The Oligomerization Domain of the Asialoglycoprotein Receptor Preferentially Forms 2:2 Heterotetramers in Vitro*

Mare Bider, Johanna M. Wahlberg, Richard A. Kammerer, and Martin Spiess‡
From Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

The human hepatic asialoglycoprotein receptor is a noncovalent hetero-oligomer composed of two homologous subunits, H1 and H2, with an as yet unknown stoichiometry. Ligand specificity and binding affinity depend on the arrangement of the subunits in the complex. An 80-amino acid segment connecting the transmembrane and the carbohydrate binding domains contains heptad repeats characteristic of α-helical coiled coil structure. We expressed and purified corresponding heptad repeats of the receptor subunits to form an α-helical coiled coil stalk on top of which the carbohydrate binding domains are exposed for ligand binding. We propose that the functional asialoglycoprotein receptor is a 2:2 heterotetramer.

The asialoglycoprotein (ASGP) receptor is a C-type lectin of hepatocytes that recognizes desialylated glycoproteins for endocytosis and lysosomal degradation (1–3). Optimal ligands are heptas, and hydrophilic residues at a and d positions and hydrophilic residues elsewhere are characteristic for proteins in which subunit oligomerization is mediated by α-helical coiled coils. The presence of heptad repeats in HL-1 and HL-2 suggested that this segment of the stalk domains of the human ASGP receptor by forming a coiled coil stalk. In this study, we tested the ability of the stalk domains of the human ASGP receptor subunits, H1 and H2, to form α-helical coiled coil structure and determined their preferred oligomerization state and stoichiometry in vitro. The results suggest that the functional receptor is a 2:2 heterotetramer.

*This work was supported by Grant 31-43483.95 from the Swiss National Science Foundation. 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. Tel.: 41-61-267-2164; Fax: 41-61-267-2149; E-mail: spie@ubacu.unibas.ch.


de-107

150 amino acids that specifically binds terminal galactose and N-acetylgalactosamine with low affinity (K\text{D} in the millimolar range). Simultaneous expression of the receptor subunits produced homodimers and heterotrimers of HL-1 as well as heterodimers and heterotrimers (9). Based on these results, a minimal model of the ASGP receptor was composed of a core trimer of HL-1 and an associated HL-2 subunit (8).

The polypeptide segment connecting the transmembrane anchor with the CRD contains heptad repeats (10). Heptad repeats of the general type (abcdefgh\text{m}) with hydrophobic residues at a and d positions and hydrophilic residues elsewhere are characteristic for proteins in which subunit oligomerization is mediated by α-helical coiled coils. The presence of heptad repeats in HL-1 and HL-2 suggested that this segment of the subunits is primarily responsible for the oligomerization of the ASGP receptor by forming a coiled coil stalk. In this study, we tested the ability of the stalk domains of the human ASGP receptor subunits, H1 and H2, to form α-helical coiled coil structure and determined their preferred oligomerization state and stoichiometry in vitro. The results suggest that the functional receptor is a 2:2 heterotetramer.

EXPERIMENTAL PROCEDURES

DNA Constructs—The sequence of the stalk domain of ASGP receptor subunit H1 (codons 65–144) was amplified by polymerase chain reaction (PCR) from the wild-type H1 cDNA (11). Primers (GGCCG-GATCTGCGAAATCTAACAAGCCTGACGGAGAG and CGCGAAATCCTTATTAGAGCGCCGCCATCTGAGAGCTCAG) were designed to add the amino acid sequence GSCEK with a BamHI site to the 5’ end and two stop codons followed by an EcoRI site to the 3’ end. The second primer also changed TGC of 9 to the 5’ end and TGC to the 3’ end. The PCR product was ligated at the BamHI/EcoRI sites into the bacterial expression vector pETP-T (12).

Wild-type H2 cDNA (4) was used as a template for the PCR amplification of a DNA fragment coding for residues 83–162 using the primers GCCGGATCTGCGAATCTAACAAGCCTGACGGAGAG and CGCGAAATCCTTATTAGAGCGCCGCCATCTGAGAGCTCAG) were designed to add the amino acid sequence GSCEK with a BamHI site to the 5’ end and two stop codons followed by an EcoRI site to the 3’ end. The PCR product was ligated at the BamHI/EcoRI sites into the bacterial expression vector pETP-T (12).

