Oligomerization of Peptides Analogous to the Cytoplasmic Domains of Coatomer Receptors Revealed by Mass Spectrometry†

Thilo A. Fligge,‡ Constanze Reinhard,§ Cordula Harter,§ Felix T. Wieland,§ and Michael Przybylski*,‡

Fachbereich Chemie, Universität Konstanz, 78457 Konstanz, Germany, and Biochemie-Zentrum Heidelberg (BZH), Ruprecht-Karls-Universität Heidelberg, 69120 Heidelberg, Germany

Received September 29, 1999; Revised Manuscript Received February 3, 2000

ABSTRACT: Members of the p24 family of type I transmembrane proteins are involved in budding of coat protein type I (COPI)-coated vesicles. They serve as coat protein receptors, binding via their cytoplasmic domains to coatomer, a stable cytosolic protein complex that represents the major coat component of these vesicles. Experimental evidence suggest that p23, a member of the p24 family, binds to coatomer in an oligomeric state and that this binding triggers polymerization of the coat protein. Toward an understanding of this process at the molecular level, formation of noncovalent complexes and their relative stabilities were analyzed by Fourier transform ion cyclotron resonance mass spectrometry using nanoelectrospray ionization. Specificity and stability of oligomers formed were established to depend on characteristic peptide sequence motifs and were confirmed by mass spectrometric competition experiments with control peptides. Mutations in the peptide sequence caused decreased interaction and destabilization of the noncovalent complexes. The formation and relative stabilities of dimeric and tetrameric complexes were assessed to be formed by cytoplasmic tails of coatomer receptors. The direct molecular identification provided by mass spectrometry correlates well with biochemical results. Thus, electrospray ionization mass spectrometry proves to be a powerful tool to investigate physiologically relevant peptide complexes.

COPI-coated[1] vesicles mediate transport of proteins within the early secretory pathway (*1*−*3*). Budding of these vesicles involves a bivalent interaction with the Golgi membrane of coatomer, their major coat component that consists of seven subunits (α−ζ *co*at *p*roteins, COPs) (*4*): coatomer interacts (i) via its β- and γ-subunits with membrane anchored ADP-ribosylation factor 1 (ARF 1) (*5*, *6*) and (ii) via its γ-subunit with the cytoplasmic domain of specific type 1 transmembrane proteins, the so-called p24 family (*7*). p23, a member of this family, is abundant in COPI-coated vesicles and present in a ratio to coatomer of approximately 4:1 (*8*). The cytoplasmic domain of p23 (YLRRFFKAKKLIE) is structurally similar to a classical dilysine motif (KKXX) for retrieval to the endoplasmic reticulum (ER) (*9*−*11*), known to bind to coatomer (*12*), and binds the complex with the same efficiency as the KKXX motif (*8*). p23 binding, however, depends on its phenylalanine residues as well as its lysine residues. The three components described above, coatomer, membrane-bound ARF1, and p23, are all that is needed to form a COPI-coated vesicle as recently shown by reconstitution from chemically defined liposomes (*13*). Moreover, in vitro studies suggest that a tetramer of p23 induces a conformational change of coatomer leading to a polymerization of the complex (*14*). Polymerization of coatomer on the surface is likely to represent the driving force to shape the membrane into a bud (*14*). Tetramerization of the p23 tail domain is in agreement with a 4:1 stoichiometry of p23 and coatomer in COP1-coated vesicles and supports the hypothesis that tetramers of the p24 family function in vivo. In the resting donor Golgi p23, p24, and other members of the p24 family form hetero-oligomeric complexes (*15*−*18*).

The molecular basis of these oligomerizations is still unclear. Therefore, we have established a method to investigate specificity and stability of supramolecular complexes formed by synthetic p23 and p24 tail domains (Figure 1A). We demonstrate here that new "soft-ionization" methods of mass spectrometry provide key information about peptide complexes such as those formed by the cytoplasmic tail of p23. Electrospray ionization mass spectrometry (ESI-MS) has been found suitable to study noncovalent biomolecular complexes at near-physiological solution conditions (*19*−*22*). Although gas-phase species are measured in ESI-MS, intact noncovalent complexes are maintained that are characteristic for the solution phase structure (*22*−*24*). The sensitivity of this technique was substantially increased with the introduction of the nanoelectrospray ionization (nanoESI) source (*25*−*27*). Furthermore, instrument conditions, especially the spray voltage, are optimized in nanoESI-MS to

† Supported by grants from the Deutsche Forschungsgemeinschaft (SFB 352 to F.W.), the Fonds der chemischen Industrie (to M.P. and F.W.), and the European Union (network "Peptide and Protein Structure Elucidation by Mass Spectrometry" to M.P.).

