taken together these results are consistent with the recombinant fibrinogen being a glycoprotein that is not of the high mannose type but contains an asialo-galactosylated biantennary oligosaccharide.

The absence of sialic acid was confirmed by assaying the recombinant yeast fibrinogen by the thiobarbituric acid method (27). Plasma fibrinogen, used as a control, yielded about 1.25 \( \mu \)mol of sialic acid/\( \mu \)mol of fibrinogen, but sialic acid was not detected in yeast fibrinogen. Also a monosaccharide composition assay, performed by Glyko Inc., failed to detect sialic acid (data not shown). It was not possible, however (because of a high background of glucose, which was also present in the blank sample and is possibly due to contamination from the dialysis membranes), to accurately determine the molar concentration of the sugars (data not shown).
Secretion of Fibrinogen—After induction with galactose for 16 h the media of INVSCIA-Bβγ cells was collected and neutralized, and the amount of fibrinogen was determined. Quantitation was performed using two different monoclonal antibodies with specificities to different domains of fibrinogen. One of the monoclonal antibodies (1–8C6) reacted with Bβ chain at amino acid residues 1–21, and the other (Fd4–7Bc) recognizes a plasmin digest fragment of fibrinogen (fragment D). Using both antibodies, human plasma fibrinogen and recombinant fibrinogen gave identical curves (Fig. 5). Transformed cells (10⁶ cells/ml) secreted 25–30 μg/ml after 16 h of induction with galactose.

In some cases the secreted fibrinogen was isolated from the incubation medium by affinity chromatography using protamine sulfate conjugated to Sepharose 6B. The fibrinogen was eluted at pH 4.5. As a control, fibrinogen from protaminesulfate was purified by adsorption on a protamine-Sepharose column. The position of cross-linked fibrinogen was shown in panel A, Western blot reacted with fibrinogen chain/γ-dimer antibody (4–2). Panel B, lane 4 also showed that these chains are present in the fibrin clot. The results are shown in Fig. 7. As controls, reduced plasma fibrinogen and untreated recombinant fibrinogen are shown in lanes 2 and 3. The Aα chain of plasma and recombinant fibrinogen, as is often the case, was partially degraded (panel A). On treatment with thrombin, in the absence of factor XIIIa, a similar pattern to the control samples was noted. Removal of fibrinopeptides A and B was not expected to show a marked difference in electrophoretic mobility of the α and β chains as compared with Aα and Bβ. Western blots with antibody to the β chain (panel B, lane 4) and to γ chain (panel C, lane 4) also showed that these chains are present in the fibrin clots. On treatment with factor XIIIa, the γ chain was not detected by Coomassie Blue staining (panel C, lane 5) and was markedly reduced as determined by Western blot with antibody to γ chain. There was a concomitant appearance of γ-dimer (panel C, lane 5). As a control it was noted that factor XIIIa had no effect on the β chain (panel B, lane 5).
addition, tunicamycin and endoglycosidase H treatment suggest that initial N-linked glycosylation of recombinant fibrinogen occurs in a manner similar to that in hepatocytes. Tunicamycin treatment only affected the processing of Bβ and γ chains, and digestion with endoglycosidase H indicated that mannose-rich fibrinogen precursors are present in the ER and are processed before secretion occurs. Carbohydrate analysis demonstrated that recombinant fibrinogen, unlike plasma fibrinogen, does not contain terminal sialic acid but otherwise may be similar in composition and sequence to plasma fibrinogen (30). These results are in keeping with the synthesis of N-linked glycans by yeast. The early stages of N-glycosylation in yeast and animal systems are similar, but further oligosaccharide processing, which occurs in the Golgi, differs. In yeast, mannose-rich oligosaccharides are usually formed, although galactose and N-acetylglucosamine residues may be added (31). Our studies indicate that recombinant yeast fibrinogen is not of the high mannose variety and is similar but not identical to that of plasma fibrinogen, since it lacks terminal sialic acid.

Biological activity of recombinant fibrinogen was shown by its ability to form a thrombin-induced clot and to undergo factor XIIIα-catalyzed cross-linking of fibrin chains. In addition to the response of recombinant fibrinogen to thrombin and factor XIIIα, fibrinogen shows that recombinant fibrinogen has a structure similar to that of plasma fibrinogen. Cleavage of fibrinopeptides A and B by thrombin, polymerization, and correct alignment of α chains, are indications that fragments D and E are produced when recombinant fibrinogen is digested with plasmin. Fragments D and E are characteristic products of fibrinogen that are distinct from other products of fibrin(ogen) that is treated with plasmin and can only be produced when fibrinogen is digested with plasmin in the correct configuration. Together these results demonstrate that recombinant fibrinogen forms a thrombin-induced clot and undergoes factor XIIIα-catalyzed cross-linking.

