A Membrane Setting for the Sorting Motifs Present in the Adenovirus E3-13.7 Protein Which Down-regulates the Epidermal Growth Factor Receptor*

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The adenovirus E3-13.7 protein interferes with endosomal protein sorting to down-regulate the epidermal growth factor receptor and related tyrosine kinase receptors. The cytoplasmic C terminus of this protein contains three protein sorting motifs which are related to the function of E3-13.7. In this study, the structure of a 23-residue polypeptide corresponding to this domain was examined using solution NMR and CD spectroscopic methods. The peptide was observed to exist in a mostly random structural state in aqueous solution but underwent high affinity association with dodecylphosphocholine micelles, where it adopted an ordered structure. The affinity of this peptide for the micellar surface and the structure of the bound peptide were independent of pH variation, surface charge, or attachment of a myristoyl anchor to the N-terminal. Studies with phospholipid vesicles suggested that the micellar structural results can be extrapolated to a true lipid bilayer. On the micellar surface all three sorting motifs are closely associated with the water/apolar interface; 72-YLRH and 87-LL lie within interfacial amphipathic helices, while 76-HPQY is non-helical and dimples just above the surface. These results contribute to the development of an understanding of the basis for specificity in recognition of sorting motifs by components of the cellular protein trafficking machinery.

A 13.7-kDa protein expressed from the “Early 3” genome region of adenovirus (E3-13.7) down-regulates the EGFR and related tyrosine kinase receptors during infection of epithelial cells and fibroblasts (1, 2). For EGFR it has been shown that E3-13.7 exerts its role by altering sorting at the endosomal level (3) such that the ratio of receptor destined for lysosomal degradation relative to receptor destined for recycling to the plasma membrane surface is increased. The down-regulatory action of E3-13.7 is believed to be associated with the constitutive recycling pathway of EGFR and does not require ligand stimulation of the receptor to be operative. An enhanced understanding of the down-regulatory mechanism of E3-13.7 is desirable because of light it will shed both upon the cell biology of protein trafficking pathways and upon the molecular biology of adenoviral infections.

As outlined in Fig. 1, E3-13.7 is an integral membrane protein comprised of an N-terminal transmembrane helix followed by an extracellular/luminal loop, a second transmembrane segment, and a C-terminal cytoplasmic domain (1). While the process by which E3-13.7 down-regulates tyrosine kinase receptors is incompletely understood, it is known that the C-terminal cytoplasmic domain (E3-CyD) is critical to its function. Within this 23-residue domain there are three different sequence motifs (Fig. 1) known to be broadly relevant to the cellular trafficking of membrane proteins via clathrin-mediated internalization pathways (4–6). One of these is the “dileucine” (87-LL) motif (Refs. 7–9 and references therein). While a functional role for this motif in E3-13.7 has yet to be established, it is of probable significance because it and adjacent residues (LRLIL) are also present in the juxtamembrane cytoplasmic domain of EGFR where it has been shown to be critical to proper sorting of the receptor.‡ The other two motifs present in the E3-CyD represent the tyrosine-based motifs YXXN (refs. 8 and 10). Carlin and co-workers§ have established that mutation of either tyrosine to alanine in E3-13.7 leads to a loss of EGFR down-regulatory activity in adenovirus-infected cells. They have also shown that the 72-YLRH motif is required for high affinity binding of the adaptin AP-2 by the E3-CyD in vitro while both tyrosine motifs are critical for binding by AP-1.

In this paper, the conformation of the cytoplasmic domain of E3-13.7 is examined. Its structure is of relevance both because of specific insight it may provide into the mode of receptor down-regulatory action by E3-CyD and thereby into mechanisms of endosomal receptor sorting. More generally, structural information for E3-CyD may shed light upon the general mechanisms by which sorting motifs are recognized by components of cellular protein trafficking networks. While sorting motifs have previously been subjected to structural character-
ization (12–18), the present study extends previous work in two crucial aspects. First, because the E3-CyD contains three different sorting motifs within a fairly short sequential span, this study will provide insight into the structural consequences of sorting signal multimerization. Clustering of signals is relevant to E3-13.7 function³ and has been observed to be important in the sorting of other proteins (7, 9). Second, most previous studies have focused on the structures of motif-containing polypeptides in aqueous solution. However, since sorting motifs are often found in sequentially juxtamembrane domains (as for E3-13.7) we considered the possibility that membrane interactions might sometimes play an important role in defining the local structure of a motif-containing segment.