* To whom correspondence should be addressed at Fachbereich Chemie, Universität Konstanz, 78457 Konstanz, Germany. Phone: +49 7531 88 2249. Fax: +49 7531 88 3097. E-mail: Michael.Przybylski@uni-konstanz.de.

‡ Universität Konstanz.

§ Ruprecht-Karls-Universität Heidelberg.

[1] Abbreviations: COPI, coat protein type I; ARF1, ADP-ribosylation factor 1; ER, endoplasmic reticulum; ESI-MS, electrospray ionization mass spectrometry; FT-ICR, Fourier transform ion cyclotron resonance; SWIFT, stored waveform inverse Fourier transform; IRMPD, infrared radiation multiphoton dissociation.

10.1021/bi9922751 CCC: $19.00
Published on Web 06/30/2000

A

YLRR **FFKAKK**LIE	p23wt-m	1712.1 Da
YLRR **AA**KAKKLIE	p23AK-m	1559.9 Da
YLRR FFKA**SS**LIE	p23FS-m	1629.9 Da
YLRR **AA**KA**SS**LIE	p23AS-m	1477.7 Da
YLKR FFEVRRVV	p24wt-m	1612.0 Da
SSVG KKLETFKKTN	Wbp1-m	1566.8 Da
C YLRR FFKAKKLIE C YLRR FFKAKKLIE	p23wt-d	3628.5 Da
C YLKR FFEVRRVV C YLKR FFEVRRVV	p24wt-d	3428.2 Da
C YLRR FFKAKKLIE C YLKR FFEVRRVV	p23p24	3528.3 Da
C YLRR AAKASSLIE C YLRR AAKASSLIE	p23AS-d	3159.7 Da

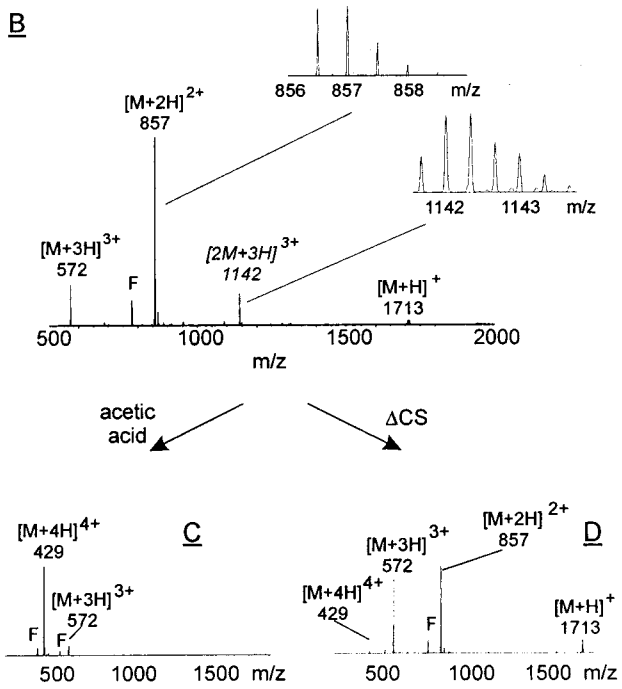

FIGURE 1: Peptides used in this study (A) and ESI mass spectrometric identification of noncovalent p23 peptide complexes (B−D). p23wt-m represents the cytoplasmic domain of p23, whereas p23FS-m, p23AK-m, and p23AS-m lack one or both of the Lys−Lys or Phe−Phe motifs. Dimerization in p23wt-*d* is effected by disulfide formation of a cysteine introduced at the N-terminus. Peptide Wbp1-m represents the cytoplasmic domain from the yeast N-oligosaccharyl transferase complex containing a KKXX ER retrieval motif. Masses in panel A are average molecular masses, and *m*/*z* values in FT-ICR spectra represent monoisotopic masses. Mass determination accuracy was <5 ppm for all FT-ICR measurements. (B) ESI mass spectrum of a 50 μM solution of p23wt-m in 10 mM ammonium acetate, pH 6, containing 10% methanol. Inserts show the isotopically resolved doubly charged ion of the monomer and the triply charged ion of the homodimer, respectively; (C) dissociation of the p23wt-m homodimer in 2% acetic acid, pH 2.5 (ΔCS 10 V); (D) dissociation of homodimer of p23wt-m at increased ΔCS 20 V in 10 mM ammonium acetate, pH 6. F denotes an impurity due to a peptide analogue missing an arginine residue.

particularly mild conditions that are most suitable for the analysis of noncovalent interactions.

In this study, Fourier transform ion cyclotron resonance (FT-ICR) ESI-MS is demonstrated as a new approach that has been applied to the characterization of noncovalent interactions of biopolymers only in a few studies (recently reviewed in ref *28*). In this study, we describe the rapid and sensitive evaluation of peptide complex stabilities at high resolution. The noncovalent interactions were studied by a combination of stored waveform inverse Fourier transform (SWIFT) and subsequent infrared multiphoton dissociation (IRMPD) (*29*−*31*). Stabilities of noncovalent complexes in the gas phase were also assessed using conventional nano-ESI-Tandem-MS. These results correlate well with solution association and stability assessed by ESI-MS and by in vitro precipitation studies of coatomer−peptide complexes (*14*). Hence, the techniques applied in this study constitute powerful tools to analyze formation and stabilities of peptide complexes.