This paper is available on line at http://www-jbc.stanford.edu/jbc/
in-frame BamHI site, to yield the plasmid pET-H2S.

To generate a cDNA encoding a secretory form of H2 (H2exo), the exoplasmic portion of H2 with a 5' BamHI site was amplified using the primer ACGCGGATCCGCACAGCTGCAAG and a second primer complementary to a sequence in the vector. The PCR product was ligated to the signal sequence of influenza hemagglutinin (13) in the eukaryotic expression vector pECE (14). Ligation of the same PCR product to an artificial signal anchor with the sequence MGPRL19 followed by a BamHI site (15) generated a plasmid encoding H2exoLeu19.

**Cell Culture, Transfection, and Immunofluorescence**—Culture conditions for Madin-Darby canine kidney (MDCK) cells and the preparation of M1Δ cells were cotransfected with vector alone (A and B), with H2 (C), H2exo (D), or with H2exoLeu19 (E) and processed for indirect immunofluorescence microscopy with (A) or without (B, C, D, and E) permeabilization and stained using an antiserum specific for H1.

**Expression and Purification of H1S and H2S Peptides**—pPEP-H1S and pET-H2S were transformed into *Escherichia coli* host strains BL21(DE3) (Novagen) and into JM109(DE3), respectively. Expression and purification of the recombinant precursor peptides with a 6-His tag was performed according to the manufacturer’s protocols (Novagen). Briefly, expressing cells were lysed by sonication in 8 M urea, and the peptides were purified by Ni²⁺-chelate chromatography. The stalk peptides H1S and H2S were released by thrombin digestion and separated from the His-tagged fragments by a second chelate chromatography. Peptide concentration was determined by the bicinchoninic acid procedure (Pierce Chemical Co.) using bovine serum albumin as standard and using a correction factor determined by amino acid analysis (17).

**Oxidative Disulfide Cross-linking**—Purified H1S and H2S were reduced with 10 mM dithiothreitol for 30 min at 37 °C, precipitated with 95% (H1S) or 50% (H2S) ammonium sulfate, and redissolved in 200 mM Tris-HCl, pH 8, 200 mM NaCl, and 1 mM EDTA at a peptide concentration of 2 mg/ml. Oxidized and reduced glutathione were added to final concentrations of 9 mM and 0.9 mM, respectively. To analyze the formation of hetero-oligomers, the peptides were mixed in the presence of 0.9 mM reduced glutathione, heated to 37 °C for 10 min, and then cooled to 4 °C at a rate of 0.05 °C/s. Oxidation was started by addition of 9 mM oxidized glutathione. After incubation at 4 °C, remaining free cysteines were blocked by the addition of 100 mM iodoacetamide for 15 min. Products were analyzed by Tricine-SDS-polyacrylamide gel electrophoresis (18) under nondenaturing conditions and visualized by Coomassie staining.

To alkylate the peptides, they were completely reduced for 4 h at 37 °C with 10 mM dithiothreitol followed by incubation for 20 min at room temperature with 50 mM iodoacetamide. Excess of iodoacetamide was quenched by addition of 60 mM 2-mercaptoethanol. Alkylated peptides were dialyzed against 5 mM sodium phosphate buffer, pH 7.4, and 150 mM NaCl at 4 °C.

**Circular Dichroism**—CD spectra were recorded on a Cary 61 spectropolarimeter equipped with a thermostated quartz cell of 0.1- or 0.2-cm path length.