Structure of Recombinant Fibrinogen

Digestion—Plasmin digestion yielded well-defined fragments that represent the diversity of fibrinogen and the chains. To determine whether plasmin treatment with plasmin, yielded fragments derived from the terminal and central domains of dimeric fibrinogen, purified recombinant fibrinogen was digested with plasmin. The digest was subsequently fractionated by affinity chromatography using antibodies specific to fragments D and E. Adsorbed proteins were further characterized by SDS-PAGE followed by Coomassie Blue staining and Western immunoblot analyses. Panel A, protein stain; panel B, immunoblot reacted with antibody to fragment D; panel C, immunoblot reacted with antibody to fragment E. Lane 1, plasmin digest of yeast fibrinogen; lane 2, material absorbed by antibody to fragment D; lane 3, material absorbed by antibody to fragment E.

**DISCUSSION**

Human fibrinogen has been expressed in a number of different recombinant systems (8–11). Although these procedures usually only produce small amounts of secreted fibrinogen, they can be scaled up, using cells in suspension and roller bottles, to yield sufficient quantities to study structure/function relationships. The yeast system offers an advantage in that it is more easily adaptable to express and secrete milligram quantities of fibrinogen. The fibrinogen expressed in yeast is biologically active in that it forms a thrombin-induced clot and undergoes factor XIIIα cross-linking. Carbohydrate processing, composition, and sequence was determined by several different methods. Periodic acid-Schiff staining of the separated chains, treatment of transformed yeast cells with tunicamycin, and endoglycosidase H digestion of intracellular and secreted fibrinogen showed that only Bβ and γ chains are glycosylated. In conjunction with other observations, these results demonstrated the similarity of yeast-produced fibrinogen to that of plasma fibrinogen. Cleavage of fibrinopeptides A and B by thrombin, polymerization, and correct alignment of α chains, are indications that fragments D and E are produced when recombinant fibrinogen is digested with plasmin. Fragments D and E are characteristic products of fibrinogen that are distinct from other products of fibrin(ogen) that is treated with plasmin and can only be produced when fibrinogen is digested with plasmin in the correct configuration. Together these results demonstrate that recombinant fibrinogen forms a thrombin-induced clot and undergoes factor XIIIα-catalyzed cross-linking.

Structure of Recombinant Fibrinogen

Digestion—Plasmin digestion yielded well-defined fragments that represent the diversity of fibrinogen and the chains. To determine whether plasmin treatment with plasmin, yielded fragments derived from the terminal and central domains of dimeric fibrinogen, purified recombinant fibrinogen was digested with plasmin. The digest was subsequently fractionated by affinity chromatography using antibodies specific to fragments D and E. Adsorbed proteins were further characterized by SDS-PAGE followed by Coomassie Blue staining and Western immunoblot analyses. Panel A, protein stain; panel B, immunoblot reacted with antibody to fragment D; panel C, immunoblot reacted with antibody to fragment E. Lane 1, plasmin digest of yeast fibrinogen; lane 2, material absorbed by antibody to fragment D; lane 3, material absorbed by antibody to fragment E.

**DISCUSSION**

Human fibrinogen has been expressed in a number of different recombinant systems (8–11). Although these procedures usually only produce small amounts of secreted fibrinogen, they can be scaled up, using cells in suspension and roller bottles, to yield sufficient quantities to study structure/function relationships. The yeast system offers an advantage in that it is more easily adaptable to express and secrete milligram quantities of fibrinogen. The fibrinogen expressed in yeast is biologically active in that it forms a thrombin-induced clot and undergoes factor XIIIα cross-linking. Carbohydrate processing, composition, and sequence was determined by several different methods. Periodic acid-Schiff staining of the separated chains, treatment of transformed yeast cells with tunicamycin, and endoglycosidase H digestion of intracellular and secreted fibrinogen showed that only Bβ and γ chains are glycosylated. In
REFERENCES