MATERIALS AND METHODS

Peptide Synthesis. Peptides were prepared by automated solid-phase synthesis using Fmoc strategy (*32*) and were purified by HPLC. The sequences of the synthetic peptides are shown in Figure 1*A*. The disulfide-bridged peptides were prepared by oxidation of cysteine residues introduced at the N-terminus, in aqueous 20% dimethyl sulfoxide for 48 h. Subsequently, the dimers were isolated by HPLC and characterized by ESI mass spectrometry.

Electrospray Ionization Mass Spectrometry. ESI mass spectra were obtained with a Vestec A201 quadrupole mass spectrometer (PerSeptive Biosystems, Framingham, MA) (*33*). Samples were infused with a self-constructed nanospray system (*34*), with a voltage at the capillary tip of 1.1−1.3 kV. The declustering potential (ΔCS) was generally set to 10 V for noncovalent complexes and was varied from 2 to >10 V in ΔCS-dependent dissociation studies (*34*). The temperature in the ESI source was measured by a thermocouple adjacent to the capillary and was 40 °C. In temperature-dependent studies, a thermostatic bath was used for controlling the ESI source temperature between 35 and 70 °C (*35*).

Dissociation Experiments in Solution. Stock solutions of the peptides (1.2 mM) in water were stored at −20 °C. Peptide concentrations of the solutions used for ESI-MS were kept at 50 μM during all experiments. For studying the pH-dependent complex formation, peptides were dissolved in 10 mM ammonium acetate containing 10% methanol to a concentration of 50 μM. The pH of the solution was adjusted by acetic acid and ammonia. For dissociation in solution, the peptides were dissolved in 2% acetic acid containing 10% methanol. Competition experiments were carried out with varying amounts of the control peptide (NPS KYIRT IYNRV-$CONH_2$).

Evaluation of Gas-Phase Complex Stabilities. (1) FT-ICR Mass Spectrometry. FT-ICR spectra were obtained with a 9.4-T external source electrospray FT-ICR instrument (*36*). Typical ESI conditions were 1700 V needle voltage, and 1 A current of the heated capillary. Ions were accumulated in a linear octapole trap for 1 s and then transmitted to a Penning trap through a second octapole guide. For gas-phase dissociation experiments, ions were stored in the cylindrical cell and irradiated with a Synrad CO_2 laser (10.6 μm, unfocused) with a laser power of 2 W. The pulse length and power were controlled by an Odyssey data system (Finnigan Corp., Madison, WI). The analytical methodology used for the investigation of complex stabilities is depicted in Figure 3. The procedure is based on the capability of the FT-ICR

instrument to perform *m*/*z*-selective ion accumulation, isolation, and low energy dissociation. By creating a stored waveform inverse Fourier transformation, all ions of the specified *m*/*z* ranges were simultaneously excited (*29*). Ions outside the *m*/*z* range of noncovalent complexes were ejected by this excitation method, which is greatly facilitated by use of the high magnetic field. The stored ions of the noncovalent complex in the resulting spectrum are then irradiated by IR laser photons causing a "slow heating" of the complex.

(2) Product Ion Analysis by ESI-Tandem-MS. Equimolar concentrations of peptides (50 μM in 10 mM ammonium acetate at pH 6 containing 10% methanol) were investigated. The gas-phase stabilities of homodimer complexes of disulfide-bridged peptides were determined by product ion analysis with a triple quadrupole ESI mass spectrometer (Quattro II, Micromass, Manchester, U.K.) equipped with a nanoESI source. Typical ESI parameters were 1.7 kV needle voltage and 30 V cone potential. The complex signals were selected by the first quadrupole and dissociated dependent on the acceleration voltage in the collision cell. Argon at a pressure of 1.1×10^{-6} mbar was used as collision gas.