**RESULTS**

A Membrane-anchored Exoplasmic Portion of H2 Is Sufficient for Oligomerization with H1—Formation of H1-H2 het-
ero-oligomers is essential to generate a functional receptor. To study which domains in H2 are required for oligomerization with H1, we took advantage of a mutant MDCK cell line, M1Δ, expressing a mutant form of H1 with a truncated cytoplasmic domain, H1(Δ4–33A). The mutant protein is not transported to the cell surface but is retained in the trans-Golgi (15). Coexpression of H1(Δ4–33A) with wild-type H2 was found to rescue H1(Δ4–33A) to the cell surface, which reflected the formation of hetero-oligomers. The immunofluorescence staining in Fig. 1 shows the characteristic Golgi pattern for H1(Δ4–33A) in perinuclear, mock-transfected M1Δ cells (panel A) and the absence of any specific staining on the surface of these cells (panel B). In contrast, upon cotransfection with H2 cDNA, H1(Δ4–33A) was clearly detectable on the surface of M1Δ cells (panel C).

To test the role of the exoplasmic domain of H2 in oligomerization we constructed two variants (Fig. 1, top): H2exo is a secreted form in which the exoplasmic portion of the protein is preceded by a cleavable signal sequence, whereas H2exoLeu19 is a membrane-anchored form of the exoplasmic domain fused to a generic type II signal-anchor sequence Met-Gly-Pro-Arg-(Leu)19. Transfection of these constructs into M1Δ cells showed that H2exo failed to rescue H1(Δ4–33A) to the cell surface (panel D) and was mostly retained in the endoplasmic reticulum and degraded (not shown). The membrane-anchored version, H2exoLeu19, however, produced clear surface staining for H1(4–33A) (panel E). These results indicate that neither the cytoplasmic domain nor the specific sequence of the transmembrane segment of H2 are necessary for oligomerization with H1, but that the exoplasmic portion, when anchored to the membrane, is sufficient. Anchoring to the lipid bilayer may be required in order to reach a minimal concentration of the subunits on the surface of the ER membrane for efficient oligomerization to occur. These in vivo results support the hypothesis that the heptad-repeat sequence connecting the transmembrane segment and the CRD is involved in oligomerization.

Recombinant Stalk Peptides of H1 and H2 Form α-Helical Coiled Coils—To directly test the ability of the stalk sequences of H1 and H2 to form a coiled coil structure, peptides corresponding to the 80 amino acid residues comprising the stalk segment of H1 and H2 (shown in Fig. 2) were expressed in E. coli and purified. As shown in Fig. 2, the sequences can be aligned and grouped into heptads such that hydrophobic residues predominate in the positions a and d of the heptads. There are two positions where the heptad alignment is interrupted by “stutters” of four residues (marked with xxxxx in Fig. 2).

In each peptide the stalk sequence was immediately preceded by the sequence GSCECK. The two cysteines were potentially useful for disulfide cross-linking of associated peptides. To facilitate interpretation of cross-linked products, the single cysteine in the original stalk sequence (position 81 in H1S and 89 in H2S) was altered to serine (underlined in Fig. 2). To be able to distinguish between H1S and H2S by gel electrophoresis and to identify the composition of mixed cross-linking products, H2S was designed to be larger by 8 residues at the amino terminus and by another 17 residues at the carboxyl terminus.

The purified peptides H1S and H2S were 90% and 95% pure (insets in Fig. 3, A and C). Contaminating smaller peptides were most likely degradation products. To assess the formation of coiled coils, the secondary structure of the peptides was examined by CD spectroscopy. Fig. 3A shows the CD spectrum of H1S after complete reduction and alkylation. The spectrum recorded at 8°C (bold line) displays minima around 222 nm and 208 nm characteristic for α-helical structure. Based on the ellipticity at 222 nm, a helix content of 35% was calculated (according to Ref. 19). Upon heating to 60°C, the negative ellipticity at 222 nm largely disappeared, and the spectrum changed to one characteristic for random coil peptides (thin line). The melting process was >95% reversible upon cooling back to 8°C (not shown). Oxidative conditions generated by the addition of oxidized and reduced glutathione at a ratio of 10:1 resulted in extensive cross-linking of oligomers (inset to Fig. 3B). CD analysis of the oxidized sample showed an increased helix content of approximately 50%.