The cytoplasmic domain of E3-13.7 was investigated in two forms (Fig. 1): (i) an N-acetylated 23-residue peptide which will be referred to as E3-CyD (E3 Cytoplasmic Domain). (ii) An N-myristoylated 25-residue peptide, referred to as E3-mCyD, where the myristoyl tail serves (ideally) as a surrogate transmembrane domain.

In this paper we describe structural studies of these two peptides in both aqueous solution and in aqueous detergent micelles. Micelles were used to model biological membranes. The detergent used in most of our studies was dodecylphosphocholine (DPC, see Ref. 19). DPC has the same zwitterionic phosphocholine head group as the dominant phospholipid species of most eukaryotic membranes, phosphatidylcholine (20). DPC micelles have also been recently demonstrated to provide a favorable balance between desirable NMR properties and biochemical compatibility with membrane proteins, properties which are often at odds with each other (21).

**EXPERIMENTAL PROCEDURES**

**Sample Preparation**—The two peptides illustrated in Fig. 1 were obtained from Quality Controlled Biochemicals Inc. (Boston, MA). Both peptides were specified to be >95% pure according to the company documentation, but we found that the myristoylated peptide, E3-mCyD required further purification. This was accomplished by size-exclusion chromatography using Bio-Gel P2 (Bio-Rad) and water with 20% isopropanol alcohol and 0.5% formic acid as an eluant. E3-CyD powder was dissolved either in an aqueous buffer or was premixed with detergents DPC and/or SDS in perdeuterated form from Cambridge Isotopes Lab (Andover, MA). E3-mCyD peptide had a high tendency to aggregate in water and required sonication to be dissolved in the presence of micelles.

Standard sample preparations contained 3 mM E3-CyD in (85% H2O, 15% D2O) 50 mM buffers (sodium acetate, pH 4.5, or sodium phosphate, pH 7.6), 100 mM NaCl, 1 mM EDTA. For micellar samples the following concentrations of detergent were used: 50–60 mM DPC for E3-CyD and 150–400 mM for E3-mCyD (plus 10 mol % of SDS in some cases). Bilayer-containing samples were prepared by mixing the peptides with 50-nm unilamellar POPC vesicles which were formed by repeatedly forcing aqueous POPC dispersions through 50-nm nylon filters using a LIPOSOFAST extruder (Avestin, Ottawa, ON).

**NMR Methods**—NMR experiments were carried out using a Varian UNITY-Plus 600 MHz NMR spectrometer equipped with Varian 5-mm 1H/X and 1H/13C/15N indirect z axis pulsed field gradient probes. For most of the samples, the following experiments were executed: one-dimensional 1H temperature-variation, DQF-COSY (double-quantum-filtered) (22), TOCSY (23), and NOESY (using pulsed field gradients for water suppression) (24). The TOCSY mixing time was 60–70 ms. Several NOESY mixing times were used for micellar samples: 80, 200, and 300 ms, while for detergent-free samples a single NOESY mixing time of 200 ms was used. Translational diffusion coefficients were measured using the water-suppressed longitudinal eddy-current delay pulse sequence as described by Altieri et al. (25).

**J-Coupling Determination**—The method of Kim and Prestegard (26) was utilized which allows accurate calculation of scalar coupling from measurement of separations of extrema in dispersive and absorptive plots of rows through cross-peaks. This method is effective in analyzing spectra with moderate-to-high signal-to-noise ratios. Additionally, traces through the antiphase DQF-COSY cross-peaks were deconvoluted with two antiphase Lorentzian resonances using VNMR. Coupling constants derived by these two methods were compared and only

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**Fig. 1. The 13.7-kDa adenovirus E3 protein and the two peptides examined in this study.**

**A** E3-CyD in water, 4°C, pH 4.5

| Signal Sequence | Lumenal/ Extracellular Domain (residues 1-22) | Transmembrane Domain (residues 22-35) | Cytoplasmic Domain (residues 37-68) |
|-----------------|---------------------------------------------|--------------------------------------|----------------------------------|
| E3-CyD peptide | Acetyl-R1A-YLRH-RDTRITA-LRLRQ-COONH4 |                                      |                                  |
| E3-mCyD peptide | Myristoyl-R1A-YLRH-RDTRITA-LRLRQ-COONH4 |                                      |                                  |

**Fig. 2. Summary of NMR data for E3-CyD in water (A) and micelles (B).**
values which were determined to be in agreement were accepted.