Precipitation of Coatomer. Coatomer was isolated as described previously (*37*). Precipitation experiments were performed according to ref *14* and are described in brief. Soluble coatomer (0.09 μM in 25 mM HEPES−KOH, pH 7.4, and 100 mM KCl) was incubated with increasing concentrations of peptides (100−1000 μM) for 1 h at room temperature. Precipitates were pelleted by centrifugation at 40000*g* for 15 min at 4 °C. Pellets and supernatants were analyzed by using SDS/PAGE and immunoblotting using anti-COP antibodies. Quantification of precipitated and soluble coatomer was performed by scanning (in a linear range) the γ-COP signal detected by enhanced chemiluminescence (ECL, Amersham Pharmacia)

RESULTS

Mass Spectrometric Identification of p23 Peptide Oligomerization States. A noncovalent peptide complex composed of identical polypeptide chains is directly identified by a molecular ion which, when divided by the postulated number of polypeptide chains of the complex, yields an odd charge number. In contrast, even charge states may not differentiate monomer and oligomers, unless the charge state is identified by isotopic resolution. At high resolution, the oligomer formation is directly identified at any given charge state (e.g., $\Delta m = 0.33$ amu for charge state 3+). Figure 1B shows the ESI FT-ICR mass spectrum obtained from a 50 μM solution of p23wt-m in 10 mM ammonium acetate (pH 6), containing 10% methanol. The charge state and the isotopic distribution reveals signals of the peptide monomer (M) and a homodimer complex (2M). The macroion $[2M + 3H]^{3+}$ at *m*/*z* 1142 identifies the dimeric peptide complex. The peaks at *m*/*z* 857 and 1713 can originate from the peptide monomer and/or the homodimer. The isotopic resolution obtained by FT-ICR MS reveals that both peaks predominantly represent monomeric peptide (see insert for $m/z = 857$ in Figure 1B). There was no indication of a complex formed from three or more peptide chains (Figure 1B).

To establish the specificity of complex formation, solution phase parameters, i.e., pH, temperature, and peptide concentration were varied. At pH 2.6 (2% acetic acid), the charge state distribution shifted to higher charges, and the homodimer ion signal disappeared (Figure 1C). Dissociation of the homodimer was also obtained at increased declustering potential (Figure 1D), which is generally used to identify noncovalent interactions in ESI-MS (*21*). Correspondingly, dissociation induced by increased declustering potential was obtained for all homodimers of the investigated peptides.

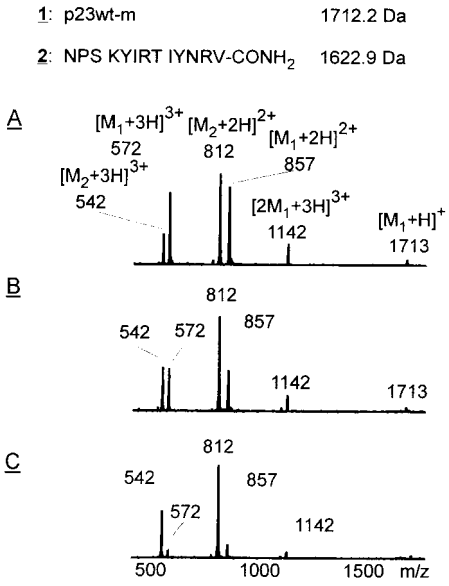

FIGURE 2: ESI mass spectra of a mixture of p23wt-m **1** (50 μM) with increasing concentrations of the nonbinding control peptide **2** (NPS KYIRT IYNRV-CONH$_2$, 1622.9 Da). (A) 25 μM **2**, (B) 50 μM **2**, and (C) 250 μM **2**.

Specificity of Complex Formation of p23 Peptides. The specificity of homodimer formation of the p23 peptides was established by ESI mass spectrometric studies with mixtures of peptides completely unrelated to the cytoplasmic domain of p23. Figure 2 shows the ESI spectra of peptide mixtures of p23wt-m (**1** in Figure 2) with a control peptide (**2** in Figure 2), NPS KYIRT IYNRV-CONH$_2$ (1622.9 Da), at different molar ratios. The concentration of p23wt-m was kept constant at 50 μM. At a concentration of 25 μM of the control peptide (Figure 2A), the peaks at *m*/*z* 542 and 812 are assigned to molecular ions of **2**, whereas all other signals correspond to p23wt-m. The peak at *m*/*z* 1142 is identified as the triply protonated homodimer of p23wt-m. A mixture of equimolar concentrations (50 μM) of both peptides yielded a similar spectrum, with increased abundances of the ions for peptide **2** (Figure 2B). Even at a 5-fold molar excess of the control peptide, the ions of p23wt-m remained unchanged (Figure 2C). No signals indicative of a dimer of the control peptide **2** or heterodimer formation between p23wt-m and **2** were observed. Specificity of homodimer formation was established in a similar way for all peptides (data not shown).