The spectrum of reduced and alkylated H2S peptide (panel C) showed little helix content at 8°C and did not change significantly at increasing temperature up to 40°C. Above 40°C, the peptide underwent an irreversible change in conformation resulting in a CD spectrum characteristic for β-sheet conformation (not shown). Therefore, in the following experiments, we did not heat samples containing reduced and alkylated H2S above 40°C. Despite its low helix content, H2S was readily cross-linked to covalent oligomers by mild oxidation (panel D, inset). Upon oxidation, the CD spectrum of H2S indicated a helix content of approximately 55%. These results support the hypothesis that the stalk sequences of H1 and H2 are able to form α-helical coiled coils. This structure is significantly less stable for H2S than for H1S, but it is stabilized in both sequences by intermolecular cross-linking via the amino-terminal cysteines.

**H1S and H2S Form Different Homo-oligomers**—To determine the size of the homo-oligomers formed by H1S and H2S, the reduced peptides were incubated at 4°C with 9 mM oxidized glutathione and, to allow redox shuffling, 0.9 mM reduced glutathione. Samples were taken after different times, and free sulfhydryl groups were alkylated to stop further oxidation. The size of the cross-linked peptides were then analyzed by SDS-gel electrophoresis under nonreducing conditions. As shown in Fig. 4A, H1S was rapidly cross-linked, predominantly to trimers. Most importantly, no higher oligomers than trimers were produced. In contrast, higher oligomers were readily generated by oxidation at 75°C (lane 5), a temperature at which the peptide is mostly in a random coil conformation, as expected for unspecific cross-linking of monomeric peptides in solution. The specific formation of dimers and trimers at 4°C thus indicates that three H1S molecules are associated in a complex.

Oxidative cross-linking of H2S at 4°C was slower and produced tetramers as well as some dimers and trimers, but no higher oligomers (Fig. 4A, lanes 1–4). The fraction of tetramers continually increased whereas the fraction of dimers and trimers...
ers decreased with time. After 29 h, tetramers accounted for the most abundant species. Oligomers larger than tetramers were only generated at elevated temperature (lane 5).

H1S and H2S Preferentially Associate to 2:2 Heterotetramers—To investigate the formation of hetero-oligomeric structures between H1S and H2S, the peptides were separately reduced, mixed in various ratios, and heated to 37 °C to unfold existing homo-oligomers. The samples were then slowly cooled to 4 °C and oxidized with glutathione for 5 h. After oxidation, the cross-linked peptide complexes were separated by electrophoresis on nonreducing SDS gels (Fig. 5). Several products were generated which were distinct from the homo-oligomers of H1S and H2S alone. Since H2S was larger than H1S by 25 residues, it was possible to deduce the stoichiometry of each species by comparing the electrophoretic mobility of the new complexes with the ones corresponding to the homo-oligomers of H1S (lane 1) and H2S (lane 5).

Most significantly, the highest hetero-oligomeric complexes formed were tetramers with H1S:H2S ratios of 2:2 or 1:3. Their abundance depended on the initial amount of subunits. The 2:2 complex was the predominant tetrameric species generated when H1S was in excess and when both peptides were present in equal amounts. With an excess of H2S in the mixture, the major species had a 1:3 stoichiometry, but a significant amount of 2:2 tetramers was made as well.

Since the amount of HL-1 on the surface of HepG2 cells and human and rat hepatocytes exceeds that of HL-2 approximately 3-fold, we chose a corresponding 3:1 molar mixture of H1S and H2S for a time course study of oxidative cross-linking. Fig. 6 shows the fraction of tetrameric (panel A) and trimeric species (panel B) produced in percent of the total peptides in the reaction mixture at different time points. According to these results, the 2:2 heterotetramer was the major tetrameric species formed. Among all products, only homotrimers of H1S (3:0), which was in excess, were more abundant (Fig. 6B). The fraction of covalent 2:2 heterotetramers increased significantly during the 170 h of the experiment, whereas the fraction of 2:1
and 1:2 heterotrimers remained constant or decreased with time. The heterotrimers thus largely represent intermediates in the formation of 2:2 oligomers. These results show that the stalk peptides H1S and H2S, mixed at a ratio similar to the abundance of the ASGPR receptor subunits H1 and H2 in HepG2 cells, preferentially formed 2:2 heterotetramers and H1S homotrimers.