1. Blomback, B., and Blomback, M. (1972) Ann. N. Y. Acad. Sci. 202, 77–79
2. Doolittle, R. F. (1984) Annu. Rev. Biochem. 53, 195–229
3. Henschen, A., Lotspeich, F., Kehl, M., and Southan, C. (1983) Ann. N. Y. Acad. Sci. 408, 28–43
4. Moseson, M. W. (1992) Semin. Hematol. 29, 177–188
5. Hantgan, R., Francis, C. W., and Marder, V. J. (1994) Hemostasis and Thrombosis: Basic Principles and Clinical Practice (Colman, R., Hirsh, J., Marder, V., and Salzman, E., eds) pp. 277–300, J.B. Lippincott Co., Philadelphia
6. Crabtree, G. (1987) The Molecular Biology of Blood Diseases (Stamatoyanopoulos, G., Nienhuis, A., Leder, P., and Majerus, P., eds) pp. 631–661, W.B. Saunders Co., Philadelphia
7. Weisel, J. W., Stauffacher, C. V., Bullitt, E., and Cohen, C. (1985) Science 230, 1388–1391
8. Roy, S. N., Procyk, R., Kudryk, B., and Redman, C. M. (1991) J. Biol. Chem. 266, 4758–4765
9. Hartwig, R., and Danishefsky, K. J. (1991) J. Biol. Chem. 266, 6578–6585
10. Fisher, D. H., Mulvihill, E. R., Huang, S., Chung, D. W., and Davie, E. W. (1991) Biochemistry 30, 9414–9420
11. Binnie, C. G., Hettasch, J. M., Strickland, E., and Lord, S. T. (1993) Biochemistry 32, 107–113
12. Bolyard, M. G., and Lord, S. T. (1988) Gene (Amst.) 66, 183–192
13. Bolyard, M. G., and Lord, S. T. (1989) Blood 73, 1202–1206
14. Lord, S. T., and Fowlkes, D. M. (1989) Blood 73, 166–171
15. Bolyard, M. G., and Lord, S. T. (1991) Biochem. Biophys. Res. Commun. 174, 853–860
16. Binnie, C. G., and Lord, S. T. (1993) Blood 81, 3186–3192
17. Farrel, D. H., Thigaranjan, P., Chung, D., and Davie, E. W. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10729–10732
18. Farrel, D. H., and Thigaranjan, P. (1994) J. Biol. Chem. 269, 226–231
19. Yu, S., Sher, B., Kudryk, B., and Redman, C. M. (1983) J. Biol. Chem. 258, 13407–13410
20. Yu, S., Sher, B., Kudryk, B., and Redman, C. M. (1984) J. Biol. Chem. 259, 10574–10581
21. Roy, S., Yu, S., Banerjee, D., Overton, O., Mukhopadhyay, G., Oddoux, C., Grienzinger, G., and Redman, C. (1992) J. Biol. Chem. 267, 23151–23158
22. Sambruk, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Rose, M. D., Winston, F., and Hieter, P. (1990) Methods in Yeast Genetics: A Laboratory Course Manual, pp. 39–52, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Danishefsky, K., Hartwig, R., Banerjee, D., and Redman, C. (1990) Biochem. Biophys. Acta 1048, 202–208
25. Kudryk, B., Grossman, Z. D., McAfee, J. G., and Rosebrough, S. F. (1989) Monoclonal Antibodies as Probes for Fibrinogen Proteolysis: Monoclonal Antibodies in Immunoscintigraphy (Chatel, J.-F., ed) pp. 365–398, CRC Press, Inc., Boca Raton, FL
26. Warren, L. (1963) Methods Enzymol. 6, 463–465
27. Procyk, R., Bishop, P. D., and Kudryk, B. (1993) Thromb. Res. 71, 127–138
28. Procyk, R., Medved, L., Engelke, K. J., Kudryk, B., and Blomback, B. (1992) Biochemistry 31, 2273–2278
29. Townsend, R. R., Hilliker, E., Li, Y.-T., Laine, R. A., Belil, W. R., and Lee, Y. C. (1982) J. Biol. Chem. 257, 9704–9710
30. Kukuruzinska, M. A., Bergh, M. L. E., and Jackson, B. J. (1987) Annu. Rev. Biochem. 56, 915–944
31. Kudryk, B., Okada, M., Redman, C. M., and Blomback, B. (1982) Eur. J. Biochem. 125, 673–682
32. Huang, S., Mulvihill, E. R., Farrell, D. H., Chung, D. W., and Davie, E. W. (1993) J. Biol. Chem. 268, 8919–8926
33. Zhang, J. Z., and Redman, C. M. (1994) J. Biol. Chem. 269, 652–658
34. Normington, K., Kohno, K., Kozutsumi, Y., Gething, M.-J., and Sambrook, J. (1989) Cell 57, 1223–1236
35. Rose, M. D., Misra, L. M., and Hieter, P. (1989) Cell 57, 1211–1221
36. Tachibana, C., and Stevens, T. H. (1992) Mol. Cell. Biol. 12, 4601–4611
37. D’Enfert, C., Lila, T., and Schekman, R. (1991) J. Cell Biol. 114, 663–670
38. Danishefsky, K., Hartwig, R., Banerjee, D., and Redman, C. (1991) J. Cell Biol. 114, 717–726
39. Wilson, D. W., Whiteheart, S. W., Wiedmann, M., Brunner., and Rothman, J. E. (1992) J. Cell Biol. 117, 531–538