Gas-Phase Stabilities of Complexes by FT-ICR Mass Spectrometry and by ESI-Tandem-MS. To investigate the gas-phase stabilities of coatomer binding complexes, a laser pulse dissociation originally employed to produce peptide fragments in primary structures of protein ions was used (Figure 3). At low laser energy, the peptide backbone remains intact, but partial dissociation is obtained of the peptide complexes by using increasing irradiation times. The isotopically resolved peak of p24wt-m at *m*/*z* 1075.8 clearly shows that a homodimer complex is the only molecular species con-

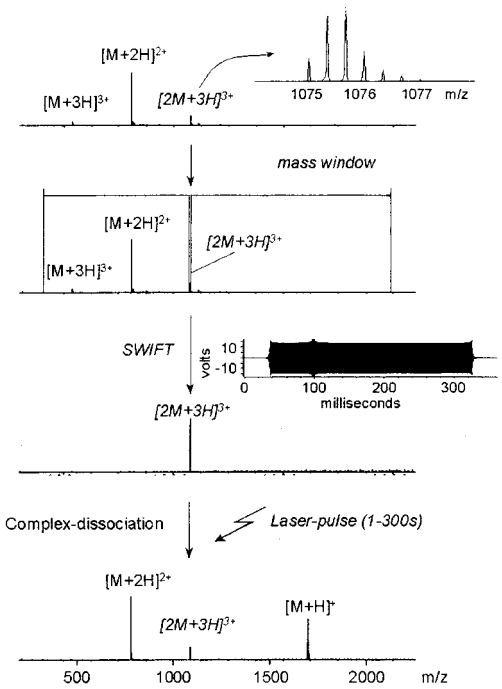

FIGURE 3: Analytical procedure for evaluation of gas-phase stabilities for coatomer peptide complexes by high resolution FT-ICR nanoESI-MS. The triply protonated ion of the homodimer complex is selected using a stored waveform inverse Fourier transformation by ejection of all ions in the *m*/*z* ranges 400−1070 and 1080−2200. Isolated ions are irradiated by IR laser photons inducing slow heating of the complex.

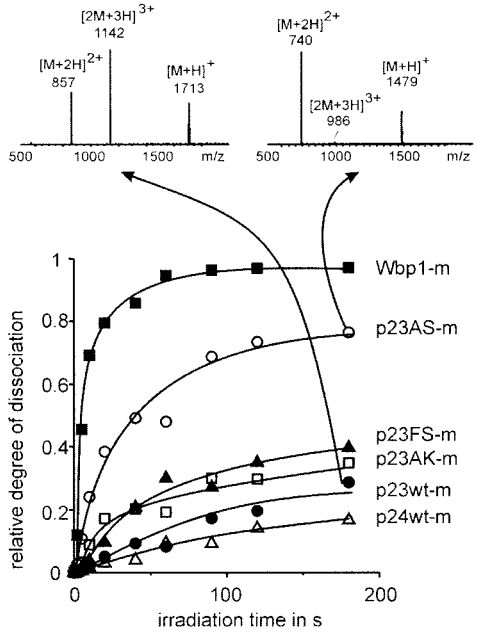

FIGURE 4: Comparison of the relative gas-phase stabilities of homodimer complexes of coatomer-binding peptides and peptide analogues determined by infrared multiphoton dissociation FT-ICR nanoESI-MS. A laser energy of 2 W was employed in all experiments. Inserts show ESI FT-ICR mass spectra of p23wt-m and p23AS-m after 300 s irradiation, respectively. Signals for the triply protonated molecular ions of the homodimer complexes were isolated by SWIFT.

tained in the signal. For the characterization of complex stability, the triply protonated ion was selected by SWIFT, and varying laser irradiation times of up to five minutes were applied. To isolate the signal of the triply protonated molecular form, the ions in the *m*/*z* ranges 400−1070 and 1080−2200 were ejected by the SWIFT excitation method. The resulting spectrum (Figure 3) showed exclusively the signal of the noncovalent complex at *m*/*z* 1075.8. Since the ion storage time applicable in the cyclotron cell increases with the magnetic field (*29*), the ions in this study could be stored up to five minutes, without significant loss of intensity. Without laser irradiation, no signals of dissociation products were obtained, indicating stability of the homodimer in the gas phase. Irradiation of the stored ions for various times resulted in partial dissociation to monomers of the homodimer (Figure 3). Stabilities of various peptide dimers were compared by varying laser irradiation times (Figure 4). To quantitatively describe the dissociation process, the single, isotopically resolved charge states were integrated and, assuming that the peak intensities reflect ion concentrations in the gas phase, the extent of dissociation was measured by the ratio of dimer ions divided by the sum of the monomer ions. Using this approach, the gas-phase stabilities of the peptide complexes (Figure 4) were found to correlate with their coatomer-binding activities (*8*). As an example, after 180 s, p23wt-m still showed a significant dimer signal. In contrast, a peptide with amino acid residues exchanged that are essential for coatomer binding (p23AS-m) was almost completely dissociated. Even after 5 min irradiation, p23wt-m still gave a significant signal of the dimer (data not shown). The peptides corresponding to the cytoplasmic domains of p23 and p24 consistently showed the highest yield of homodimer upon laser irradiation, indicating the high stability of these dimers.