**DISCUSSION**

The Stalk Domains of the ASGPR Receptor Subunits Form an \( \alpha \)-Helical Coiled Coil—The formation of \( \alpha \)-helical coiled coil structures is a common mechanism of subunit assembly both of homo- and of hetero-oligomers. Well characterized examples are myosin, intermediate filaments, laminin, and transcription factors (20–23). The two subunits of the ASGPR receptor contain such heptad repeats in the segment connecting the transmembrane anchor and the CRD in the exoplasmic portion of the protein (Ref. 10 and Fig. 2). Consistent with a role of this segment in subunit oligomerization, we found that the exoplasmic portions of H1 and H2 are necessary for complex formation in vivo. We used CD spectroscopy and oxidative cross-linking to show that the stalk peptides form \( \alpha \)-helical structures and that the stalk peptides associate to produce specific homo- and hetero-oligomers. These results represent strong evidence that the ASGPR receptor subunits oligomerize by forming an \( \alpha \)-helical coiled coil structure with their stalk domains. The CRDs are thus bundled and exposed on top of a stem with an approximate length of 12 nm. The heptad repeats of both stalk sequences are interrupted by two stutters of four additional residues each. In other systems, such stutters were shown to result in a reduced superhelicity of the coils (24, 25).

Both H1S and H2S independently assume at least in part an \( \alpha \)-helical conformation, which is extended and stabilized by covalent cross-linking. The helix content and thus the stability of coiled coils in non-cross-linked H2S is clearly lower than in H1S. This correlates with the fate of the respective receptor subunits in vivo. When expressed separately, H1 is transported to the cell surface quite efficiently, whereas only \( \sim \)10% of H2 escapes degradation in the ER and reaches the plasma membrane (6, 26). The majority of H2 may thus not oligomerize efficiently but is retained in the ER and subsequently degraded.

It is notable that the helical structure of both non-cross-linked H1S and H2S have melting points well below physiological temperature (data not shown). The stalk sequences alone could thus not account for oligomerization of receptor subunits in vivo. Intermolecular interactions between the flanking domains, i.e. the CRD and the membrane anchor, probably add stability to the oligomer. In mannose-binding protein, a significant amount of interface between subunits is formed via the CRDs (27). Furthermore, an important contribution is likely made by the transmembrane domain, since it further extends the segment of heptad repeats into the membrane. Coiled coil structures which are formed within the transmembrane domain have been suggested to occur in the phosphoprotein regulator protein phospholamban (28). In addition, by anchoring the polypeptide at one end in the lipid bilayer, it is concentrated on the exoplasmic surface of the membrane and favors parallel interaction between stalk sequences. The importance of the membrane anchor for oligomerization is confirmed by the experimental finding that a secretory form of H2 (H2exo) did not associate with H1S(4–33A) and did not exit the ER, whereas an anchored form of H2 (H2exoLeu19) did (Fig. 1). **Determination of Oligomer Stoichiometry by Oxidative Cross-***

**FIG. 5. Stoichiometry of heterocomplex formation by the stalk peptides H1S and H2S.** Reduced H1S and H2S were mixed in the ratios indicated on the top of the gel and incubated for 10 min at 37 °C. After cooling to 4 °C, oxidation was carried out for 5 h using 9 mM oxidized and 0.9 mM reduced glutathione. Samples were alkylated and analyzed by SDS-gel electrophoresis under nonreducing conditions and stained with Coomassie Blue. The stoichiometry of covalent complexes are indicated on the right as H1:H2, and the positions of molecular mass markers on the left.