Determination of Peptide Partition Coefficients from Diffusion Measurements—The NMR-based method used in this work to determine the partition coefficient of the peptides in micelles is similar to that described elsewhere (27–30). In the present case, a pulsed field gradient longitudinal eddy-current delay experiment was used instead of the stimulated spin-echo experiment.

Circular Dichroism—CD spectra were acquired using a Jasco-600 spectrometer. Samples for far-UV studies were prepared by dissolving about 0.5 mg/ml peptides powder in 0–320 mM dodecylphosphocholine (Anatrace, Maumee, OH) in buffer. The exact concentration was determined by measuring the solution absorbance at 280 nm using a calculated extinction coefficient of 2840 [M⁻¹ cm⁻¹] for both peptides. Quartz cuvettes with a light path-length of 1 mm were used. The parameters employed were: bandwidth = 1 nm; sensitivity = 50 mDeg; time constant = 0.5 s. Spectra acquired represented an average of 4 scans. Reference spectra, obtained with the samples containing all components except the peptide, were recorded before each spectrum and subtracted from the peptide spectrum during acquisition. All spectra were recorded in mDegree units. The instrument was routinely calibrated with a standard solution of recrystallized (1R)(−)-10-camphorsulfonic acid, ammonium salt at 290 nm (ρ = 7910).

RESULTS

The structures of E3-CyD and E3-mCyD were examined in aqueous solution and in aqueous micelles. Measurements were made at both pH 4.5 and 7.5 for two reasons. First, this allowed data to be taken at pH above and below the pKᵢ of the histidine side chains present in both tyrosine sorting motifs, thereby allowing us to investigate whether the structure of E3-CyD is sensitive to the state of protonation of these residues. Second, while studies at the higher pH might be more physiologically relevant, it is much easier to extract structurally useful NMR data at mildly acidic pH where all amide protons can be directly observed due to depressed rates of chemical exchange with water protons.

Structure of E3-CyD in Aqueous Solution—Total backbone ¹H NMR resonance assignments were made for E3-CyD in water at 4, 15, and 25 °C at pH 4.5 and 4 °C for pH 7.6. One-dimensional proton and CD spectra were routinely used as qualitative structural indicators while NOE and J-coupling measurements were used as a source of more quantitative

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**Fig. 3.** Circular dichroism spectra of E3-CyD and E3-mCyD in various solutions. A, E3-CyD at 4 °C in water and micelles (10 mM DPC); B, E3-mCyD at 25 °C in micelles (80 mM DPC). The peptide concentration in each case was 0.5 mg/ml. The buffers used were: 10 mM sodium acetate, pH 4.5, and 20 mM sodium phosphate, pH 8.5.
It was observed that E3-CyD in water at 25 °C is mostly unstructured at both acidic and neutral pH. Only by lowering the temperature to 4 °C at pH 4.5 were we able to detect some residual α-helical/turn structure as reflected by $d_{NN}(i, i+1)$ cross-peaks in the NOESY spectrum (Fig. 2A) and negative intensity at 220 nm in the CD spectrum (Fig. 3A). Even in this case, the helical content appears to be minimal. Few intermediate and no long-range NOEs were observed. Moreover, while strong sequential $d_{NN}(i, i+1)$ connectivities were present there was a lack of deviations by chemical shifts from their random-coil values ("fingerprint region" Fig. 4A). Vicinal $J_{NN}$ coupling constants fell mostly inside the range typical for extended random coil conformations. Taken together, these results indicate the lack of a defined conformation for this peptide in water under any conditions examined.

Affinity of the E3 Cytoplasmic Domain for Membranes—When E3-CyD was dissolved in DPC micelles it showed a major change in its conformational state. Chemical shifts were much more dispersed and a dramatic increase in line widths was observed relative to the peptide in solution at the corresponding temperatures (Fig. 5). The amide proton frequency of Arg$^1$ is shifted about 0.8 ppm downfield while amide proton frequencies of several of the C-terminal residues (Arg$^{21}$, Ile$^{22}$, and...
Some general structural information can be gleaned from temperature-induced changes in the amide-aromatic region of one-dimensional 1H NMR spectra of 3 mE3-CyD plus 50 mM DPC at pH 4.5 as a function of temperature. Impurities from deuterated DPC are labeled with CyD and that the membrane-associated structure of the cytoplasmic domain of E3–13.7 can be independent of an N-terminal membrane anchor.