Gas-Phase Stabilities of Complexes By Product Ion Analysis. Members of the p24 family have been shown to form hetero-oligomeric complexes (*15*−*18*). To study a possible contribution of the cytoplasmic domains in stabilizing such complexes, a mixture of monomeric peptides corresponding to the cytoplasmic domains of p23 and p24 were analyzed by ESI-Tandem-MS. In addition to the molecular ions of the peptide monomers, signals for the corresponding homodimers and a heterodimer were detected (Figure 5A). As outlined above, the stoichiometry between p23 and coatomer was found to be approximately 4:1 (*8*, *14*). Therefore, we investigated a possible formation of tetramers in solution. To this end, we used covalently linked dimers of cytoplasmic domains for the following reasons: since direct tetramer formation from a monomer in solution is unlikely to occur according to the law of mass action (tetramolecular reaction), a covalently linked dimer was considered to increase the tetramer formation by association as a bimolecular reaction. Dimerization of covalently dimerized peptides was studied by product ion analysis in ESI-Tandem-MS. Signals specific for dimerized dimer were selected in the first quadrupole, and complex dissociation was obtained under particularly mild dissociation conditions in the collision cell (not affecting any covalent linkage). The extent of complex formation depends on the acceleration voltage applied and correlated with peptide sequences as observed by FT-ICR MS. As shown in Figure 5B, a noncovalent homodimer complex formed by the covalently linked peptide p23wt-*d* (equivalent to four cytoplasmic domains of p23) showed the highest stability, followed by the corresponding complex of p24. A heterologous complex

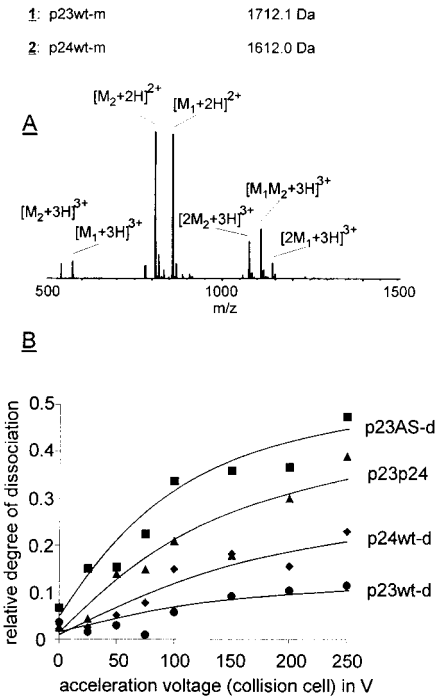

FIGURE 5: (A) ESI mass spectra of an equimolar mixture of p23wt-m (**1**) and p24wt-m (**2**) (50 μM in 10 mM ammonium acetate containing 10% methanol); (B) comparison of the relative gas-phase stabilities of homodimer complexes of disulfide-bridged coatomer-binding peptides and peptide analogues determined by mild collision-induced dissociation.

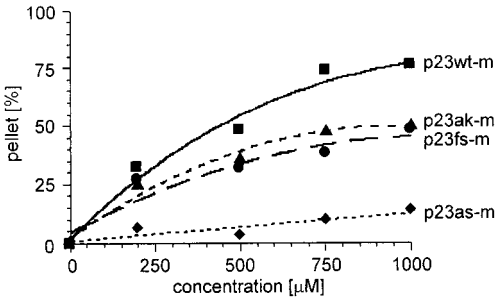

FIGURE 6: p23 tail peptide-induced polymerization of coatomer. Coatomer (0.09 μM) was incubated with increasing concentrations of various peptides and centrifuged, and the amounts of coatomer were determined in the pellet. Peptides investigated included the cytoplasmic domain of p23 (p23wt-m, filled circles), mutants with one binding motif exchanged (p23AK-m, filled rhombus, and p23FS-m, filled triangles), and a peptide (p23AS-m, filled squares) with all amino acid residues essential for coatomer binding exchanged.

and a complex of a noncoatomer binding mutant peptide still showed measurable gas-phase stabilities, although at a significantly reduced level. Interestingly, a covalent dimer of the Wbp1 tail domain, known to bind efficiently to coatomer, did not yield any detectable dimerization signal.

Peptide-Induced Polymerization of Coatomer. To study sequence-specific interaction of p23-derived peptides with isolated coatomer, the complex was incubated with different synthetic peptides. After centrifugation, a polymerization of coatomer was observed that was clearly most prominent for the cytoplasmic peptide, p23wt-m (Figure 6). In contrast, sequence mutations showed decreased coatomer precipitation, and the mutation p23AS-m, where all amino acid residues essential for coatomer binding are exchanged, did not cause any appreciable precipitation. These results correlate well with the stability differences observed of peptide complexes as evaluated by mass spectrometry.