**FIG. 6. Kinetics of covalent heterotetramer and -trimer formation.** The fraction (expressed as percent of the total) of covalent tetrameric (panel A) and trimeric species (panel B) present in the reaction mixture at different time points were quantified by densitometric scanning of SDS-gels stained with Coomassie Blue. The experiment was performed as the one shown in Fig. 5 using a molar ratio of H1S:H2S of 3:1.
To allow cross-linking via the formation of disulfide bonds, the amino acid tag CEK was fused to the amino terminus of the stalk peptides. This sequence naturally occurs in the trimeric coiled coil domain of cartilage matrix protein, where the cysteines have been shown to form intermolecular disulfide bonds (29). Similarly closely spaced cysteine residues are also found in other extracellular coiled coil proteins (e.g. cartilage oligomeric matrix protein, which forms covalent pentamers) where they are involved in the connection of subunits by disulfide bond formation. Separately, the stalk peptides rapidly formed specific covalent oligomers: trimers for H1S and tetramers for H2S. Since these were the predominant cross-link products in the complete absence of higher ones, they very likely reflect the oligomeric structure of the coiled coils. Yet, it cannot be entirely excluded that higher complexes were present and that for some reason cross-linking was limited to a subset of the subunits.

When the two stalk peptides were combined in oxidation experiments, mixed cross-link products were readily formed. Two types of heterotetrameric complexes were observed with an H1S:H2S ratio of 2:2 and 1:3. However, covalent tetramers composed of three H1S and one H2S (3:1), corresponding to previous models for the ASGP receptor, were not detectable at any peptide ratio. The kinetics of formation of the different hetero-oligomers suggested that all but the tetramers were intermediates of cross-linking rather than end products. In reaction mixtures containing equal amounts of H1S and H2S, or an excess of H1S, the 2:2 hetero-oligomers represented the predominant tetrameric species produced.

A Model for the Structure of the ASGP Receptor—Our in vitro studies of the peptides H1S and H2S provide evidence that the ASGP receptor complex is assembled by an α-helical coiled coil structure composed of the stalk sequences of the receptor subunits. Although it is probable that flanking sequences like the transmembrane segment and/or the CRD contribute to subunit assembly, it appears unlikely that their interactions can induce the formation of an oligomer that is clearly disfavored by the stalk domain. It thus seems improbable that the receptor is composed of three H1 and one H2 subunit, since this stoichiometry was not observed for the stalk peptides. Among the tetrameric stoichiometries produced by the stalk peptides only the 2:2 stoichiometry is consistent with the photoaffinity cross-linking studies by Rice et al. (7) on rat hepatocytes which demonstrated that the ligand binding site consists of two HL-1 subunits and one HL-2 subunit. We therefore propose that the functional ASGP receptor is a 2:2 heterotetramer.

To reconcile this model and the observed subunit ratio of ~3:1 in hepatocytes, the existence of at least two major receptor complexes have to be assumed: 2:2 heterotetramers that can bind triantennary ligands with high-affinity and H1 homotrimers that cannot. The latter are known to reach the cell surface quite efficiently (6) and to be constitutively endocytosed and recycled (30). However, in binding assays using the typical ligands like asialooligosaccharides, H1 homotrimers will go undetected. Based on co-immunoprecipitation studies, Herzig and Weigel (31) also suggested the presence of homooligomeric HL-1 in rat hepatocytes. In chemical cross-linking experiments using 1,6-difluoro-2,4-dinitrobenzene, covalent homodimers and -trimers and heterodimers and -trimers have been detected (6, 9). (In these experiments, the cross-linking efficiency generally did not allow the detection of larger products.) According to our model, the covalent homotrimers are derived from the H1 homo-oligomers, and the covalent heterodimers and -trimers from the 2:2 heterotetrameric receptors. An intriguing consequence of the hypothesis is the possibility that the H1 homotrimers in hepatocytes might recognize other specific ligands than the ones identified so far. Most binding studies with natural and synthetic glycans were performed as competition experiments in which the inhibition of the binding of a radioactive, triantennary, desialylated N-linked glycan to the hetero-oligomeric receptor was assessed. Recently, transgenic mice deficient in the minor HL-2 subunit were generated (32). Analysis of these mice showed no detectable defect. The as yet unknown ligands of HL-1 homotrimers may be the physiologically important ligands of the ASGP receptor system, and their binding and uptake would hence not be affected in these mice.