We also examined the question of whether E3-CyD binds to a "real" lipid bilayer with the same high affinity as DPC micelles and whether it adopts the same structure in both systems. We therefore examined the NMR spectrum of E3-CyD when mixed with unilamellar POPC vesicles in the liquid crystalline phase. POPC is a major component of eukaryotic membranes and vesicles comprised of this phospholipid are often used to mimic biological bilayers (20). The NMR spectra of E3-CyD in POPC vesicles are similar to spectra of the peptide in micelles but with much larger line widths in the former case (data not shown). The breadth of the lines prohibits detailed structural characterization in vesicles, but the general similarity of the one-dimensional spectra confirms that the peptide does indeed have a high affinity for actual lipid bilayers and strongly suggests that its structural state in bilayers is similar to its structure in micelles.

The results of this section established that E3-CyD binds with high affinity to DPC micelles in a manner which appears to be similar to its interaction with bilayers and which is independent of the presence of either an N-terminal membrane "anchor" or of a net negative charge in the interface. We therefore proceeded to a detailed structural characterization of E3-CyD in DPC micelles.

**CD and NMR Structural Measurements for E3-CyD Associated with DPC Micelles**—Some general structural information can be gleaned from temperature-induced changes in the am-
ide-aromatic region of one-dimensional $^1$H NMR spectra of micellar E3-CyD (Fig. 5). At 4 °C the spectrum is extremely broad and more highly dispersed than in the absence of detergent. When the temperature of the micellar sample is increased, peaks become sharper and less dispersed, suggestive of a loss of structural order. Some peaks undergo more changes compared with His 8. This suggests that in the ordered (low temperature) state and only a few $^1$H$_{\text{Na}}$ coupling constants could be unambiguously measured. Nevertheless, backbone assignments were made at 40 °C at both pH values and, while quantitative structure calculations were unfeasible, it was possible to determine a semiquantitative structural model for the ordered form of the peptide based upon these data.

Fig. 2B summarizes NOE and $\beta$-coupling data obtained for E3-CyD in DPC at 40 °C and acidic pH from two-dimensional NMR experiments. There are a number of intermediate-range NOEs representing the ordered population, typical for $\alpha$-helical like structure between residues 1–6 and 17–21 (d$^\alpha$($i$, $i+3$), d$^\alpha$($i$, $i+2$), d$^\alpha$($i$, $i+1$)). The deviation of $\alpha$-proton chemical shifts ($\delta^\alpha$) from their random coil values and measured vicinal $J_{\text{Na}}$ couplings are consistent with modeling these regions as $\alpha$-helical. This indicates that 11 of 23 residues are $\alpha$-helical, in good agreement with the 4 °C CD data (Fig. 3A) estimate of about 40%. The following NOEs were also observed: Pro$^{\beta}$-His$^5$-NH, Pro$^{\beta}$-Gln$^{10}$-NH, Pro$^{\beta}$-Tyr$^{11}$-NH, Pro$^{\beta}$-Gln$^{10}$-NH, and Pro$^{\beta}$-Gln$^{10}$ side chain NH$_2$. These are indicative of a loop or $\beta$-turn involving residues His$^5$-Tyr$^{11}$. Furthermore, His$^5$ and His$^8$ exhibit amide-water exchange peaks, indicating that they are water accessible. Measurements at 25 °C yielded data which generally confirmed the structural conclusions gleaned from the 40 °C data although spectral quality at 25 °C was not as high as at 40 °C.

An additional source of information on the structure of the bound peptide is differential broadening of resonances from various peaks seen in the DQF-COSY and TOCSY spectra. Even at 40 °C, some side chain peaks (such as those from Ile$^5$, Asp$^{13}$, Ile$^{16}$, and Thr$^{15}$) are so broad that they cannot be observed, while others (Ala$^7$, Tyr$^{4}$, Ala$^{17}$, and Asp$^{18}$) are clearly more broad than others. The most probable explanation is that the side chains of these residues are imbedded in the micellar interface resulting in shorter transverse relaxation times due to restrained local motion.