DISCUSSION

Members of the p24 family are suggested to play a role in budding of COPI-coated vesicles (*8*) and to possibly function as tetramers in vivo. However, the molecular basis of their oligomerization is still unclear. Therefore, we have established a method to investigate specificity and stability of p23 and p24 tail peptide complexes. Using FT-ICR MS and nanoESI-Tandem-MS we obtained direct molecular evidence that the cytoplasmic domains of p23 and p24 undergo specific oligomerizations under near-physiological solution conditions. ESI mass spectrometry provided the identification of specific homodimers of the wild-type peptides without signals indicating higher unspecific aggregation products, and specificity of complex formation was ascertained at different solvent conditions. Furthermore, competition experiments with control peptides confirm that the homodimer formation results from specific interaction. Peptides analogous to the wild-type sequences of the p23 and p24 cytoplasmic domains showed stabilities significantly higher than mutant peptides in which either their diphenylalanine or dilysine motifs were exchanged. Exchange of both motifs lead to a dramatic decrease of homodimer stability, indicating that the combination of both diphenylalanine and dilysine motifs contributes to intermolecular interactions within the various complexes.

The relative stabilities found for the various peptide species analyzed were the following: dimers, p24wt = p23wt > p23AK = p23FS > p23AS > Wbp1; tetramers, p23wt > p24wt > p23p24 > p23AS $\gg$ Wbp1. This correlates well with the idea that p23 is part of the budding machinery of COPI vesicles and that it is its tetramer form that binds coatomer and triggers polymerization of the complex. In line with this hypothesis, ESI signals of a peptide complex analogous to the cytoplasmic domain of Wbp1 (*38*), an ER-resident transmembrane protein that is not part of the transport machinery but rather cargo for retrieval (*11*), showed dramatically reduced stability of the homodimer complex in the gas phase; this peptide did not cause precipitation of the coatomer complex (*14*). These relative stabilities are also consistent with binding and stoichiometry data (*8*, *14*). Unpublished data from our laboratory indicate that this also holds not only for p23 but also for the cytoplasmic domain of p24.

Coatomer is able to bind to such peptide domains in solution without any additional component added, i.e., its binding site for the cytoplasmic tails is available. In contrast, coatomer does not bind to donor Golgi membranes containing ample amounts of these proteins. Only after interaction with membrane-bound ARF-GTP, coatomer can bind to a Golgi membrane. Two alternative mechanisms may explain this strict ARF-GTP dependence: (i) The p24-family in the nonactivated Golgi may reside in non-homo-oligomerized forms, most likely in a heterologous complex, consistent with biochemical results (*15*, *17*, *18*). This then implies that a function of ARF-GTP binding to the donor membrane is to release individual p24 proteins to enable them to oligomerize (a process that might well involve their luminal domains)

and thus to create a productive binding site for coatomer. (ii) ARF-GTP may regulate the thermodynamic equilibrium of coatomer between a soluble and a membrane-associated form by binding to coatomer via its β- and γ-subunit (*5*, *6*). This concentration of coatomer at the Golgi membrane is needed for an efficient interaction with p24 protein cytoplasmic domains.

In summary, FT-ICR MS and ESI-Tandem-MS have been demonstrated to be powerful tools to study the formation and relative stabilities of noncovalent peptide complexes in the gas phase, indicative of biochemical function of such complexes.

ACKNOWLEDGMENT

We wish to thank Dr. Alan Marshall for access to the FT-ICR instrumentation at the National High Magnetic Field Laboratory, Tallahassee, FL, Rüdiger Pipkorn for peptide synthesis, and Mark Emmett and Michael Freitas for helpful discussions.