Acknowledgments—We thank T. Mini for amino acid analysis, N. Beuret for technical assistance, and Dr. C. Suzuki for critically reading the manuscript.

References
1. Spiess, M. (1990) Biochemistry 29, 10009–10018
2. Drickamer, K., and Taylor, M. E. (1993) Annu. Rev. Cell Biol. 9, 237–264
3. Weigel, P. H. (1994) Bioessays 16, 519–524
4. Spiess, M., and Lodish, H. F. (1986) Cell 44, 177–185
5. McPhaul, M., and Berg, P. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8863–8867
6. Shia, M. A., and Lodish, H. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1158–1162
7. Rice, K. G., Weiss, O. A., Barthel, T., Lee, R. T., and Lee, Y. C. (1990) J. Biol. Chem. 265, 18429–18434
8. Lodish, H. F. (1991) Trends Cell Biol. 16, 374–377
9. Bischoff, J., Libresco, S., Shia, M. A., and Lodish, H. F. (1988) J. Cell Biol. 106, 1074–1077
10. Beavil, A. J., Edmeades, R. L., Gould, H. J., and Sutton, B. J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 753–757
11. Spiess, M., Schwartz, A. L., and Lodish, H. F. (1985) J. Biol. Chem. 260, 1979–1982
12. Brandenberger, R., Kammerer, R. A., Engel, J., and Chiquet, M. (1996) J. Cell Biol., in press
13. Schmid, S. R., and Spiess, M. (1988) J. Biol. Chem. 263, 16886–16891
14. Ellis, L., Clausser, E., Morgan, D. O., Edery, M., Roth, R. A., and Rutter, W. J. (1986) Cell 45, 721–732
15. Wahlberg, J. M., Geffen, I., Reymond, P., Simmen, T., and Spiess, M. (1995) J. Cell Biol. 130, 285–297
16. Cullen, B. R. (1987) Methods Enzymol. 152, 684–704
17. Knecht, R., and Chang, J. Y. (1986) Anal. Chem. 58, 2375–2379
18. Schägger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
19. Chen, Y. H., Yang, J. T., and Chau, K. H. (1974) Biochemistry 13, 3550–3559
20. Chew, M. W., and Squire, J. M. (1995) J. Struct. Biol. 115, 233–249
21. North, A. C. T., Steger, M., and Parry, D. A. D. (1994) Proteins 20, 174–184
22. Hunter, I., Schulthess, T., and Engel, J. (1992) J. Biol. Chem. 267, 6006–6011
23. Harbury, P. B., Zhang, T., Kim, P. S., and Alber, T. (1993) Science 262, 1401–1407
24. Bullough, P. A., Hughson, F. M., Skehel, J. J., and Wiley, D. C. (1994) Nature 371, 37–43
25. Lapan, A., Muller, S., Goldie, K., Engel, A. M., Engel, A., and Baumeister, W. (1995) J. Mol. Biol. 248, 180–189
26. Fuhrer, C., Geffen, I., Hugl, K., and Spiess, M. (1994) J. Biol. Chem. 269, 3277–3282
27. Weis, W. I., and Drickamer, K. (1995) Structure 3, 1227–1240
28. Simmerman, H. K. B., Kobayashi, Y. M., Autry, J. M., and Jones, L. R. (1996) J. Biol. Chem. 271, 5941–5946
29. Haudenschild, D. R., Tondravi, M. M., Hofer, U., Chen, Q., and Goetinck, P. F. (1995) J. Biol. Chem. 270, 23150–23154
30. Geffen, I., Wessels, H. P., Roth, J., Shia, M. A., and Spiess, M. (1989) EMBO J. 8, 2855–2862
31. Herzig, M. C. S., and Weigel, P. H. (1990) Biochemistry 29, 6437–6447
32. Ishibashi, S., Hammer, R. E., and Herz, J. (1994) J. Biol. Chem. 269, 27593–27596
33. Leung, J. O., Holland, E. C., and Drickamer, K. (1985) J. Biol. Chem. 260, 12523–12527