Increasing the pH to 7.6 did not significantly change the pattern of the NOESY fingerprint region (Fig. 4, B and C).
However, some amide proton frequencies shifted at residues (in decreasing order) Thr$^{15}$, Ile$^{16}$, Arg$^{14}$, Gln$^{10}$, Ala$^{17}$, Leu$^{19}$, and His$^{8}$ without dramatic changes of intermediate-range NOE pattern. The major difference in the NOE pattern (data not shown) was a reduction in intensity of NN-cross-peaks for Arg$^{6}$-His$^{7}$, Leu$^{20}$-Arg$^{21}$, and Asp$^{18}$-Arg$^{21}$, suggestive of a population decrease for the ordered state versus the disordered one. This conclusion is supported by the CD spectrum showing a lower helical population at the higher pH. These results suggest that changing the pH does not change the overall structure of the peptide, but rather changes the stability of the ordered conformation.

Identification of Amphipathic Helices in Micelle-associated E3-CyD—Additional insight into the probable mode of the peptide-surface interaction was provided by mapping the distribution of the hydrophobicity in the helical segments. We examined helical wheel diagrams of E3-CyD to identify possible amphipathic helices in the peptide which would be likely sites of insertion into the micellar interface. These diagrams (not shown) indicated that regions 1–7 and 17–21 are certainly amphipathic if helical. The residues comprising the hydrophobic faces of these helices correspond to the side chain resonances which exhibited the most broad NMR resonances (above section).

**DISCUSSION**

Structure of Micelle-associated E3-CyD—Based upon the data presented under “Results,” a model can be constructed for E3-CyD incorporated in DPC micelles. Key components of this model are as follows and represented pictorially in Fig. 6. 1) Residues 1–7 adopt a helical conformation, with Ile$^{6}$, Ala$^{3}$, and Leu$^{5}$ constituting the hydrophobic face imbedded into the micellar surface. 2) Residues 8–11 form a b-turn or loop facing aqueous solution. 3) Residues 12–16 may interact with the micellar surface, perhaps with Arg$^{17}$ and Asp$^{18}$ interacting with the phosphocholine moiety of DPC. However, these residues do not adopt a helical structure and this is the most uncertain part of the structural model. 4) Residues 17–21 are a-helical, with residues Ala$^{17}$, Ile$^{18}$, Leu$^{19}$, and Leu$^{20}$ forming a hydrophobic face imbedded into the interface. 5) The last two residues, Ile$^{22}$ and Leu$^{23}$, are in random coil configuration.

According to this model, the first (YLRH) and the third (LL) sorting motifs exist in surface-inserted amphipathic helices, while the second one (HPQY) forms a solvent-exposed turn or loop. This model is equally valid under both mildly basic and acidic conditions, is independent of the presence of a net negative charge on the micellar surface, does not require an N-terminal hydrophobic anchor, and likely can be extended to negative charge on the micellar surface. This model is equally valid under both mildly basic and acidic conditions.

It has sometimes been observed that residue spacing between the transmembrane domain and the sorting motifs present in the juxtamembrane cytoplasmic domains of membrane proteins is critical to recognition of the motif by sorting molecules such as adaptins (10, 31): removal or addition of residues from the linking region can disrupt normal sorting. While such experiments have yet to be carried out with the intact E3-13.7, it is interesting to note that if the cytoplasmic domain within native E3-13.7 does indeed lie on the membrane surface then there must be a roughly 90° conformational turn at the linkage between the transmembrane domain and the cytoplasmic domain in the native protein. Addition or removal of additional residues to this linkage could profoundly affect the structure and/or membrane interactions of the cytoplasmic domain in a manner which might have little to do with vertical spacing from the membrane surface, representing instead the perturbation of an important conformational linkage.

Implications for E3-13.7 Recognition—It is now known that the 72-YLRH motif of E3-13.7 is critical to high affinity binding of the protein by the clathrin adaptin AP-2 and that both 72-YLRH and 76-HPQY motifs are critical to AP-1 recognition and binding. The affinity of the adaptins for these motifs in native and mutant form within the E3-CyD correlates with the efficiency of EGFR down-regulation by the corresponding E3-13.7 mutants in vivo. Therefore, interaction of these adaptins with the tyrosine motifs are critical to E3-13.7’s in vivo function, probably to ensure appropriate trafficking of the viral protein to the endosomes. Once in endosomes it is quite possible that these or other adaptin-like molecules also interact with the sorting motifs as part of the process by which the viral protein perturbs normal receptor sorting.