REFERENCES

1. Rothman, J. E. (1994) *Nature 372*, 55−63.
2. Rothman, J. E., and Wieland, F. T. (1996) *Science 272*, 227−234.
3. Schekman, R. (1996) *Science 271*, 1526−1533.
4. Wieland, F., and Harter, C. (1999) *Curr. Opin. Cell Biol.*, in press.
5. Zhao, L. Y., Helms, J. B., Brugger, B., Harter, C., Martoglio, B., Graf, R., Brunner, J., and Wieland, F. T. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 4418−4423.
6. Zhao, L., Helms, J. B., Brunner, J., and Wieland, F. T. (1999) *J Biol. Chem. 274*, 14198−203.
7. Harter, C., and Wieland, F. T. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 11649−11654.
8. Sohn, K., Orci, L., Ravazzola, M., Amherdt, M., Bremser, M., Lottspeich, F., Fiedler, K., Helms, J. B., and Wieland, F. T. (1996) *J. Cell Biol. 135*, 1239−1248.
9. Nilsson, T., Jackson, M., and Peterson, P. A. (1989) *Cell 58*, 707−718.
10. Jackson, M. R., Nilsson, T., and Peterson, P. A. (1990) *EMBO J. 9*, 3153−3162.
11. Jackson, M. R., Nilsson, T., and Peterson, P. A. (1993) *J. Cell Biol. 121*, 317−333.
12. Cosson, P., and Letourneur, F. (1994) *Science 263*, 1629−1631.
13. Bremser, M., Nickel, W., Schweikert, M., Ravazzola, M., Amherdt, M., Hughes, C. A., Sollner, T. H., Rothman, J. E., and Wieland, F. T. (1999) *Cell 96*, 495−506.
14. Reinhard, C., Harter, C., Bremser, M., Brugger, B., Sohn, K., Helms, J. B., and Wieland, F. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 1224−1228.
15. Dominguez, M., Dejgaard, K., Fullekrug, J., Dahan, S., Fazel, A., Paccaud, J. P., Thomas, D. Y., Bergeron, J. J., and Nilsson, T. (1998) *J. Cell Biol. 140*, 751−765.
16. Blum, R., Pfeiffer, F., Feick, P., Nastainczyk, W., Kohler, B., Schafer, K. H., and Schulz, I. (1999) *J. Cell Sci. 112*, 537−548.
17. Gommel, D., Orci, L., Emig, E. M., Hannah, M. J., Ravazzola, M., Nickel, W., Helms, J. B., Wieland, F. T., and Sohn, K. (1999) *FEBS Lett. 447*, 179−185.
18. Füllekrug, J., Suganuma, T., Tang, B. L., Hong, W., Storrie, B., and Nilsson, T. (1999) *Mol. Biol. Cell 10*, 1939−1955.
19. Schwartz, B. L., Gale, D. C., and Smith, R. D. (1996) *Methods Mol. Biol. 61*, 115−127.
20. Loo, J. A. (1997) *Mass Spectrom. Rev. 16*, 1−23.
21. Przybylski, M., and Glocker, M. O. (1996) *Angew. Chem., Int. Ed. Engl. 35*, 806−826.
22. Przybylski, M., Schnaible, V., Kast, J., Bühler, S., Michels, J., Wattenberg, A., Fligge, T. A., Forst, D., Diederichs, K., Zeth, C., Glocker, M. O., and Welte, W. (1998) in *New Methods for the Study of Biomolecular Complexes* (Ens, W., Ed.), pp 17−43, Kluwer Academic Publishers, Amsterdam.
23. Aplin, R. T., Robinson, C. V., Schofield, C. J., and Westwood, N. J. (1994) *J. Chem. Soc., Chem. Commun.*, 2415−2417.
24. Mirza, U. A., and Chait, B. T. (1997) *Int. J. Mass Spectrom. Ion Processes 162*, 173−181.
25. Wilm, M., and Mann, M. (1996) *Anal. Chem. 68*, 1−8.
26. Wilm, M. S., and Mann, M. (1994) *Int. J. Mass Spectrom. Ion Processes 136*, 167−180.
27. Fligge, T. A., Bruns, K., Hägele, K., and Przybylski, M. (1997) 14th International Mass Spectrometry Conference, Vol. 14, CD-ROM, Tampere, Finland.
28. Last, A. M., and Robinson, C. V. (1999) *Curr. Opin. Chem. Biol. 3*, 564−570.
29. Marshall, A. G., Hendrickson, C. L., and Jackson, G. S. (1998) *Mass Spectrom. Rev. 17*, 1−35.
30. Colorado, A., Shen, J. X., Vartanian, V. H., and Brodbelt, J. (1996) *Anal. Chem. 68*, 4033−4043.
31. Cheng, X., Chen, R., Bruce, J. E., Schwartz, B., Anderson, G. A., Hofstadler, S. A., Gale, D. C., Smith, R. D., Gao, J., Sigal, G. B., Mammen, M., and Whitesides, G. M. (1995) *J. Am. Chem. Soc. 117*, 8859−8860.
32. Atherton, E., Fox, H., Harkiss, D., Login, C. J., Sheppard, R. C., and Williams, B. J. (1978) *J. Chem. Soc. Chem. Commun.*, 537−539.
33. Allen, M. H., and Vestal, M. L. (1992) *J. Am. Soc. Mass Spectrom. 3*, 18−26.
34. Fligge, T. A., Bruns, K., and Przybylski, M. (1998) *J. Chromatogr. B 706*, 91−100.
35. Fligge, T. A., Kast, J., Bruns, K., and Przybylski, M. (1999) *J. Am. Soc. Mass Spectrom. 10*, 112−118.
36. Senko, M. W., Hendrickson, C. L., Pasa-Tolic, L., Marto, J. A., White, F. M., Guan, S., and Marshall, A. G. (1996) *Rapid Commun. Mass Spectrom. 10*, 1824−1828.
37. Pavel, J., Harter, C., and Wieland, F. T. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 2140−2145.
38. te Heesen, S., Janetzky, B., Lehle, L., and Aebi, M. (1992) *EMBO J. 11*, 2071−2075.

BI9922751