The close degree of membrane association we observed for E3-CyD suggests three possible modes by which adaptins and possibly other molecules recognize the sorting motifs present in E3-CyD. First, it is possible that the sorting motifs of E3-13.7 are recognized and bound only during low probability excursions of the cytoplasmic tail of the membrane surface and into solution. This seems improbable because of its inefficiency and because it ascribes no functional significance to intimate membrane association. A second possibility is that the sorting motifs are directly recognized at the interface. This cannot be ruled out, although one might then guess that the adaptins would be integral membrane proteins, which they are not (4). A third possibility is that sorting molecules may not recognize the sorting motifs of the cytoplasmic domain when intimately surface-associated, but that some event can occur in vivo which results in translocation of the domain off of the surface and into solution where it could then be recognized. The sort of “events” which could be imagined to bring about such a change might be post-translational modification of the domain (for which there is presently no evidence, see Ref. 1), modulation by an unidentified membrane protein, or a dramatic shift in the tilt, conformation, or membrane placement of the flanking transmembrane domain resulting in dissociation of the cytoplasmic domain from the surface. This is an intriguing possibility because it would suggest a transmembrane regulatory mechanism governing key events of E3-13.7’s perturbation of normal receptor trafficking. Further experimental work will clearly be needed to establish which of the above possibilities is closest to reality, but the power of structural information to generate experimentally testable hypotheses is hopefully evident.

Implications for the Sorting of Other Membrane Proteins—In general, it is now well appreciated that the recognition of sorting motifs with membrane proteins by components of the cellular protein trafficking machinery is often dependent upon local contextual factors such as the residue spacing between a...
given motif and an adjacent transmembrane domain or the presence of certain residues sequentially adjacent to the sorting motif (9, 10, 31). The present study provides a case study in the structural consequences of motif context. For E3-CyD the fact that the sorting motifs are intimately placed near or at the membrane interface is a function of the overall affinity of the polypeptide for a membrane surface. This affinity arises not only from the residues present in the sorting motifs, but also from hydrophobic residues adjacent to the motifs: it is unlikely that either the 72-FLRH or 87-LL motif would bind tightly to a membrane surface if flanked on both sides by polar residues. Furthermore, the helical secondary structure observed for both the 72-FLRH and 87-LL motifs is almost certainly highly coupled to the fact that these motifs are set within the context of longer amphipathic helices.

How likely is it that the membrane affinity and structural characteristics observed for the E3-CyD are mirrored for sorting motif-containing segments of the many other membrane proteins containing such motifs? We examined this question by carrying out some very simple helical wheel modeling to see if NPYX, YXXZ, and LL motifs present in other proteins may also exist within longer amphipathic helices. As summarized in Table II, some but not all LL and YXXZ motifs are potentially part of amphipathic helices while NPYX is almost never flanked by a putative amphipathic helix (as for E3-13.7). These simple observations suggest that the structural conclusions drawn for E3-CyD should not be overgeneralized. Instead, the results for E3-CyD would appear to indicate that membrane affinity and placement of sorting motifs represents one of multiple possible means by which such motifs can be endowed with local structural context allowing for highly protein-specific sorting behavior in a given cell under given conditions.

REFERENCES
1. Hoffman, P., Yaffe, M. B., Hoffman, B. L., Yei, S., Wold, W. S., and Carlin, C. (1992) J. Biol. Chem. 267, 13480–13487
2. Kuivinen, E., Hoffman, B. L., Hoffman, P. A., and Carlin, C. R. (1993) J. Cell Biol. 120, 1271–1279
3. Hoffman, P., and Carlin, C. (1994) Mol. Cell. Biol. 14, 3696–3706
4. Kirchhausen, T. (1993) Curr. Opin. Struct. Biol. 3, 182–188
5. Gruenberg, J., and Maxfield, F. R. (1995) Curr. Opin. Cell. Biol. 7, 552–563
6. Mellman, I. (1996) Annu. Rev. Cell. Dev. Biol. 12, 575–625
7. Pond, L., Kuhn, L. A., Teyton, L., Schutze, M.-P., Tainer, J. A., Jackson, M. R., and Peterson, P. A. (1995) J. Biol. Chem. 270, 19989–19997
8. Trowbridge, I. S., Collawn, J. F., and Hopkins, C. R. (1993) Annu. Rev. Cell. Biol. 9, 129–161
9. Arneson, L. S., and Miller, J. (1995) J. Cell Biol. 129, 1217–1228
10. Marks, M. S., Ohno, H., Kirchhausen, T., and Bonifacino, J. S. (1997) Trends Cell. Biol. 7, 124–128
11. Chang, C. P., Lazar, C., Walsh, B., Komuro, M., Collawn, J., Kuhn, L., Tainer, J., Trowbridge, I., Farquhar, M., Rosenfeld, M., Wiley, H. S., and Gill, G. N. (1993) J. Biol. Chem. 268, 19312–19320
12. Arueti, B., Rosen, P. A., Kunz, I. D., Cohen, F. E., and Mostov, K. E. (1993) J. Cell Biol. 123, 1149–1169
13. Sandoval, I. V., Arrendondo, J. J., Alcalde, J., Noriega, A. G., Vandekerckhove, J., Jimenez, M. A., and Rico, M. (1994) J. Biol. Chem. 269, 6622–6631
14. Lehmann, L. E., Eberle, W., Krull, S., Prill, V., Schmidt, B., Sander, C., von Figura, K., and Peters, C. (1992) EMBO J. 11, 4391–4399
15. Wilde, A., Dempsey, C., and Banting, G. (1994) J. Biol. Chem. 269, 7131–7136
16. Bansal, A., and Gierrasch, L. M. (1991) Cell 67, 1195–1201
17. Eberle, W., Sander, C., Klaus, W., Schmidt, B., von Figura, K., and Peters, C. (1991) Cell 67, 1203–1209
18. Kroenke, C. D., Ziemnicki-Kotula, D., Xu, J., Kotula, L., and Palmer, A. G. (1997) Biochemistry 36, 8145–8152
19. Lauterwein, J., Bosch, C., Brown, L. R., and Wuthrich, K. (1979) Biochim. Biophys. Acts 556, 244–264
20. Gennis, R. (1989) Bioembranes, 533 pp., Springer-Verlag, New York
21. Vinogradova, O., Sonnichsen, F., and Sanders, C. R. (1998) J. Biol. Chem. 273, 7566–7567
22. Banaszek, A., and Gierasch, L. M. (1991) J. Magn. Res. 95, 13750–13757
23. Bax, A., and Davis, D. G. (1985) J. Magn. Reson. 65, 355–366
24. Pietro, M., Saudek, V., and Sklenar, V. (1992) J. Biol. Chem. 267, 361–366
25. Aller, J. A., Hinton, D. P., and Byrd, R. A. (1993) J. Am. Chem. Soc. 117, 7566–7567
26. Kim, Y., and Prestegard, J. H. (1989) J. Magn. Reson. 84, 9–13
27. Gao, X., and Wong, T. C. (1998) Biophys. J. 74, 1871–1888
28. Stilbs, P. (1982) J. Colloid Interface Sci. 87, 385–394
29. Soderman, O., and Stilbs, P. (1994) Prog. NMR Spectr. 26, 445–482
30. Jonsson, B., Wennerstrom, H., Nilsson, P. G., and Linse, P. (1986) Colloid Polymer Sci. 264, 77–84
31. Rohrer, J., Schweizer, A., Russel, D., and Kornfeld, S. (1996) J. Cell Biol. 133, 565–576
32. Letourneur, F., and Klausner, R. D. (1992) Cell 69, 1143–1157
33. Pumey, C. (1994) EMBO J. 13, 2963–2967
34. Canfield, W. M., Johnson, K. F., Ye, R. D., Gregory, W., and Kornfeld, S. (1991) J. Biol. Chem. 266, 5682–5688
35. Collawn, J. F., Stang, M., Kuhn, L. A., Esekogwu, V., Jing, S., Trowbridge, I. S., and Tainer, J. A. (1990) Cell 63, 1061–1072
36. Williams, M. A., and Fukuda, M. (1990) J. Cell. Biol. 111, 955–966
37. Breitfeld, P. P., Casonova, J. E., McKinney, W. C., and Mostov, K. E. (1996) J. Biol. Chem. 265, 13750–13757